JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Significance of Cooking Oil to Bioaccessibility of Dichlorodiphenyltrichloroethanes (DDTs) and Polybrominated Diphenyl Ethers (PBDEs) in Raw and Cooked Fish: Implications for Human Health Risk

Xiu-Bo Mi,[†,#] Yang Su,[†,#] Lian-Jun Bao,[*,‡] Shu Tao,[§] and Eddy Y. Zeng[†,‡]

[†]State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

[‡]School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China

[§]Laboratory of Earth Surface Processes, College of Urban and Environmental Science, Peking University, Beijing 100871, China

[#]University of Chinese Academy of Sciences, Beijing 100049, China

Supporting Information

ABSTRACT: The present study examined the bioacessibility of DDTs and PBDEs in cooked fish (yellow grouper; *Epinephelus awoara*) with and without heating using the colon extended physiologically based extraction test. The bioaccessibility of DDTs and PBDEs increased from 60 and 26% in raw fish to 83 and 63%, respectively, after the addition of oil to raw fish. However, they decreased from 83 to 66% and from 63 to 40%, respectively, when oil-added fish were cooked. Human health risk assessment based on bioaccessible concentrations of DDTs and PBDEs in fish showed that the maximum allowable daily fish consumption rates decreased from 25, 59, and 86 g day^{-1} to 22, 53, and 77 g day^{-1} for children, youths, and adults, respectively, after fish were cooked with oil. These findings indicated that the significance of cooking oil to the bioaccessibility of DDTs and PBDEs in food should be considered in assessments of human health risk.

KEYWORDS: *bioaccessibility, polyborminated diphenyl eithers, dichlorodiphenyltrichloroethanes, fish consumption, health risk assessment*

INTRODUCTION

The importance of organic contaminant bioavailability has increasingly been recognized worldwide as the potential risk to human and ecological health assessed with total contaminant concentrations may be overestimated.[1,2] Risk assessment based on total concentrations assumes all target compounds are soluble in the gastrointestinal tract and can be absorbed into the systemic circulation. This assumption overestimates the actual intake of target compounds because only portions of the total amounts can reach the systemic circulation (bioavailability). Bioavailability can be determined by animal-based in vivo assays and in vitro models if the results derived from the in vitro models have been well correlated with the in vivo data. However, in vivo assays are expensive, time-consuming, and ethically questionable.[3] To address this issue, bioaccessibility is used as a surrogate, which represents the potential available fraction of a compound for uptake.[4] Although bioaccessibility does not reflect true bioavailability, practically it is close to the actual fraction of a chemical that can be available for uptake by biota or humans and therefore can be used for a more accurate assessment of health risk.[5]

Bioaccessibility is often estimated with in vitro models, which have been used for heavy metals and persistent organic pollutants (POPs)[6−8] among others. As in vitro models may reduce the need for use of laboratory animals and account for matrix effects in risk assessment,[9] they have been used to estimate the bioaccessibility of POPs in different environmental media by many researchers.[5,10−12] For example, the bioaccessibility of POPs in food was estimated by a spiking approach to minimize potential analytical uncertainty;[13,14] however, it was unclear whether the result was altered artificially due to the use of the spiking method. Fish used for assessing bioaccessibility of POPs are often raw,[15−17] but fish consumed are largely cooked. Cooking was shown to alter the concentrations and compositions of POPs in fish.[18−20] In addition, cooking oil has been used in a variety of food preparation procedures. Previous studies found that the bioaccessibility of POPs and POPs-like compounds (e.g., polycyclic aromatic hydrocarbons (PAHs)) in soil and fuel soot increased with the addition of lipid-containing materials (e.g., soybean oil and milk) in digestive models.[21,22] Therefore, the impact of cooking oil on the bioaccessibility of target compounds in fish is critical for assessing human health risk from dietary uptake of cooked fish. Such information has remained limited.

To address the above-mentioned knowledge gap, we evaluated the bioaccessibility of dichlorodiphenyltrichloroethanes (DDTs) and polybrominated diphenyl ethers

Received: February 1, 2017
Revised: April 1, 2017
Accepted: April 6, 2017
Published: April 6, 2017

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

(PBDEs) in native and spiked common edible yellow grouper (*Epinephelus awoara*) caught from typical fishing areas in the Pearl River Delta of southern China. The selection of DDTs and PBDEs as the target compounds was based on previous findings that they were ubiquitous in aquatic products from the sampling areas.[23−25] The objectives of the present study were to (1) examine whether there was significant difference in the bioaccessibility derived from DDTs in native and spiked fish; (2) compare the bioaccessibility of DDTs and PBDEs in raw and cooked fish; and (3) assess the potential human health risk based on the bioaccessibility of DDTs and PBDEs in raw and cooked fish.

MATERIALS AND METHODS

Materials. Standards of individual target analytes, including *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD, *o,p′*-DDD, *p,p′*-DDMU, and *p,p′*-DDNU and the internal standard, 2,2′,3,3′,4-pentachlorobiphenyl (PCB-82), were purchased from AccuStandard (New Haven, CT, USA). *o,p′*-DDT-d_8 used as a surrogate standard, and *p,p′*-DDT-d_8, *p,p′*-DDD-d_8 and *p,p′*-DDE-d_8 used in spiking experiments were purchased from C/D/N Isotopes (Quebec, Canada). Eight individual BDE congeners, including BDE-28, -47, -99, -100, -153, -154, -183, and -209, surrogate standards (BDE-51, BDE-115, and 4′-fluoro-2,2′,3,3′,4,5,5′,6,6′-nonabromodiphenyl ether (F-BDE-208), and internal standards (BDE-69, 3-fluoro-2,2′,4,4′,5,6-hexabromodiphenyl ether (F-BDE-139), and 4′,6-difluoro-2,2′,3,3′,4,5,5′,6′-octabromodiphenyl ether (F-BDE-201) were purchased from AccuStandard. SX-3 Bio-Beads used in gel permeation chromatography were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Mucin from porcine stomach (type Π), bile salts, peptone from casein, tryptone from vegetable, and guar gum were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other in vitro materials were obtained from J&K Scientific (Beijing, China). Hexane of HPLC grade was acquired from Honeywell Burdick & Jackson (Morristown, NJ, USA), whereas dichloromethane (DCM) and acetone were obtained locally and further purified by double distillation before use. Silica gel (80−100 mesh) was purchased from Qingdao Haiyang Chemical (Qingdao, China). Corn oil (Jinlongyu brand; Yihai Kerry, Shenzhen, China) was bought from Xingan Supermarket in Guangzhou, and the saturated, monounsaturated, and polyunsaturated fats were 15, 32, and 53 g in 100 g of corn oil, respectively. Other materials, such as gases and in vitro materials, and pretreatment processes of anhydrous sodium sulfate and silica gel are detailed in the Supporting Information (SI).

