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Concentration, dietary exposure and health risk estimation of polycyclic aromatic hydrocarbons (PAHs) in youtiao, a Chinese traditional fried food

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ABSTRACT

Youtiao, or oil stick, is a typical, traditional and widely-consumed fried food in China. The concentration of polycyclic aromatic hydrocarbons (PAHs) in youtiao from different origins was determined. The dietary exposure and cancer risk associated with benzo[a]pyrene equivalents from youtiao consumption were estimated using Monte Carlo simulation. Analysis of 16 PAHs in youtiao was completed by gas chromatography-mass spectrometry (GC-MS). Concentrations of the sum of 16 PAHs were between 9.90 and 89.97 μg/kg. The sum concentrations of PAH4, including benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF) and benzo[*a*]pyrene (BaP), ranged from 1.41 to 26.56 μg/kg. The median dietary exposure of BaPeq concentrations from youtiao for children, adolescents, adults and seniors in China, were 0.0147, 0.0101, 0.0561 and 0.0106 ng/(kg·day), respectively. Health risk estimates expressed as the 95th percentile incremental lifetime cancer risks (ILCRs) with respect to PAHs indicated a slight potential carcinogenic risk for children in northern China and adults in both the north and south.
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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of well-known toxic environmental and food processing contaminants containing two or more fused aromatic rings. Based on the number of condensed aromatic rings, they can be divided into light (2–4 rings) and heavy (5 or more rings) PAHs. It has been known for a long time that a number of PAHs have carcinogenic, mutagenic and teratogenic properties. The heavy PAHs, such as benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenzo[*a,h*]anthracene, and benzo[*g,h,i*]perylene, are more stable and toxic than the light ones. Other PAHs which are not defined as carcinogens may act as synergists (Plaza-Bolaños, Frenich, & Vidal, 2010). The metabolism of PAHs has also been studied in a number of laboratory animals, human cells and tissues, and shown to have substantial contribution to several types of human cancers, such as breast, pancreas, lung, and colon cancer (Anderson et al., 2005; Armstrong, Hutchinson, Unwin, & Fletcher, 2004; SCF, 2002; Xia et al., 2013). Some PAHs undergo metabolic activation to diol-epoxides, which are capable of binding covalently to DNA (Rengarajan et al., 2015). The U.S. Environmental Protection Agency (EPA) selected 16 priority PAHs based on their occurrence and carcinogenicity. In recent years, studies have found that benzo[*a*]pyrene is not a suitable marker for PAHs occurrence in foods, since it is not a good indicator of the concentration of other carcinogenic PAHs. Thus, the use of the sum of eight genotoxic PAHs, benzo[*a*]pyrene equivalents (BaPeq), as well as the sum of four PAHs, including benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene was recommended (Alomirah et al., 2010; EFSA, 2008; Purcaro, Moret, & Conte, 2013).

Human beings are exposed to PAHs mostly through intake of food, apart from smoking and occupational exposure (Falcó et al., 2003; Martorell et al., 2010; Xia et al., 2010). Food contaminated with PAHs generally arises from environmental contamination, food processing, and direct contact with non-food grade mineral oil and contaminated packaging (Purcaro et al., 2013). Heating is an important cause for PAHs formation in food. A high PAHs level is

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found in cooked foods after processes such as frying, smoking, grilling, and roasting (Farhadian, Jinap, Faridah, & Zaidul, 2012; Lin, Weigel, Tang, Schulz, & Shen, 2011; Perelló, Martí-Cid, Castell, Llobet, & Domingo, 2009; Škaljac et al., 2014).

Youtiao, or oil stick, is a long golden-brown deep-fried stick of dough widely consumed in China. As a traditional breakfast food, youtiao is very popular with all ages of the population. Many reports have revealed the occurrence of PAHs in fried meats and French fries (Chen & Chen, 2003; Janoszka, 2011; Purcaro, Navas, Guardiola, Conte, & Moret, 2006). Very few studies, however, have examined the PAHs content in youtiao and dietary exposure due to its consumption. Moreover, the cancer risk assessment pertaining to PAHs in youtiao has been rarely investigated. Therefore, the objectives of this study were to 1) measure the concentration of the 16 USEPA priority PAHs in commercial and lab-made youtiao samples, 2) estimate the dietary exposure to PAHs through youtiao consumption in northern and southern China using Monte Carlo simulation, and 3) quantify the incremental lifetime cancer risk (ILCR) caused by PAHs dietary exposure from youtiao. Sensitivity and uncertainty analyses were conducted to identify the accuracy of critical input variables.

