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Oral Bioaccessibility of Polycyclic Aromatic Hydrocarbons (PAHs) through Fish Consumption, Based on an in Vitro Digestion Model

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An in vitro gastrointestinal digestion model was used to evaluate bioaccessibility of PAHs in 20 fish species collected from Hong Kong markets. The average bioaccessibilities of PAHs were 24.3 and 31.1%, respectively, in gastric and intestinal conditions. When bioaccessibility was taken into consideration, the values of potency equivalent concentrations (PEC) decreased from 0.53 to 0.18 ng g^{-1} for freshwater fish and from 1.43 to 0.35 ng g^{-1} for marine fish. This indicated that bioaccessibility should be taken into account for health risk assessment with regard to PAH contamination in fish. The relative accumulation ratios (R_{nn}) of PAH congeners were significantly correlated with their physicochemical parameters and their corresponding concentrations reported in subcutaneous fats of Hong Kong residents. The data suggest that R_{nn} values calculated in the present study could effectively reflect the accumulations of PAHs in the human body.

KEYWORDS: Bioaccessibility; PAHs; freshwater fish; marine fish; gastrointestinal digestion model; Hong Kong

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are primarily derived from incomplete combustion of fossil fuels and burning of vegetation and other organic materials (*1*). Due to their carcinogenic properties and acute toxicity, 16 congeners of PAHs have been listed as priority control pollutants by the U.S. Environmental Protection Agency (EPA) (*2*). Food intake is the major route for the general population's exposure to PAHs (*3*). Seafoods, particularly freshwater and marine fish, are the major components of the diet for Hong Kong residents. The average Hong Kong person consumes fish or shellfish four or more times per week, at about 164.4 g per day (*4*). Earlier studies indicated that PAH concentrations in fish collected from the Pearl River delta (PRD) were rather high: 1050−4260 ng g^{-1} (dry weight, dw) in wild fish (*5*), 62−196 ng g^{-1} (dw) in freshwater fish (*6*), and 46.5−354 ng g^{-1} (wet weight, ww) in market fish (*7*) respectively.

The use of total extractable PAHs from contaminated foods for general health risk assessment may result in an overestimation of the amount of contaminants absorbed via oral intake (*3*). Some in vivo experiments were carried out to analyze the intake efficiency of PAHs. It was observed that the absorption rate of benzo(*a*)pyrene (BaP) was only 33% in pigs (*8*) and 12% in lactating goats (*9*). Therefore, for assessing health risks posed by PAHs, bioavailability should be taken into consideration (*10*). However, in vivo studies are expensive, laborious and time-consuming, and can pose ethical dilemmas (*11*), so in vitro methods have been adopted for estimating the bioaccessibility of contaminants in food items (such as selenium and mercury in fish (*11*) and polychlorinated biphenyl (PCBs) in vegetables and fish (*12*)).

The bioaccessibility of a contaminant in foods is defined as the fraction of the contaminant mobilized from the food matrices during gastrointestinal digestion (*13*). In terms of PAHs, there are only a few studies concerning their bioaccessibility in soil samples. Holman et al. (*14*) observed substantial enhancement of the solubility of hydrophobic petroleum hydrocarbons in the fat digestion condition relative to the fasted condition, presumably contributint to the lowering of their surface tension by bile salts and the formation of "mixed micelles". Van de Wiele et al. (*15*) observed that the release of PAHs in stomach digests was only 0.44% of the total PAHs present in soils. Tang et al (*16*) revealed that the oral bioaccessibility of total PAHs ranged from 9.2 to 60.5%, as a means of assessing human exposure to PAHs in soils.

However, there is a lack of information concerning the bioaccessibility of PAHs in foods. Therefore, the bioaccessibilities of PAHs in several species of common market fish were investigated in the present study. More specifically, the objectives were (1) to develop an in vitro digestion method for analyzing the bioaccessibility of PAHs contained in fish and (2) to assess the accumulation ratios for the oral intake of PAHs for Hong Kong residents though fish consumption. The associated potential health risk assessment was assessed. To our knowledge, this is the first study to investigate the bioaccessibility of PAHs in foods using an in vitro digestion method.

MATERIALS AND METHODS

Sample Collection and Treatment. Twenty species of commonly consumed fish were purchased from local fish markets from May to

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Table 1. Fish Species Obtained from Local Markets in Hong Kong from May to November 2009