Sample Collection and Preparation. Field sampling was conducted in October 2014, at four mariculture zones along the coast of Guangdong province, southern China (Figure S1). Fish individuals were collected from three sampling sites in each mariculture zone, resulting in 20 samples of three fish species (Table S1). All samples were wrapped with aluminum foil, placed in plastic zipper-closed bags onsite, cooled with ice during transport to the laboratory, and stored at −20 °C until analysis.

Yellow grouper samples for assessing bioaccessibility of the target compounds in native and spiked fish were prepared right before the experiment was conducted, according to a previously published (but slightly modified) protocol.[13] Briefly, each edible muscle sample was homogenized, freeze-dried, and ground into fine powder. A 0.5-g dry fish powder sample was spiked with a 1 mL of DCM solution, which was prepared by diluting a 20 μL of hexane stock solution containing *p,p′*-DDT-d_8, *p,p′*-DDD-d_8, and *p,p′*-DDE-d_8 at 5 μg mL^{-1}, BDE-28, -47, -99, -100, -153, -154, and -183 at 1 μg mL^{-1}, and BDE-209 at 10 μg mL^{-1} with DCM in an 1 mL volumetric flask. The spiked fish powder sample was homogenized and placed under a fume hood overnight to allow solvent to evaporate. In addition, approximately 2 g (wet weight) of fresh fish was ground into debris with a stand mixer and subsequently spiked by the same levels of target analytes in an acetone solution. After solvent evaporation, the spiked fish sample was cooked as follows.[18] A 250-mL glass bottle (60 mm i.d. × 140 mm height) containing 0.5 g of fish powder sample or 2 g of fresh fish and 0.5 g of corn oil was placed in a heating block (Ansai, Shenzhen, China) preheated at 200 °C for 15 min. At the same time, a control test with the same type of sample mixed with the same cooking oil but without heating was also conducted. The corn oil was free of DDTs and PBDEs as demonstrated in trial testing prior to use.

Determination of Bioaccessibility of DDTs and PBDEs. The in vitro digestion model used was modified from a recently developed colon-extended physiologically based extraction method (CE-PBET).[14,26] Briefly, the CE-PBET model consists of three compartments, that is, the stomach, small intestine, and colon. An aliquot of uncooked yellow grouper (0.5 g dry weight or 2 g wet weight) was accurately weighed into a labeled glass bottle. A 50-mL gastric solution was added to the labeled glass bottle, which was sealed with a polytetrafluoroethylene membrane-sealed screw cap. For the cooked fish sample, the gastric solution was directly added into the 250-mL glass bottle used during the cooking process. The headspace was purged and filled with ultrapure nitrogen (≥99.999%; Guangdong Huate Gas, Guangzhou, China) to create an anaerobic environment.[27] The bottle was agitated at 80 rpm with a thermostatic oscillator at 37 °C for 1 h. The gastric solution was transformed to a small intestine solution, by adding approximately 0.6 g of $NaHCO_3$ to adjust pH (7.1 ± 0.1) and 5 mL of water solution containing 17.8 g L^{-1} of bile salts and 5 g L^{-1} of pancreatin at a ratio of 10:1 (stomach to water solution in volume). Finally, the bottle was resealed and incubated in a shaker (Bluepard THZ-100; Shanghai, China) for 4 h. The transition between small intestine and colon solutions was achieved by a physical transfer; that is, the test substrate was recovered by centrifugation (4000*g* for 5 min, Sigma 3K15, Darmstadt, Germany), added to a 50-mL prewarmed colon solution at a ratio of 100:1 (solution/sample weight; v/m), and incubated for 8 h.

Sample Extraction. After incubation, a suspended solution from each bottle for small intestine and colon compartments was centrifuged to obtain supernatant (∼50 mL). Fifty milliliters of ultrapure water and 50 mL of acetone containing the surrogate standards were added to the supernatant, which was liquid−liquid extracted three times with 50 mL of DCM each. The solution was rigorously shaken for 5 min and separated into aqueous solution and DCM by centrifugation at 4000*g* for 5 min. The underlying DCM layer was collected with a dropper, and three extracts were combined and concentrated to 1 mL with a Zymark TurboVap 500 (Hopkinton, MA, USA) for purification with a silicon column. The subsequent procedures were the same as those of fish samples.

The spiked fish samples were dosed with a 20-μL surrogate standard solution containing *o,p′*-DDT-d_8 at 5 μg mL^{-1}, BDE-51 and BDE-115 at 1 μg mL^{-1}, and F-BDE-208 at 10 μg mL^{-1} and sonicated three times, each with 40 mL of a mixture of hexane, dichloromethane, and acetone (2:2:1 in volume).[28] The sonicated fish mixture was centrifuged at 4000*g* for 5 min, after which the supernatants were collected. Each extract was concentrated to 1−2 mL, mixed with ∼15 mL of hexane, and further concentrated to approximately 1 mL. Each extract was purified with a glass column packed with 1 cm of anhydrous sodium sulfate, 10 cm of neutral silica gel, and 1 cm of anhydrous sodium sulfate. The fraction containing DDTs and PBDEs was eluted with 15 mL of hexane and concentrated to approximately 1 mL. The final extract was concentrated to 200 μL and spiked with the internal standards before instrumental analysis.

To determine the background levels of DDTs and PBDEs, 5 g of dry fish (∼20 g wet weight) sample was extracted following the same extraction procedure as described above. Each extract was concentrated, solvent-exchanged to DCM and hexane (1:1 in volume), and further concentrated to approximately 1 mL. Each extract was loaded onto the gel permeation chromatograph (with a 30 cm × 1.0 cm i.d. glass column packed with 6 g of Bio-Beads SX-3) and washed with 15 mL of a mixture of DCM and hexane (1:1 in volume). The portion containing DDTs and PBDEs was eluted with 15 mL of the same solution. The eluent was concentrated, solvent-exchanged to hexane, and purified with a silicon column following the same purification procedure described above. The final extract was concentrated to 100