2. Materials and methods

2.1. Chemicals and materials

All solvents used in this study were of HPLC grade. *n*-Hexane, acetonitrile, acetone, and dichloromethane were purchased from CNW Technologies GmbH (Darmstadt, Germany). Acetone, methanol, and toluene were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Water was purified with a Milli-Q water purification system (Millipore Co., Milford, USA). A standard mixture containing 16 PAHs (catalog no. 47940-U) with concentrations of 10 μg of each compound in 1 ml of acetonitrile was purchased from Supelco Inc. (Bellefonte, PA). This mixture contained the following PAHs: naphthalene (NA), acenaphthylene (Ap), acenaphthene (Ac), fluorine (F), anthracene (Ant), phanthrene (Phe), fluoranthene (Fl), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*k*]fluoranthene (BkF), benzo[*b*]fluoranthene (BbF), benzo[*a*]pyrene (BaP), indeno[1,2,3-*c,d*]pyrene (Ip), dibenzo[*a,h*]anthracene (DBahA), benzo[*g,h,i*]perylene (BghiP). C18 solid phase extraction (SPE) cartridges (2 g, 12 ml) and Florisil SPE cartridges (1 g, 6 ml) were purchased from Supelco Inc. (Bellefonte, PA).

2.2. Sampling and sample preparation

No. 1 to 8 youtiao samples were randomly purchased from eight different locations including food factories, popular chain restaurants, school cafeterias, and representative supermarkets in Shanghai, China, from the years 2012 to 2014. We collected 3–5 batches of samples from each location for analysis. Samples from each batch were mixed prior to analysis. No. 9 and 10 samples were prepared in the laboratory. Origins and frying oils of the samples are shown in Table 1.

The ingredients for lab-made youtiao included 500 g of wheat flour, 8.5 g of salt, 9.5 g of sodium bicarbonate, 8.6 g of aluminum potassium sulfate, and 270 ml of deionized water. Solutions of salt, sodium bicarbonate, and aluminum potassium sulfate in deionized water were added to the flour. All ingredients were kneaded for 5–6 min to form a soft elastic structure and smooth skin. Then the dough was conditioned at 20 °C for 4–6 h. After conditioning, the dough was rolled out and cut into pieces of 8 cm length and 2.5 cm width. Every two pieces were stacked, pressed on the center with a wooden rod, stretched to 25 cm long from both ends, and then deep-fried at 180 °C for 130 s.

Table 1
The origins of youtiao samples.

No.	Origin	Frying oils
1	Factory	soybean oil[a]
2	Restaurant	soybean oil
3	Restaurant	palm oil
4	Cafeteria	soybean oil
5	Cafeteria	soybean oil
6	Supermarket	soybean oil
7	Supermarket	soybean oil
8	Supermarket	soybean oil
9	Lab-made	soybean oil
10	Lab-made	palm oil

[a] Samples obtained in factory are precooked frozen products.

Both the purchased and the lab-made youtiao samples were vacuum-dried (DZF-6020, Shanghai, China) at 60 °C for 2 h after shearing into small pieces with a scissor. Approximately 100 g of dried sample was extracted with 350 ml of *n*-hexane in an ultrasound bath for 1 h. The oil sample was obtained after concentrating the extraction solution with a rotavapour (RE-52AA, Shanghai, China) at 40 °C. The oil samples were placed in sealed glass bottles and stored in the dark at 4 °C before use.

2.3. Extraction and clean-up

Sample extraction and clean-up were performed using the procedure reported by Wu and Yu (2012) with some modifications. Approximately 2.5 g of the oil sample was weighed into a stoppered centrifuge tube, and 10 ml acetonitrile/acetone mixture (3:2, v/v) was added. The sample was agitated for 30 s, ultra-sonicated for 5 min, and centrifuged at 2311 g for 5 min. The upper phase was collected in a new centrifuge tube. The extraction was repeated two more times, and the combined extracts were then concentrated to approximately 200–800 mg in a water bath at 35 °C under a nitrogen flow. The concentrated oil residue was extracted again three more times with 2 ml acetonitrile/acetone mixture (3:2, v/v), shaken for 15 s and centrifuged for 30 s at 2311 g, and the top layer was transferred onto a C18 SPE cartridge, which had been activated with 2 × 12 ml of methanol and then 2 × 12 ml of acetonitrile. Another 5 ml acetonitrile/acetone (3:2, v/v) was eluted through the cartridge. All of the collected eluate was evaporated to approximately 50 mg under a stream of nitrogen and dissolved in 1 ml *n*-hexane. Then the solution was transferred onto a Florisil SPE cartridge, which had been activated with 2 × 6 ml of dichloromethane and 2 × 6 ml of *n*-hexane. Another 5 ml *n*-hexane and 5 ml *n*-hexane/dichloromethane (95:5, v/v) were eluted through the cartridge and discarded. The PAHs were then eluted with 5 ml of *n*-hexane/dichloromethane (1:2, v/v). The eluate was concentrated under a nitrogen flow to approximately 20 μl and diluted with acetonitrile to 250 μl.

The prepared samples were stored in the dark at −18 °C before GC-MS analysis. Standards of different concentrations were added to the samples prior to extraction to estimate the recoveries.

2.4. GC-MS analysis of PAHs

A gas chromatograph-mass spectrometer (Agilent 7890A-5975C, USA) was used for analytical determination. Separation of compounds was performed on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) from J&W Scientific (Folsom, CA, USA). The column temperature was held at 80 °C for 2 min, then increased to 140 °C at 20 °C/min, and then to 305 °C at 3 °C/min. The temperatures of injector, transfer line and ion source were set at 300, 280 and 230 °C, respectively. Helium (purity > 99.999%) was used as carrier gas at a constant flow rate of 1 ml/min. The extract

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