	common name	scientific name	n	length (cm)	weight (g)	producing area	feeding mode	food items
				Freshwater Fish				
1	tilapia	*Oreochromis mossambicus*	10	27.0 ± 2.07	432 ± 8.38	Shunde, PRD	omnivorous	plant tissue, small fish, shrimp, detritus, and sediment
2	snakehead	*Channa asiatiea*	12	31.6 ± 0.38	449 ± 25.0	Shunde, PRD	carnivorous	crustaceans, insect larvae, and fish
3	spotted snakehead	*Channa maculate*	10	28.8 ± 0.14	254 ± 12.5	Shunde, PRD	carnivorous	crustaceans, large insects, fish, and even frogs
4	rice field eel	*Monopterus albus*	14	58.6 ± 3.70	279 ± 17.3	Shunde, PRD	carnivorous	crustaceans, insects, fish, and invertebrates
5	mud carp	*Cirrhina molitorella*	15	28.0 ± 1.40	414 ± 55.7	New Territories, HK	bottom feeder	algae, phytoplankton, and organic detritus
6	mandarin fish	*Siniperca kneri*	3	42.7 ± 2.08	1519 ± 119	Shunde, PRD	carnivorous	fingerling and small fish
7	grey mullet	*Mulgil cephalus*	18	30.7 ± 0.90	379 ± 10.4	Shunde, PRD	bottom feeder	zooplankton, benthic organisms, detritus, and plant material
8	grass carp	*Ctenopharyngodon idellus*	6	41.9 ± 1.37	1338 ± 106	New Territories, HK	herbivorous	grass and other submerged higher plants
9	catfish	*Clarias fuscus*	21	30.5 ± 1.01	316 ± 21.5	New Territories, HK	carnivorous	small fish, insects, crustaceans, plankton, and rotting plants
10	bighead carp	*Aristichthys nobilis*	6	36.1 ± 0.98	875 ± 38.9	New Territories, HK	filter feeder	zooplankton
				Marine Fish				
11	yellow seafin	*Acanthopagrus latus*	9	26.1 ± 0.19	417 ± 9.42	South China Sea	omnivorous	benthic invertebrates
12	yellow croaker	*Pseudosciaena crocea*	15	30.8 ± 1.70	353 ± 54.8	South China Sea	carnivorous	small fish, shrimps, and crabs
13	tongue sole	*Cynoglossus robustus*	18	32.3 ± 0.77	193 ± 20.2	South China Sea	bottom feeder	benthic invertebrates
14	snubnose pompano	*Trachinotus blochii*	9	26.5 ± 0.60	409 ± 36.1	Sai Kung, HK	carnivorous	sand mollusks and hard-shelled invertebrates
15	orange-spotted grouper	*Epinephelus coioides*	9	30.2 ± 0.33	432 ± 7.15	South China Sea	carnivorous	fish and crustaceans
16	golden threadfin bream	*Nemipterus virgatus*	15	21.9 ± 2.26	181 ± 58.3	South China Sea	carnivorous	crustaceans, fish, and cephalopods
17	goldspotted rabbitfish	*Siganus punctatus*	36	19.7 ± 1.23	122 ± 16.0	South China Sea	herbivorous	benthic algae and seagrass
18	Bleeker's grouper	*Epinephelus bleekeri*	10	30.7 ± 1.79	364 ± 58.1	Hainan Province	carnivorous	fish and crustaceans
19	bigeye	*Priacanthus macracanthus*	33	24.4 ± 2.19	201 ± 53.8	Hainan Province	carnivorous	crustaceans, small fish, and small invertebrates
20	bartail flathead	*Platycephalus indicus*	10	40.0 ± 16.0	489 ± 388	South China Sea	bottom feeder	small fish

November 2009. The scientific and common names, body length, weight, producing area, feeding mode, and food items of the fish are listed in **Table 1**. At least three individuals of similar size of each species were collected and kept frozen by storing them in an ice box and transported to the laboratory as soon as possible. There were altogether 279 fish samples. The muscle of fish (including axial and ventral muscle) was dissected, freeze-dried, and homogenized by grinding into powder prior to analyses.

Digestible Fraction. Determination of the digestible fraction followed the method described by Cabanero et al. (*11*) and Xing et al. (*12*) with slight modification. Briefly, about 2−3 g of freeze-dried samples were first placed in a 50 mL capped centrifuge tube with 30 mL of synthetic gastric juice (5% w/v pepsin in 0.15 M NaCl, acidified with HCl to pH 1.8) and shaken at 100 rpm for 1 h at 37 °C in the dark. After 1 h of gastric digestion, the mixtures were centrifuged (15 min, 37 °C, 1500 rpm), and all suspensions were transferred to new tubes for further cleanup and analyses. Twenty milliliters of artificial intestinal juice (1.5%, w/v pancreatin, 0.5%, w/v amylase, and 0.3% w/v bile salts, in 0.15 M NaCl, pH 6.8) was added, and the mixture was resuspended and shaken at 30 rpm for 5 h at 37 °C in the dark. Finally, the tubes were centrifuged at 1500 rpm at 37 °C for 15 min to separate supernatant and solid. Both gastric and intestinal supernatants were filtered though a 0.45 Millipore filter and extracted using liquid−liquid shaking method, with 50 mL of hexane twice and with 50 mL of DCM once. All of the extracts were concentrated to 1 mL using a rotary evaporator and passed through a Florisil column for purification of the extract according to Standard Method 3620B (*17*). The eluant was concentrated to 200 μL for PAH analyses. Blanks were prepared by containing the enzyme treatment in series without fish samples.