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

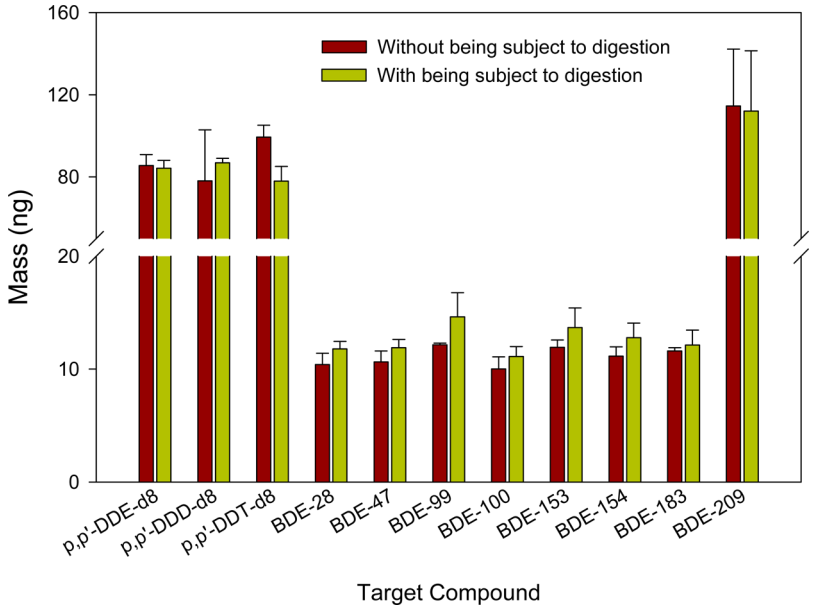

Figure 1. Mass of deuterated DDT compounds and BDE congeners in the blank spiked samples with and without being subjected to digestion process of the colon-extended physiologically based extraction method (n = 3).

μL and spiked with the internal standards prior to instrumental analysis, which is detailed in the SI.

Data Analysis. The bioaccessibility of DDTs and PBDEs in fish, defined as BA%, was calculated by the fraction of target compound mobilized from fish into the gastrointestinal phase with in vitro digestion[14,29]

$$\mathrm{BA\%} = \frac{m_{\mathrm{extracted}}}{m_{\mathrm{total}}} \times 100 \quad (1)$$

where $m_{\mathrm{extracted}}$ and m_{total} are the masses of the target compound extracted from fish and initially present in fish, respectively. It should be noted that BA% was actually calculated by the average $m_{\mathrm{extracted}}$ dividing the average m_{total}. The deviation of BA% was derived from error propagation. This approach was expected to minimize the effects of artificially pairing the masses of target analytes in fish with and without digestion on the value of BA%.

The estimated daily intake (EDI) of DDTs and PBDEs via consumption of a given fish species, noncarcinogenic and carcinogenic hazard quotient (HQ), and maximum allowable fish consumption rate based on carcinogenic effects ($\mathrm{CR_{max}}$) were estimated by the models developed by the U.S. Environmental Protection Agency (USEPA):[30]

$$\mathrm{EDI} = \frac{CR}{\mathrm{BW}} \times \sum_{m=1}^{x} C_m \times \mathrm{BA}_m \quad (2)$$

$$\mathrm{HQ} = \frac{CR}{\mathrm{BW}} \times \sum_{m=1}^{x} \frac{C_m \times \mathrm{BA}_m}{\mathrm{RfD}_m} \text{ (for noncarcinogenic effects)} \quad (3)$$

$$\mathrm{HQ} = \frac{\mathrm{CR}}{\mathrm{BW} \times \mathrm{ARL}} \times \left(\sum_{m=1}^{x} \mathrm{CSF}_m \times C_m \times \mathrm{BA}_m\right) \text{ (for carcinogenic effects)} \quad (4)$$

$$\mathrm{CR_{max}} = \frac{\mathrm{ARL} \times \mathrm{BW}}{\sum_{m=1}^{x} \mathrm{CSF}_m \times C_m \times \mathrm{BA}_m} \quad (5)$$

$$\mathrm{CR_{mm}} = \frac{\mathrm{CR_{max}} \times T_{\mathrm{ap}}}{\mathrm{MS}} \quad (6)$$

$$T_{\mathrm{dm}} = \frac{\mathrm{CR_{max}} \times T_{\mathrm{ap}}}{\mathrm{CR}} \quad (7)$$

where C_m is the concentration of target analyte m in the edible portion of fish (mg kg^{-1}); BA_m is the bioaccessibility (%) of target analyte m in raw and cooked fish; CR is the mean daily consumption rate of fish (kg day^{-1}); BW is the body weight of the consumer (kg); RfD_m is the reference dose of target analyte m for noncarcinogenic effects (mg kg^{-1} day^{-1}); ARL is the maximum acceptable lifetime risk level (unitless), which was taken as 10^{-5} from the USEPA;[30] CSF_m is the cancer slope factor of target analyte m for carcinogenic effects (mg kg^{-1} day^{-1})$^{-1}$; $\mathrm{CR_{mm}}$ is the number of allowable meals per month (meals per month); MS is the amount of fish consumed per meal (0.227 kg of fish per meal); T_{ap} is the time averaging period (365.25 days per 12 months = 30.44 days per month); and T_{dm} is the number of allowable fish consumption days per month (days month^{-1}). Values of CR and BW for different age groups are detailed in Table S4. In the present study, the average concentrations of DDTs and PBDEs in yellow grouper collected from four mariculture zones and their bioaccessible concentrations were used for health risk assessment. The CSF and RfD values for DDTs and PBDEs acquired from the USEPA[31] are listed in Table S5. Because BDE-209 is the only congener with available data for carcinogenic assessment, the CSF and RfD values of other BDE congeners were taken from BDE-209.[32] Our previous study obtained various fish consumption rates from 15.3 to 63.6 g per day for different age groups in coastal cities of Guangdong province, southern China;[33] hence, a value of 227 g per meal from the USEPA seemed not applicable in the present study. We decided to replace $\mathrm{CR_{mm}}$ with T_{dm}. If T_{dm} is higher than 30 days per month, then no obvious human health risk is associated with consumption of the fish species.

Quality Assurance and Quality Control. Two procedural blanks, three spiked blanks, and three spiked samples were analyzed for each batch of 20 samples; all of these samples were processed in triplicate. The recoveries of the surrogate standards, o,p'-DDT-d_8, BDE-51, BDE-115, and F-BDE-208, were 91 ± 17, 77 ± 5, 92 ± 10, and 71 ± 12% in fish samples and 80 ± 26, 81 ± 9, 98 ± 22, and 68 ± 16% in digestive fluid samples, respectively. In addition, a standard solution of p,p'-DDT and BDE-209 was analyzed once for every batch of 10 samples prior to instrumental analysis to ensure the degradation rates of p,p'-DDT and BDE-209 were <10%. Concentrations of DDTs and PBDEs in all samples were not corrected for the recoveries of the surrogate standards. The lowest calibration concentrations divided by the actual sample mass were defined as the limits of quantification for the target compounds, that is, 0.025 ng g^{-1} (except for DDTs and BDE-209, which was 0.25 ng g^{-1}) wet weight for 20 g fish samples and

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

0.02 ng mL^{-1} (except for DDTs and BDE-209, which was 0.20 ng mL^{-1}) for 50 mL digestive fluids. All measured concentrations were expressed on a wet weight basis.

RESULTS AND DISCUSSION

Validity of Colon Extended Physiologically Based Extraction Test. The CE-PBET method was proposed by Tilston et al.[26] to assess the bioaccessibility of PAHs in soil. To validate the method for assessing the bioaccessibility of DDTs and PBDEs in fish, blank spiked experiments (only standards) were conducted. The results indicated that there was no significant difference in the masses of individual DDT and PBDE compounds in the blank spiked samples with and without being subject to digestion (Figure 1). Furthermore, *p,p′*-DDT was believed not to degrade during the digestion processes because no metabolites of *p,p′*-DDT-d_8 were detected in the blank spiked samples. It is worth noting that there was no apparent readsorption of DDTs and PBDEs to the digestive residues after in vitro digestion. Previous studies demonstrated that if sorption of mobilized pollutants occurred on the digestive residues, their bioaccessibility would be underestimated due to readsorption.[34,35] The difference between our results and previously reported findings was probably caused by different fluid/solid ratios employed. The fluid/solid ratio was 7 and 35 in the previous studies,[34,35] whereas it was 100 in the present study. It was proposed that the concentrations of organochlorine pesticides in separated fluids increased with increasing fluid/solid ratio.[34] A previous study also found that the liquid/solid ratio had significant effects on the bioaccessibility of PBDEs in foodstuffs when it fell below 90.[13] All of these results corroborated the validity of the CE-PBET method for assessing the bioaccessibility of DDTs and PBDEs in fish.

Bioaccessibility of DDTs in Native and Spiked Fish. To examine whether there was any difference in BA% of DDTs between native and spiked fish, fish samples containing high concentrations of DDTs were desirable. One fish species (yellow grouper) from Hailing Bay contained substantially higher concentration of DDTs (110 ng g^{-1}) than other fish species (Table S2). This was consistent with the results of our previous studies.[36] Therefore, we used this sample as the digestion material.

The BA% of DDTs in native fish (48 ± 12%) in the present study was greater than those in the market fish (5−46%; mean = 23%) in a previous study.[36] This was probably due to the use of different in vitro models. The difference between the two models was the use of different model components and fluid/solid ratios. The model components were gastric, intestinal, and colon conditions, and the fluid/solid ratio was 100 in the present study, whereas the model components were gastric and intestinal conditions only and the fluid/solid ratio was 10 in the previous study.[36] Furthermore, the BA% values of *p,p′*-DDE-d_8, *p,p′*-DDD-d_8, and *p,p′*-DDT-d_8 were 48 ± 2.1, 56 ± 11, and 47 ± 11%, consistent with those of *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT (50 ± 9.1, 46 ± 15, and 47 ± 16%, respectively) in native fish. Apparently BA% values were not significantly different between native and spiked fish on the basis of either individual DDT components or total DDTs (Figure 2). This confirmed the validity of utilizing spiked samples[14,27,37] to mimic field samples in in vitro digestion experiments. At the same time, previous studies have documented that bioaccessibility was not affected by analyte concentrations at a fixed ratio of the digestive fluid volume to the mass of foodstuff.[13,38] Therefore, the spiking method was feasible for generating the required analyte concentrations for in vitro experiments.

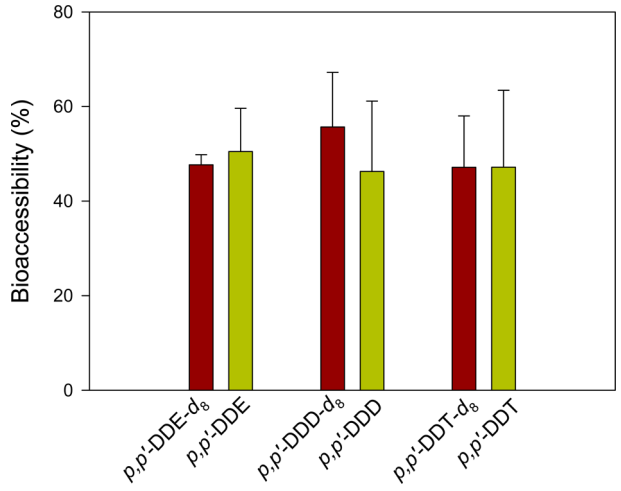

Figure 2. Bioaccessibility of deuterated and native DDT compounds in yellow grouper (*Epinephelus awoara*) by using the colon extended physiologically based extraction method (n = 3).

Bioaccessibility of PBDEs in Spiked Fish. The highest concentration of PBDEs was 0.94 ± 0.06 ng g^{-1} in silver croaker (*Pennahia argentata*) from Honghai Bay (Table S3), too low for estimating BA% values with the in vitro method. Because BA% was not significantly different between native and spiked fish for either individual DDT components or total DDTs, the BA% of PBDEs in yellow grouper was also estimated by a spiking approach. Furthermore, Yu et al.[13] examined the bioaccessibility of PBDEs (BDE-17, -28, -47, -66, -71, -85, -99, -100, -138, -153, -154, -183, and -190) in fish with spiking concentrations of 10, 20, 60, 100, 140, and 200 ng g^{-1} dry weight for each individual BDE congener. The results showed that the bioaccessibility of PBDEs was not affected by spiking concentrations if the liquid to solid (L/S) ratio was fixed in the digestive model. In addition, the BA% values of DDTs and PBDEs in untreated and treated fresh fish were not significantly different from those in untreated and treated dry fish powder except for BDE-28 and BDE-47 in raw fish and *p,p′*-DDT in cooked fish (Figure S4). This result suggested that homogenized fish samples can also be prepared from fresh fish spiked with target analytes. Because the BA% of DDTs and PBDEs in fresh fish are closely related to the transport of bioaccessible target compounds in the digestive process of fish in humans, they are further discussed in the following.