PAH Analyses. Samples (2−3 g) were extracted for 16−18 h with a mixture of acetone, dichloromethane (DCM), *n*-hexane (v/v/v 1:1:1, 120 mL) in a Soxhlet extractor, according to Standard Method 3540C (*18*). A series of chromatographic columns were applied for sample cleanup such as Florisil cleanup (EPA Standard Method 3620B) (*17*) and gel permeation cleanup (EPA Standard Method 3640A) (*19*). Deuterated PAH internal standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) were added into all extracts at a concentration of 320 ng g^{-1} prior to instrumental analyses for quantification. Concentrations of PAHs were determined with a Hewlett-Packard (HP) 6890 N gas chromatograph (GC) coupled with a HP-5973 mass selective detector (MSD) and a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J&W Scientific Co. Ltd., Folsom, CA), using Standard Method 8270C (*20*). The PAH standards (AccuStandard, New Haven, CT) consisted of 16 priority pollutant PAHs: naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (FlA), pyrene (Py), benz(*a*)anthracene (BaA), chrysene (Chry), benzo(*a*)-pyrene (BaP), benzo(*b*)fluoranthene (BbF), benzo(*k*)fluoranthene (BkF), indeno(1,2,3-*cd*)pyrene (IP), dibenz(*a*,*h*)anthracene (DahA), and benzo-(*g*,*h*,*i*)perylene (BghiP). The peaks of benzo(*b*)fluoranthene (BbF) and benzo-(*k*)fluoranthene (BkF) were extremely close and difficult to distinguish; therefore, these two compounds were combined as one, namely, B(b+k)F. The standard curve was obtained by using 0, 2, 5, 10, 20, 50, 100, and 200 ng g^{-1} PAH standards. Concentrations based on individually resolved peaks were summed to obtain the total PAH concentrations ($\sum$PAHs).

QA/QC. The limit of detection (LOD) using the present method was determined as the concentrations of analytes in a sample that gave rise to a peak with a signal-to-noise ratio (S/N) of 3, which ranged from 0.05 to 0.15 ng g^{-1}. The recoveries of individual PAHs ranged from 73.2% for Nap to 108.2% for DahA in standard reference material (SRM) 2977 (mussel tissue) form the National Institute of Standards and Technology (NIST, Bethesda, MD). For each batch of 12 field samples, a method blank (solvent), a spiked blank (standards spiked into solvent), a sample duplicate, and a standard reference material (NIST SRM 2977) sample were processed. The variation coefficients of PAH concentrations between duplicate samples were $< 10\%$. The PAH levels of digestion blank were less than the LOD.

Calculation of Bioaccessibility. The bioaccessibility of PAHs in the present study was defined as the fraction of the contaminants mobilized into the digestive juices from fish, which would be available for absorption. The bioaccessibility (% BA) of PAHs was calculated as the ratio of the amount of PAHs in liquid phase to that in the sum of liquid and solid (eq 1), which could minimize the influence of loss during the digestion process (*12*). The total PAHs for intestinal digestion were considered as equal to the total PAHs in fish samples subtracted by PAHs in gastric juice.

$$\%\ \text{BA} = (\text{BA extracted PAHs}/\text{total PAHs}) \times 100 \qquad (1)$$

Calculation of Potency Equivalent Concentration (PEC) and Screening Value (SV). The potency equivalent concentration (PEC) of carcinogenic PAHs was calculated for each sample for comparison with

Table 2. PAH Concentrations (ng g^{-1}, ww) in Hong Kong Market Fish Muscle[a]

																			PEC	
		LC (%)	Nap	Acel	Ace	Fl	PhA	An	FlA	Py	BaA	Chry	BbkF	BaP	IP	DahA	BghiP	∑PAHs	fish	digestible
									Freshwater Fish											
1	tilapia	3.29	2.82	0.13	0.14	0.55	2.61	0.13	1.75	0.63	0.43	0.88	0.89	0.15	0.44	0.12	1.14	12.8	0.40	0.13
2	snakehead	6.15	4.17	0.14	0.33	0.50	2.29	0.11	2.10	0.47	4.50	1.44	1.23	0.44	0.32	0.21	0.41	18.7	1.20	0.24
3	spotted snakehead	6.21	2.85	0.11	0.15	0.62	2.17	0.18	1.93	0.62	0.35	0.89	1.06	0.07	0.22	0.17	0.20	11.6	0.36	0.11
4	rice field eel	1.39	2.87	0.10	0.15	0.35	1.60	0.10	1.08	0.51	0.55	0.77	0.62	0.13	0.47	0.11	0.30	9.69	0.38	0.16
5	mud carp	2.34	3.10	0.13	0.19	0.57	2.47	0.15	1.84	0.73	0.69	1.09	0.92	0.27	0.75	0.21	1.80	14.9	0.67	0.18
6	mandarin fish	4.08	2.23	0.11	0.13	0.56	2.23	0.12	1.41	0.43	0.38	0.64	0.64	0.09	0.38	0.07	0.27	9.69	0.27	0.14
7	grey mullet	4.61	3.56	0.09	0.13	0.46	2.16	0.12	1.83	0.87	0.73	0.80	1.53	0.23	0.46	0.31	0.99	14.3	0.75	0.20
8	grass carp	4.07	2.07	0.08	0.11	0.41	1.88	0.09	1.20	0.43	0.26	0.49	0.44	0.08	0.23	0.07	0.65	8.49	0.22	0.09
9	catfish	6.34	7.59	0.21	0.30	0.86	4.11	0.24	2.68	1.35	1.56	3.36	1.53	0.20	0.67	0.17	2.21	27.0	0.68	0.41
10	bighead carp	1.12	2.04	0.09	0.12	0.34	1.46	0.08	1.00	0.54	0.38	0.66	0.43	0.15	0.45	0.08	0.89	8.72	0.34	0.11
									Marine Fish											
11	yellow seafin	7.05	9.41	0.29	0.66	1.28	10.27	0.41	7.34	2.74	1.78	2.43	6.55	0.76	3.35	1.86	5.27	54.4	3.49	0.27
12	yellow croaker	12.7	8.63	0.41	1.41	1.13	5.71	0.41	3.83	1.52	3.48	2.09	2.46	0.36	1.44	0.49	0.92	34.3	1.47	0.17
13	tongue sole	1.06	6.75	0.27	0.27	0.84	4.63	0.35	2.77	1.92	1.70	1.91	2.13	0.36	1.05	0.34	4.34	29.6	1.09	0.32
14	snubnose pompano	15.1	7.66	0.28	1.51	1.31	6.40	0.28	5.44	2.00	0.94	1.73	2.65	0.45	1.24	0.51	1.27	33.7	1.33	0.35
15	orange-spotted grouper	5.15	6.44	0.26	0.86	1.04	4.86	0.21	3.37	0.90	1.20	1.26	1.52	0.15	0.61	0.19	0.90	23.8	0.61	0.30
16	golden threadfin bream	5.08	8.18	0.25	0.98	1.27	5.03	0.36	4.61	1.63	1.52	2.49	3.36	0.31	1.14	0.74	1.18	33.0	1.51	0.54
17	goldspotted rabbitfish	2.52	5.31	0.23	0.32	0.98	3.46	0.38	1.79	4.09	2.85	6.47	1.25	0.55	0.64	0.22	0.69	29.2	1.20	0.38
18	Bleeker's grouper	1.67	6.12	0.19	0.82	0.73	3.13	0.17	2.12	0.94	1.22	1.70	1.46	0.23	0.67	0.23	0.56	20.3	0.73	0.38
19	bigeye	1.92	4.96	0.18	0.33	0.65	3.32	0.19	1.90	1.28	2.82	1.68	1.83	0.38	1.05	0.23	1.44	22.3	1.10	0.31
20	bartail flathead	1.34	6.72	0.34	0.32	1.03	4.42	0.31	3.11	2.23	2.27	3.12	3.32	0.45	1.20	0.74	1.88	31.5	1.73	0.46

[a] LC, lipid content in fish muscle; PEC, potency equivalent concentration.

the screening value for BaP according to the guideline of the EPA (*21*). The toxic equivalency factor (TEF) of BaP, DahA, IP, BbK, BfK, BaA, and Chry was used in accordance with the EPA (*22*). The PEC of each fish sample was calculated using eq 2 (*23*)

$$\mathrm{PEC} = \sum(\mathrm{TEF} \times C) \qquad (2)$$

where TEF is the toxic equivalency factor compared with BaP for carcinogenic PAH congeners and C is the concentration (ng g^{-1}, ww).

The screening value (SV) is defined as the concentration of chemical in edible tissue that is of potential public health concern, and it is used as threshold value against tissue residue level of contamination in similar tissues collected from the environment (*24*). The SVs for PAHs were calculated using eq 3 (*25*)

$$\mathrm{SV} = [(\mathrm{RL}/\mathrm{SF}) \times \mathrm{BW}]/\mathrm{CR} \qquad (3)$$

where SV is screening value (μg g^{-1}), RL is maximum acceptable risk level (dimensionless), SF is oral slope factor (μg g^{-1} day^{-1}), BW is body weight (kg), and CR is consumption rate (g day^{-1}). In the present study, the body weight, consumption rate, oral slope factors of PAHs, and RL was 70 kg, 142.2 g day^{-1}, 7.30 μg g^{-1} day^{-1}, and 10^{-5}, respectively (*7*). RL is defined as if a person weighing 70 kg consumed 142.2 g of fish per day with the same concentration of contaminant, for 70 years; the increased risk would be at most one additional cancer death per 100,000 persons (*26*).

Data Analyses. All of the results were reported in nanograms per gram, ww. Data analyses were performed using SPSS 13.0 for Windows. Normality was confirmed by the Kolmogorov−Smirnov test. Data of PAH concentrations and bioaccessibility in gastric and intestinal conditions were analyzed using two independent *t* tests, the Wilcoxon rank sum test, one-way ANOVA, and the Kruskal−Wallis test as the requirement.

RESULTS AND DISCUSSION

PAH Concentrations in Hong Kong Market Fish. According to **Table 2**, ∑PAHs concentrations obtained in the present study ranged from 7.41 to 58.0 ng g^{-1} with a mean of 22.4 ng g^{-1}, which were comparable with those collected from fishponds of the PRD (25.8−77.1 ng g^{-1}, ww) (*27*) and the Hong Kong market (1.57−118 ng g^{-1}, ww) (*7*). The highest ∑PAHs concentrations among freshwater and marine fish species were for catfish (27.0 ± 5.12 ng g^{-1}) and yellow seafin (54.4 ± 3.09 ng g^{-1}). The ∑PAHs concentrations in freshwater fish (7.41−28.1 ng g^{-1}, mean of 13.6 ng g^{-1}) were significantly ($p < 0.01$) lower than those in marine fish species (16.2−58.0 ng g^{-1}, mean of 31.2 ng g^{-1}).