There was no significant difference among the bioaccessibility values of individual target BDE congeners in raw fish, fish with oil added but without heating, and fish cooked with oil (Figure 3). This finding was consistent with the bioaccessibility of PBDEs in indoor dust using the same in vitro method.[39] The BA% of BDE-209 (23 ± 4.2%) in raw fish from the CE-PBET in vitro model was close to the bioavailability of BDE-209 (26%) in rat.[40] In addition, another study[15] found that the BA% of PBDEs, determined with a method simulating human gastrointestinal digestion process, ranged from 5 to 105% in animal-sourced food, and fat content was a major factor responsible for the variability of BA%. However, the BA% of total PBDEs in raw fish (26 ± 3%) in the present study was higher than that in the previous study (20 ± 1.2%) with similar fat contents (both ∼5%; Table S1).[15] Although this may be partly attributed to the characteristics of the specific fish

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

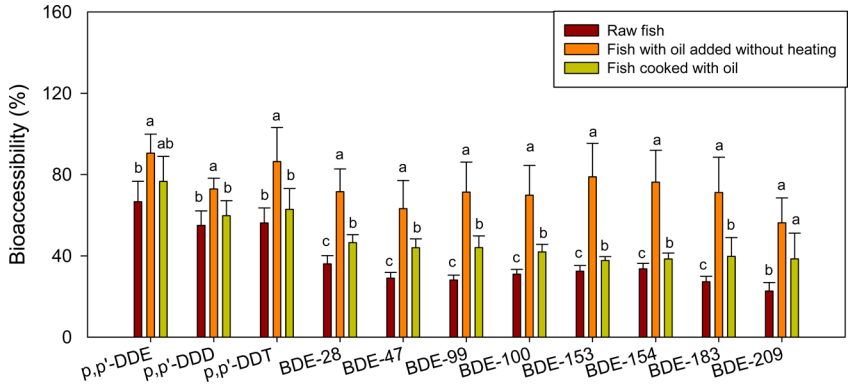

Figure 3. Bioaccessibility of DDT compounds and BDE congeners in raw and cooked yellow grouper (*E. awoara*) using the colon extended physiologically based extraction method (n = 5). Herein, fresh raw fish was used for cooking. Different letters indicate significant differences between groups ($p < 0.05$).

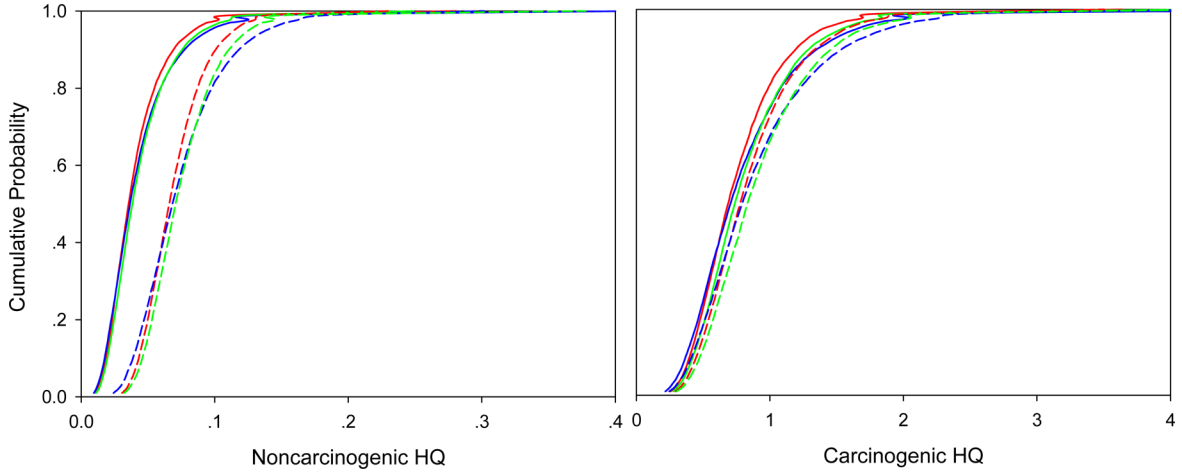

Figure 4. Noncarcinogenic and carcinogenic hazard quotient (HQ) estimated with the bioaccessible concentrations of DDTs and PBDEs in raw and cooked yellow grouper (*E. awoara*) for children, youths, and adults in Guangdong province, southern China. Red, blue, and green colors indicate children, youths, and adults, respectively, whereas solid and dashed curves represent the bioaccessible concentrations of DDTs and PBDEs in raw and cooked fish, respectively. Here, DDTs also include *p,p′*-DDNU and *p,p′*-DDMU detected in yellow grouper. Detailed results from the assessment of carcinogenic effects are presented in Tables S5 and S6.

samples examined in these two studies, the addition of an 8 h colon digestion was likely to be the major cause for this difference.[26,39]

Bioaccessibility of DDTs and PBDEs in Raw and Cooked Fish. Heating did not seem to exert any significant effect on the compositions and contents of DDTs and PBDEs in freeze-dried fish (Figure S2). However, significant decreases in the masses of DDTs and PBDEs in fresh fish were observed after frying (Figure S3). This was consistent with previous results[41,42] that the concentrations of DDTs in fish declined upon cooking. In contrast, Boer et al.[43] showed that the concentrations of DDTs increased in eel after frying due to moisture loss. The mass of PBDEs in the fresh fish also decreased during the frying process, consistent with a previous finding that small amounts of PBDEs were evaporated from cooked fish.[20] These results indicated that water present in fish is significant to mass change of organic contaminants during the cooking processes.

Figure 3 displays the changes in the bioaccessibility of DDTs and PBDEs in raw fish, fish with oil added but without heating, and fish cooked with oil. Addition of plant oil to fish substantially increased the BA% of DDTs and PBDEs with or without heating, demonstrating the significance of plant oil in mediating the bioaccessibility of DDTs and PBDEs in fish. A previous study found that the BA% of 11 PAHs (fluorene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*g,h,i*]perylene) in fuel soot increased with inclusion of lipids into digestive fluid, attributed to the combined effect of facilitated mass transfer from nonlabile to labile phase and propelled partitioning into bile acid micelles.[22] Addition of whole milk powders elevated the fractions of mobilized PAHs and polychlorinated biphenyls in different contaminated soils, from 7 to 95% and from 32 to 83%, respectively.[21] Fish fat content can also influence the bioaccessibility of contaminants. For example, a significant positive relationship (R^2 = 0.86, $p <$ 0.001) was observed between the bioaccessibility of total PBDEs (sum of BDE-17, -28, -47, -66, -71, -85, -99, -100, -138, -153, -154, -183, and -190) and fat contents of raw animal-based foods.[36] Similarly, the bioaccessibility of most PAH congeners was positively correlated with lipid contents in animal-based foods (R^2 = 0.752, p = 0.005).[44] Apparently, fish with oil added and foods with high fat contents exhibited enhanced BA% of organic compounds.

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

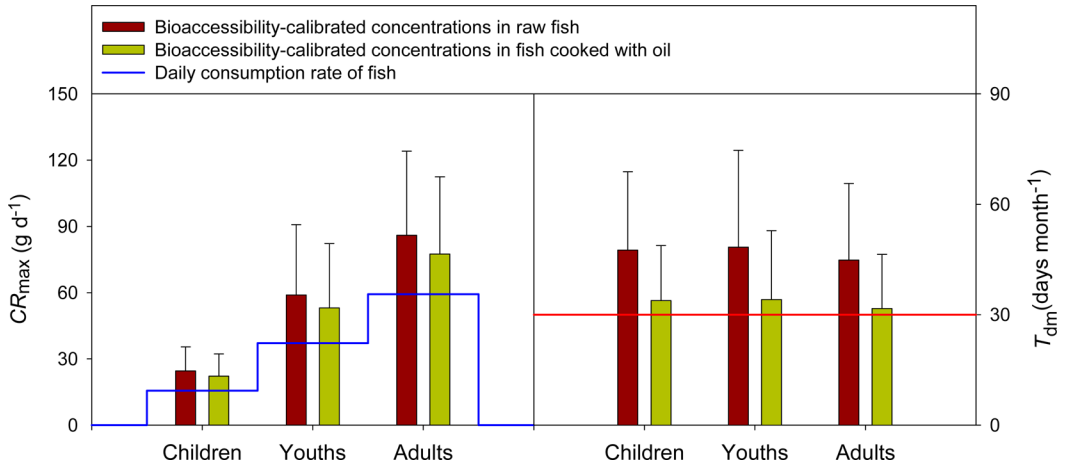

Figure 5. Maximum allowable fish consumption rate (CR_{max}) and number of days per month (T_{dm}) on the basis of carcinogenic effects estimated with the bioaccessible concentrations of DDTs and PBDEs in raw and cooked fish for children, youths, and adults in Guangdong province, southern China. The carcinogenic effect was set at 10^{-5}, acquired from the U.S. Environmental Protection Agency.[30] The daily consumption rates of fish for children, youths, and adults in Guangdong province, southern China, were derived from a dietary survey in 2006.[33] Here, DDTs also include *p,p′*-DDNU and *p,p′*-DDMU detected in yellow grouper. Detailed results from the assessment of carcinogenic effects are presented in Tables S5 and S6.

The BA% of DDTs and PBDEs decreased from 83 to 66% and from 63 to 40%, respectively, in fish subjected to heating after oil was added (Figure 3). A previous finding also found that cooking with harsh thermal conditions generally led to decreased bioaccessibility of total lipids, selenium, total mercury, and methylmercury in farmed meagre (*Argyrosomus regius*).[45] Zhang et al.[22] ascribed the increase in bioaccessibility of PAHs in soot with lipid addition to the formation of mixed lipid−bile acid micelles, in which the solubility of hydrophobic compounds was greater than in pure bile acid. On the other hand, heat can break down hydrogen bonds constructing the three-dimensional framework of proteins during the cooking processes. Protein denaturation may result in lowered water solubility and aggregation due to the exposure of hydrophobic groups. Apparently, lipid prefers to affiliate with denatured proteins, thereby reducing the formation of mixed lipid−bile acid micelles and lipid bioaccessibility. As a result, the reduction in BA% of DDTs and PBDEs in cooked fish may be attributed to protein denaturation during the frying process (temperatures in fish: 100−150 °C). Denatured proteins would aggregate and trap a portion of cooking oil[45] and consequently reduce the BA % of the targets analytes in cooked fish.

Bioaccessibility-Based Assessment of Fish Consumption Limits. The measured BA% values were used to correct the concentrations of DDTs and PBDEs in fish, so as to better examine fish consumption limits. The BA% of DDTs and PBDEs were 59 and 26%, and 83 and 63%, and 66 and 40% in raw fish, fish with oil added but without heating, and fish cooked with oil, respectively. Detailed results from the assessment of noncarcinogenic and carcinogenic HQs for local residents of different age groups are presented in Tables S5 and S6. The results showed that the noncarcinogenic HQs based on the bioaccessible concentrations for different age groups were all substantially smaller than one (Figure 4 and Figure S5), suggesting that consumption of the fish species did not pose a noncancer risk. In addition, the noncarcinogenic HQs were not significantly different among different age groups. Human exposure risk based on total concentration may be overestimated and expectedly the risk assessment can be better conducted involving BA% values of organic contaminants in cooked food. Therefore, bioaccessibility-based assessment of human health risk should take into account different cooking processes.

The carcinogenic HQs based on the bioaccessible concentrations in raw and cooked fish were also not significantly different among different age groups (Figure 4). However, enhanced carcinogenic HQs for different age groups were obtained when cooking oil was incorporated into the risk assessment, suggesting high cancer risk associated with the consumption of cooked fish. For example, the carcinogenic HQs for children with dietary uptake of raw fish increased from 0.76 (95% confidence interval (CI) of 0.34−1.39) to 0.85 (95% CI of 0.39−1.57) with cooked fish (Table S6). The fish consumption advisories with carcinogenic effects (Figure 5) were calculated for the maximum individual acceptable risk level of 10^{-5}. Local residents in Guangdong province, southern China, may be subject to carcinogenic risk if they consume cooked fish with daily consumption rates of 16, 37, and 60 g day^{-1} for children, youths, and adults, respectively, which were derived from a dietary survey in 2006.[33] To minimize carcinogenic risk, the number of days per month for consuming cooked fish by local residents should be fewer than 19 based on a worst-case scenario (95% CI of T_{dm} of 19−69). Although the general population also consumes other foodstuffs, the result presented herein represents the worst-case scenario, which can be used to establish fish consumption advisories for consumers, especially in coastal regions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b00505.

Wet weights, lengths, lipid contents, and water contents of fish; DDT and PBDE levels in fish; fish consumption and body weights for the different age groups; and noncarcinogenic and carcinogenic hazard quotient (HQ) (Tables S1−S6); map of sampling sites; mass and bioaccessibility of DDTs and PBDEs in raw and cooked fish; and cumulative probability of noncarcinogenic and carcinogenic hazard quotient (HQ) (Figures S1−S5) (PDF)

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

AUTHOR INFORMATION

Corresponding Author

*(L.-J.B.) Phone: +86-020-85226615. Fax: +86-020-85226615. E-mail: baolianjun@jnu.edu.cn.

ORCID

Lian-Jun Bao: 0000-0002-0634-0829

Shu Tao: 0000-0002-7374-7063

Funding

The present study was financially supported by the National Natural Science Foundation of China (No. 41390240) and the Department of Science and Technology of Guangdong Province (No. 2015B020237006).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Special thanks go to Chen-Chou Wu for laboratory support. This is contribution no. IS-2379 from GIGCAS.

REFERENCES

(1) Wang, H.-S.; Xu, W.-F.; Chen, Z.-J.; Cheng, Z.; Ge, L.-C.; Man, Y.-B.; Giesy, J. P.; Du, J.; Wong, C. K. C.; Wong, M.-H. In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes. *J. Hazard. Mater.* **2013**, *248*, 387−393.