The PAHs in fish muscle were bioconcentrated from water via gills, skin, and ingestion of contaminated food or sediment. The bioaccumulation rate depended mainly on their feeding preference, general behavior, and trophic level of fish (*28*). It was generally observed that the PAH concentrations in water, suspended particulate matter, sediments, and fish feeds for marine fish were significantly higher than for freshwater fish in the PRD (*29*). Therefore, the higher PAHs in marine fish could be attributed to the bioaccumulation from fish feeds as well as from the surrounding environment containing higher PAHs.

All 16 EPA priority PAHs were detected in the fish samples. The congener proportions of PAHs were comparable between freshwater and marine fishes, with low molecular weight (LMW) PAHs as the predominant congeners (28.4−62.0%, mean of 48.6%). Nap and PhA were the most prevalent parent compounds (23.2 and 16.5%, respectively). This result was in line with prior studies concerning fish samples collected from the PRD (*7*, *27*).

Oral Bioaccessibility of PAHs. Figure 1 shows that the bioaccessibility of total PAHs in the intestinal condition (12.6−42.6%, mean of 31.1%) was significantly ($p < 0.01$) higher than that in the gastric condition (12.1−37.9%, mean of 24.3%). The higher bioaccessibility of PAHs in intestinal condition was also noted in previous studies about the bioaccessibility of persistent organic pollutants (POPs) using the in vitro gastrointestinal model (PAHs (*16*); organochlorine pesticides (OCPs) (*30*)), which might be due to the micelles formed in the aqueous suspensions by bile constituents. This will be further discussed below. No significant ($p > 0.05$) difference of bioaccessibility between marine and freshwater fishes was observed in both gastric and intestinal conditions. The low bioaccessbility may indicate that the total PAH intake through fish consumption as found by conventional procedures with organic solvent has considerably overestimated human exposure to PAHs through food intake.

For PAH congeners, the oral bioaccessibility ranged from 7.22 (BghiP) to 67.3% (Nap) in gastric condition and from 12.6 (BghiP) to 64.6% (Nap) in instestinal condition (**Figure 2**). In both gastric and intestinal conditions, the bioaccessibilities of LMW PAHs were significantly ($p < 0.05$) higher than higher molecular weight (HMW) PAHs. This was in line with the result of bioaccessibility of PAHs in Beijing soil samples (*16*). The bioaccessibilities of 4−6 ring PAHs in intestinal condition were significantly ($p < 0.05$) higher than gastric condition, which was possibly due to the formation of micelles in the aqueous suspensions by bile constituents (*16*).

Contributing Factors. Former studies suggested that different bioaccessbilities of PAH congeners obtained tend to be linked to their physicochemical properties in soil samples (*15*, *16*). In the present study, the influences of physicochemical parameters including molecular weight (MW), water solubility, octanol−water partition coefficients (K_{OW}), and octanol−air partition coefficient (K_{OA}) on the bioaccessibility of individual PAHs were investigated. As shown in **Figure 3**, the results indicated that the bioaccessibilities of individual PAHs in fish samples were negatively ($p < 0.05$) correlated with their MW, K_{OW}, and K_{OA} and positively ($p < 0.05$) correlated with the water solubility in both gastric and intestinal conditions (R^2 shown in **Figure 3**). This trend was generally revealed in former studies for various organic pollutants (PAHs in soil (*16*); OCPs in soil (*30*)). The significant relationships obtained between physicochemical parameters and bioaccessibilities for individual PAHs may also indicate that the key processes controlling digestion of PAHs in fish are those concerned with the partitioning between fish muscle and digestion juice. The present results indicate that the more hydrophobic the PAH congeners are, the less they will partition to the digestion liquid, which was contradicted by the bioaccessibility of OCPs in soil that the higher hydrophobicity of DDTs was more orally bioaccessible (*30*). Therefore, the physicochemical properties are important for the sorption of mobilized PAHs on the solid to be investigated. It is important to evaluate the bioaccessibility of not just PAHs but also other organic pollutants such as PCBs and PBDEs. Further study is needed to address such effects.

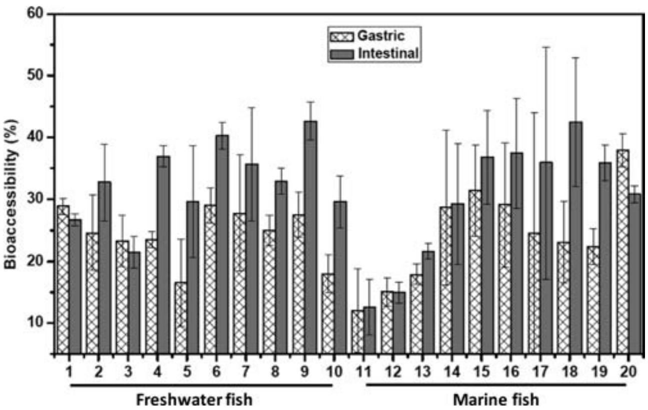

Figure 1. Bioaccessibilities of total PAHs of freshwater and marine fishes in gastric and intestinal conditions. The numbers 1−20 represent the fish species illustrated in **Table 1**.