(2) Lorenzi, D.; Entwistle, J.; Cave, M.; Wragg, J.; Dean, J. R. The application of an in vitro gastrointestinal extraction to assess the oral bioaccessibility of polycyclic aromatic hydrocarbons in soils from a former industrial site. *Anal. Chim. Acta* **2012**, *735*, 54−61.

(3) Beriro, D. J.; Cave, M. R.; Wragg, J.; Thomas, R.; Wills, G.; Evans, F. A review of the current state of the art of physiologically-based tests for measuring human dermal in vitro bioavailability of polycyclic aromatic hydrocarbons (PAH) in soil. *J. Hazard. Mater.* **2016**, *305*, 240−259.

(4) Etcheverry, P.; Grusak, M. A.; Fleige, L. E. Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Front. Physiol.* **2012**, *3*, 1−22.

(5) Collins, C. D.; Craggs, M.; Garcia-Alcega, S.; Kademoglou, K.; Lowe, S. Towards a unified approach for the determination of the bioaccessibility of organic pollutants. *Environ. Int.* **2015**, *78*, 24−31.

(6) Gouliarmou, V.; Collins, C. D.; Christiansen, E.; Mayer, P. Sorptive physiologically based extraction of contaminated solid matrices: incorporating silicone rod as absorption sink for hydrophobic organic contaminants. *Environ. Sci. Technol.* **2013**, *47*, 941−948.

(7) Collins, C. D.; Mosquera-Vazquez, M.; Gomez-Eyles, J. L.; Mayer, P.; Gouliarmou, V.; Blum, E. Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils? *Environ. Pollut.* **2013**, *181*, 128−132.

(8) Ertl, H.; Butte, W. Bioaccessibility of pesticides and polychlorinated biphenyls from house dust: in-vitro methods and human exposure assessment. *J. Exposure Sci. Environ. Epidemiol.* **2012**, *22*, 574−583.

(9) Ramesh, A.; Walker, S. A.; Hood, D. B.; Guillen, M. D.; Schneider, K.; Weyand, E. H. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *Int. J. Toxicol.* **2004**, *23*, 301−333.

(10) Zhang, Y.-Y.; Pignatello, J. J.; Tao, S.; Xing, B.-S. Bioacessibility of PAHs in fuel soot assessed by an in vitro digestive model: effect of including an absorptive sink. *Environ. Sci. Technol.* **2015**, *49*, 3905−3912.

(11) James, J.; Peters, R. E.; Laird, B. D.; Ma, W. K.; Wickstrom, M.; Stephenson, G. L.; Siciliano, S. D. Human exposure assessment: a case study of 8 PAH contaminated soils using in vitro digestors and the juvenile swine model. *Environ. Sci. Technol.* **2011**, *45*, 4586−4593.

(12) Rostami, I.; Juhasz, A. L. Assessment of persistent organic pollutant (POP) bioavailability and bioaccessibility for human health exposure assessment: a critical review. *Crit. Rev. Environ. Sci. Technol.* **2011**, *41*, 623−656.

(13) Yu, Y.-X.; Han, S.-Y.; Zhang, D.-P.; Van der Wiele, T.; Lu, M.; Wang, D.-Q.; Yu, Z.-Q.; Wu, M.-H.; Sheng, G.-Y.; Fu, J.-M. Factors affecting the bioaccessibility of polybrominated diphenylethers in an in vitro digestion model. *J. Agric. Food Chem.* **2009**, *57*, 133−139.

(14) Li, K.; Li, C.; Yu, N.-Y.; Juhasz, A. L.; Cui, X.-Y.; Ma, L. Q. In vivo bioavailability and in vitro bioaccessibility of perfluorooctanoic acid (PFOA) in food matrices: correlation analysis and method development. *Environ. Sci. Technol.* **2015**, *49*, 150−158.

(15) Yu, Y.-X.; Huang, N.-B.; Zhang, X.-Y.; Li, J.-L.; Yu, Z.-Q.; Han, S.-Y.; Lu, M.; Van der Wiele, T.; Wu, M.-H.; Sheng, G.-Y.; Fu, J.-M. Polybrominated diphenyl ethers in food and associated human daily intake assessment considering bioaccessibility measured by simulated gastrointestinal digestion. *Chemosphere* **2011**, *83*, 152−160.

(16) Xing, G.-H.; Yang, Y.; Chan, J. K. Y.; Tao, S.; Wong, M.-H. Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. *Environ. Pollut.* **2008**, *156*, 1218−1226.

(17) Harris, K. L.; Banks, L. D.; Mantey, J. A.; Huderson, A. C.; Ramesh, A. Bioaccessibility of polycyclic aromatic hydrocarbons: relevance to toxicity and carcinogenesis. *Expert Opin. Drug Metab. Toxicol.* **2013**, *9*, 1465−1480.

(18) Bendig, P.; Blumenstein, M.; Hägele, F.; Vetter, W. Hydrodebromination of decabromodiphenyl ether (BDE-209) in cooking experiments with salmon fillet. *J. Agric. Food Chem.* **2012**, *60*, 8521−8527.

(19) Bhavsar, S. P.; Zhang, X.-M.; Guo, R.; Braekevelt, E.; Petro, S.; Gandhi, N.; Reiner, E. J.; Lee, H.; Bronson, R.; Tittlemier, S. A. Cooking fish is not effective in reducing exposure to perfluoroalkyl and polyfluoroalkyl substances. *Environ. Int.* **2014**, *66*, 107−114.

(20) Bendig, P.; Hägele, F.; Blumenstein, M.; Schmidt, J.; Vetter, W. Fate of polybrominated diphenyl ethers during cooking of fish in a new model cooking apparatus and a household microwave. *J. Agric. Food Chem.* **2013**, *61*, 6728−6733.

(21) Hack, A.; Selenka, F. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. *Toxicol. Lett.* **1996**, *88*, 199−210.

(22) Zhang, Y.-Y.; Pignatello, J. J.; Tao, S.; Xing, B.-S. Bioaccessibility of PAHs in fuel soot assessed by an in vitro digestive model with absorptive sink: effect of food ingestion. *Environ. Sci. Technol.* **2015**, *49*, 14641−14648.

(23) Sun, R.-X.; Luo, X.-J.; Tang, B.; Li, Z.-R.; Wang, T.; Tao, L.; Mai, B.-X. Persistent halogenated compounds in fish from rivers in the Pearl River Delta, South China: geographical pattern and implications for anthropogenic effects on the environment. *Environ. Res.* **2016**, *146*, 371−378.