Former studies indicated that concentrations of POPs such as PAHs and PCBs in food were significantly correlated with lipid contents, and therefore high-lipid foods such as milk and fish were major contributors to human exposure to POPs (*31*). It is also observed that the bioaccessibility of organic pollutants such as PCBs in the gastrointestinal model increased in the presence of lipid through added alimentary components because of the formation of lipid/bile micelles (*15*). However, all of these calculations were based on data obtained from soil samples. For the bioaccessibility of food items, it was observed that lipids may decrease the intake of BaP in the in vitro model in everted intestinal segments from channel catfish (*32*), and high-lipid food may have a much lower bioaccessibility of PCBs than low-lipid food. This could be because lipid is difficult to digest and therefore more difficult to be released from the food matrix (*12*). It seems that the physiochemical properties of lipid content in samples could significantly affect the bioaccessibility of organic pollutants. In the present study, no linear correlation was observed between the bioaccessibliy of PAHs and lipid contents in fish muscle. The role of lipid content in food matrix on the bioaccessibility of organic pollutants needs further studies.

The oral bioaccessibility of organic pollutants could also be significantly affected by constituents of digestion juice. The ratios of bioaccessibility in intestinal condition to gastric condition of LMW PAHs were significantly ($p < 0.05$) lower than the HMW PAHs, indicating the relatively pronounced effect of bile extract on enhancing the bioaccessibility of PAHs with relatively high K_{OW} values. This is due to fact that bile extract at levels higher than the critical micelle concentration (CMC) of 0.15 g L^{-1} could reduce the surface tension of digestive juice substantially (*16*). The concentration of bile extract used in the present experiment (3 g L^{-1}) was 20 times higher than the CMC and could therefore

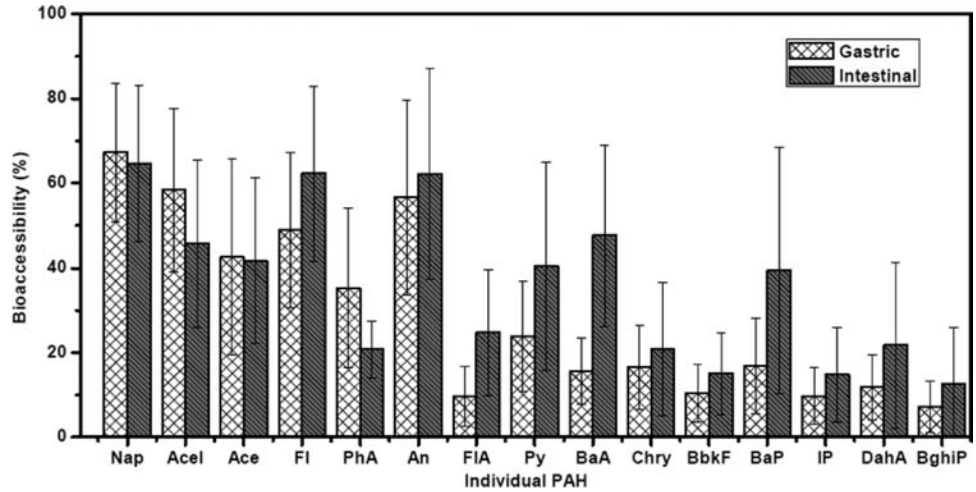

Figure 2. Average bioaccessibility of fish muscle PAH congeners in gastric and intestinal conditions.

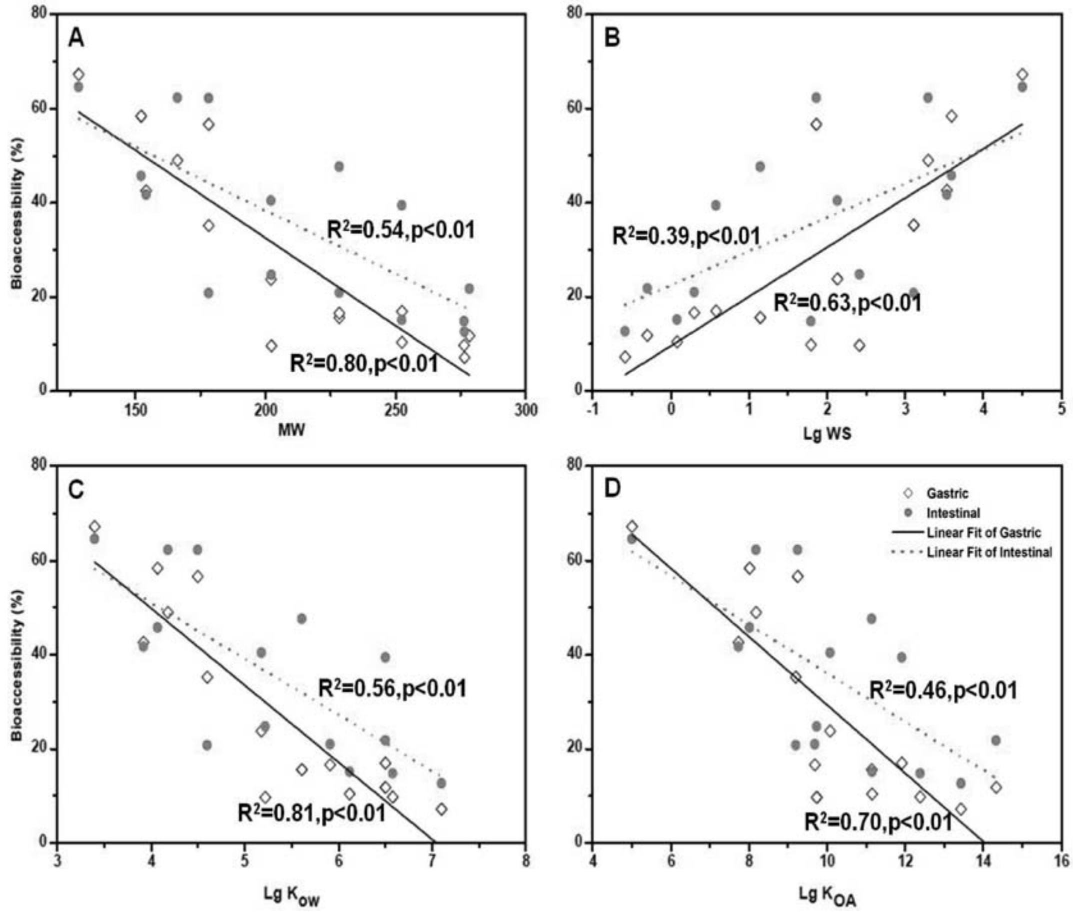

Figure 3. Dependence of the in vitro bioaccessibility of fish muscle PAHs in gastric and intestinal conditions on physicochemical properties: (**A**) molecular weight; (**B**) log water solubility (μg L^{-1}); (**C**) log K_{OW}; (**D**) log K_{OA}.

act as a surfactant-like component and promote the formation of bile micelles.

The contents of analyzed compounds, the types of samples, and constituents of digestion juices could also significantly affect the results. Considering that a number of operating parameters (i.e., temperature, pH, fluid/solid ratios) in this gastrointestinal method could greatly affect the outcome (*33*), the problem should be investigated and, hopefully, standardized procedures could be formulated in the future.

Risk Assessment Based on Bioaccessibility of Total PAHs. The diet makes a substantial contribution (>70% in nonsmokers) to PAH intake other than occupational PAH exposure (*34*). Intake via fish consumption contributes a significant proportion of total dietary intake of PAHs. Freshwater fish consumption accounted for 75% of exposure to pollutants such as DDTs (whereas other food items contribute not more than 5%) across the United States (*36*). For Hong Kong residents, it has been observed that the concentrations of DDTs in human milk collected from Hong Kong had a significant correlation with the frequency of fish consumption of the donors (*35*). A person in Hong Kong consumes fish or shellfish four or more times a week, averaging about 60 kg of fish per year, equal to 164.4 g day^{-1} (*4*), which is higher than the fish consumption rate of 142.2 g day^{-1} set by the EPA for subsistence consumers (*24*). Therefore, consumption of fish containing elevated concentrations of PAHs would be a public health concern.

On the basis of the fish consumption rate of 142.2 g day^{-1}, the guideline concentration is 0.67 ng PEC of PAHs g^{-1} (ww) for human consumption as recommended by the EPA (*24*). **Table 2** shows that the PEC values ranged from 0.16 to 3.72 ng g^{-1} (ww), with mean values of 0.53 and 1.43 ng g^{-1} for freshwater and marine fishes, respectively. Thirty percent of freshwater species and 86.7% of marine species contained elevated levels of PAHs exceeding the screening value (0.67 ng g^{-1}, ww). The yellow seafin showed significantly ($p < 0.05$) higher levels of PEC (3.49 ng g^{-1}) than other marine species, which exceeded the EPA guideline by 5.21 times. The results suggested that some freshwater fish and most marine fish available in Hong Kong market might not be suitable for continual consumption of a large amount.

It has been indicated that extraction of organic pollutants from contaminated food using solvents may considerably overestimate the absorbed amount of contaminants contributing to the general health risk (*3*). The bioavailability of various pollutants in food items is therefore an important factor for effective exposure estimation and risk assessment (*10*, *12*). Therefore, the PEC value of each fish sample was calculated with the digestible concentrations in gastrointestinal conditions (**Table 2**). The digestible PEC values ranged from 0.08 to 0.65 ng g^{-1} (mean of 0.18 and 0.35 ng g^{-1} for freshwater and marine fishes, respectively), accounting for 3.4−71.6% (mean of 35.0%) for the original total PAH PEC values, which were all below the recommended guideline (0.67 ng g^{-1}) for human consumption (*24*). Previous results on health effects concerning intake of organic pollutants (including PAHs) and heavy metals via contaminated fish assessed for Hong Kong residents based on the total pollutant concentrations (*5*−*7*) should be modified by taking bioaccessibility into account.