(24) Yu, H.-Y.; Guo, Y.; Bao, L.-J.; Qiu, Y.-W.; Zeng, E. Y. Persistent halogenated compounds in two typical marine aquaculture zones of South China. *Mar. Pollut. Bull.* **2011**, *63*, 572−577.

(25) Leung, S. Y.; Kwok, C. K.; Nie, X. P.; Cheung, K. C.; Wong, M. H. Risk assessment of residual DDTs in freshwater and marine fish cultivated around the Pearl River delta, China. *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 415−430.

(26) Tilston, E. L.; Gibson, G. R.; Collins, C. D. Colon extended physiologically based extraction test (CE-PBET) increases bioaccessibility of soil-bound PAH. *Environ. Sci. Technol.* **2011**, *45*, 5301−5308.

(27) Yu, Y.-X.; Lu, M.; Jia, W.-L.; Van der Wiele, T.; Han, S.-Y.; Zhang, D.-P.; Wu, M.-H.; Sheng, G.-Y.; Fu, J.-M. Carbon isotope effects of DDTs in carrot during the digestion process using an in vitro test. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 2803−2808.

(28) Wu, C.-C.; Bao, L.-J.; Guo, Y.; Li, S.-M.; Zeng, E. Y. Barbecue fumes: an overlooked source of health hazards in outdoor settings? *Environ. Sci. Technol.* **2015**, *49*, 10607−10615.

(29) Oomen, A. G.; Rompelberg, C. J. M.; Van de Kamp, E.; Pereboom, D. P. K. H.; De Zwart, L. L.; Sips, A. J. A. M. Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. *Arch. Environ. Contam. Toxicol.* **2004**, *46*, 183−188.

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275

(30) U.S. Environmental Protection Agency (USEPA). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Vol. 2: Risk Assessment and Fish Consumption Limits, http://www.epa.gov/sites/production/files/2015-01/documents/fish-volume2.pdf (accessed Jan 26, 2016).
(31) U.S. Environmental Protection Agency (USEPA). IRIS Advanced Search, https://cfpub.epa.gov/ncea/iris/search/index.cfm (accessed Aug 25, 2016).
(32) Yu, Y.-X.; Wang, X.-X.; Yang, D.; Lei, B.-L.; Zhang, X.-L.; Zhang, X.-Y. Evaluation of human health risks posed by carcinogenic and non-carcinogenic multiple contaminants associated with consumption of fish from Taihu Lake, China. *Food Chem. Toxicol.* **2014**, *69*, 86−93.
(33) Guo, J.-Y.; Wu, F.-C.; Shen, R.-L.; Zeng, E. Y. Dietary intake and potential health risk of DDTs and PBDEs via seafood consumption in South China. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1812−1819.
(34) Tao, S.; Lu, Y.; Zhang, D.-Y.; Yang, Y.-F.; Yang, Y.; Lu, X.-X.; Sai, D.-J. Assessment of oral bioaccessibility of organochlorine pesticides in soil using an in vitro gastrointestinal model. *Environ. Sci. Technol.* **2009**, *43*, 4524−4529.
(35) Tao, S.; Zhang, D.-Y.; Lu, Y.; Li, L.; Ding, J.-N.; Yang, Y.; Yang, Y.-F.; Wang, X.-L.; Liu, W.-X.; Xing, B.-S. Mobility of polycyclic aromatic hydrocarbons in the gastrointestinal tract assessed using an in vitro digestion model with sorption rectification. *Environ. Sci. Technol.* **2010**, *44*, 5608−5612.
(36) Wang, H.-S.; Zhao, Y.-G.; Man, Y.-B.; Wong, C. K. C.; Wong, M.-H. Oral bioaccessibility and human risk assessment of organochlorine pesticides (OCPs) via fish consumption, using an in vitro gastrointestinal model. *Food Chem.* **2011**, *127*, 1673−1679.
(37) Yu, Y.-X.; Li, J.-L.; Zhang, X.-Y.; Yu, Z.-Q.; Van der Wiele, T.; Han, S.-Y.; Wu, M.-H.; Sheng, G.-Y.; Fu, J.-M. Assessment of the bioaccessibility of polybrominated diphenyl ethers in foods and the correlations of the bioaccessibility with nutrient contents. *J. Agric. Food Chem.* **2010**, *58*, 301−308.
(38) Oomen, A. G.; Sips, A. J. A. M.; Groten, J. P.; Sijm, D. T. H. M.; Tolls, J. Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. *Environ. Sci. Technol.* **2000**, *34*, 297−303.
(39) Abdallah, M. A.-E.; Tilston, E.; Harrad, S.; Collins, C. In vitro assessment of the bioaccessibility of brominated flame retardants in indoor dust using a colon extended model of the human gastrointestinal tract. *J. Environ. Monit.* **2012**, *14*, 3276−3283.
(40) Sandholm, A.; Emanuelsson, B. M.; Wehler, E. K. Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. *Xenobiotica* **2003**, *33*, 1149−1158.
(41) Puffer, H. W.; Gossett, R. W. PCB, DDT, and benzo(a)pyrene in raw and pan-fried white croaker (*Genyonemus lineatus*). *Bull. Environ. Contam. Toxicol.* **1983**, *30*, 65−73.
(42) Zabik, M. E.; Zabik, M. J.; Booren, A. M.; Nettles, M.; Song, J.-H.; Welch, R.; Humphrey, H. Pesticides and total polychlorinated biphenyls in Chinook salmon and carp harvested from the Great Lakes: effects of skin-on and skin-off processing and selected cooking methods. *J. Agric. Food Chem.* **1995**, *43*, 993−1001.
(43) de Boer, J.; Lammertse, N.; Koekkoek, J.; van Hattum, B. PCB and organochlorine pesticide concentrations in eel increase after frying. *Chemosphere* **2013**, *90*, 139−142.
(44) Yu, Y. X.; Chen, L.; Yang, D.; Pang, Y. P.; Zhang, S. H.; Zhang, X. Y.; Yu, Z. Q.; Wu, M. H.; Fu, J. M. Polycyclic aromatic hydrocarbons in animal-based foods from Shanghai: bioaccessibility and dietary exposure. *Food Addit. Contam., Part A* **2012**, *29*, 1465−1474.
(45) Afonso, C.; Costa, S.; Cardoso, C.; Bandarra, N. M.; Batista, I.; Coelho, I.; Castanheira, I.; Nunes, M. L. Evaluation of the risk/benefit associated to the consumption of raw and cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid and docosahexaenoic acid, total mercury, and methylmercury determined by an in vitro digestion model. *Food Chem.* **2015**, *170*, 249−256.

DOI: 10.1021/acs.jafc.7b00505
J. Agric. Food Chem. 2017, 65, 3268−3275