Individual PAH Accumulation and Health Risk Assessment. To compare the relative differential accumulation of individual PAHs during the digestion process, the relative accumulation ratios (R_{nn}, **Figure 4**) based on the following equation (*12*, *37*)

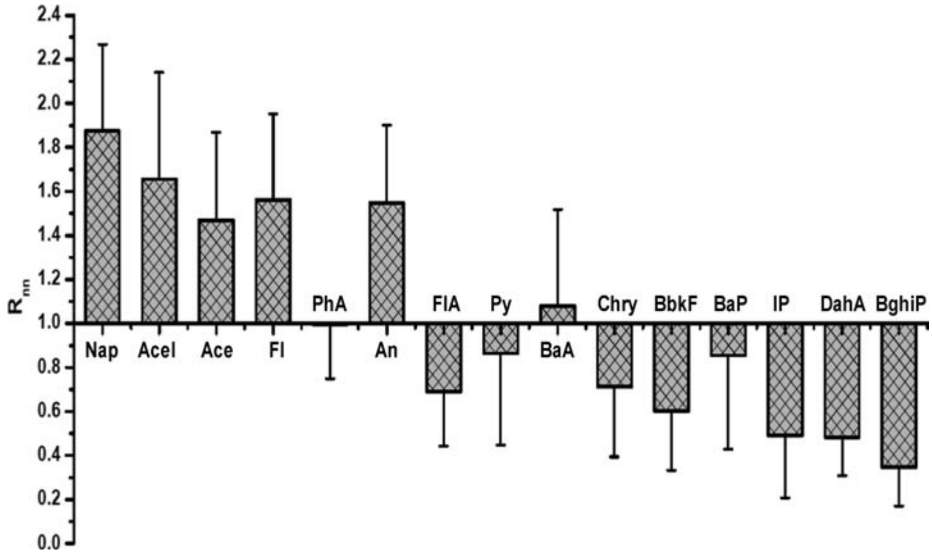

Figure 4. Relative accumulation ratios (R_{nn}) for PAHs in fish muscle (R_{nn} > 1 indicates relative accumulation; R_{nn} < 1 indicates relative dilution).

were calculated: $R_{nn} = P_{n,1}/Pn,w$, where R_{nn} is the relative accumulation ratio of congener n in Sample n. $P_{n,1}$ and $P_{n,\mathrm{w}}$ is the composition percentage of PAH congener n in digestion liquid and whole sample, respectively.

For fish muscle samples, the R_{nn} values of all 2−3 ring PAH congeners (including Nap, Acel, Ace, Fl, PhA, and An) and BaA (4 rings) were > 1, indicating relative accumulation during digestion. The R_{nn} values of LMW PAHs were significantly ($p < 0.01$) higher than HMW PAHs, suggesting that the greater benzene rings of the PAH congeners, the lower the relative accumulation ratios. The R_{nn} values of PAH congeners were significantly ($p <$ 0.05) correlated with their physicochemical parameters such as MW ($R^2 = 0.84$), water solubility ($R^2 = 0.66$), K_{OW} ($R^2 = 0.85$), and K_{OA} ($R^2 = 0.76$). The results were consistent with our previous study that the relative accumulation ratios of PCB congeners were significantly correlated with their physicochemical parameters (*12*).

The results of the in vitro digestion model that the relative accumulation ratios of PAH congeners were significantly correlated with their physicochemical parameters were consistent with the previous in vitro and vivo studies concerning the PAHs' bioavailability. Cavret et al. (*38*) observed that transepithelial permeability of LMW PAHs (PhA, 9.5%) was significantly higher than that of HMW PAHs (BaP, 5.2%) in Caco-2 cell lines after a 6 h of exposure. For the transfer pathways of PAHs from food to animals, it was observed that the absorption rates of PAH congeners were positively correlated with their water solubility and low lipophilicity in pigs (*8*) and negatively correlated with molecular size in goats (*9*). These indicated that the bioavailability of PAHs could be evaluated on the basis of the bioaccessibility results and that in vitro digestion model is effective for the evaluation of PAH bioavailability.

Recent studies have suggested that fish and seafood were the major dietary sources of POPs such as DDTs and PBDEs for Hong Kong residents (*39*, *40*). For example, fish was the major dietary source of PBDEs of secondary school students and contributed 38.5% to PBDE exposure among foods of animal origin (*40*). The regression analysis was performed between the R_{nn} values of PAH congeners and their corresponding concentrations in subcutaneous fat of Hong Kong residents (*41*). A significant ($R^2 = 0.58$, $p < 0.05$) correlation was observed between the concentrations of different PAH congeners in human adipose tissue and their respective R_{nn} values, with two outliers (Py and BaA, **Figure 5**), which may further confirm that the digestion intake of PAHs from foods such as fish is a major pathway for their accumulation in the body of Hong Kong residents. Considering that other food items such as roast duck and barbecued meats may also contain high levels of PAHs, further studies concerning the bioaccessible daily intake of PAHs of these popular food items would be necessary.

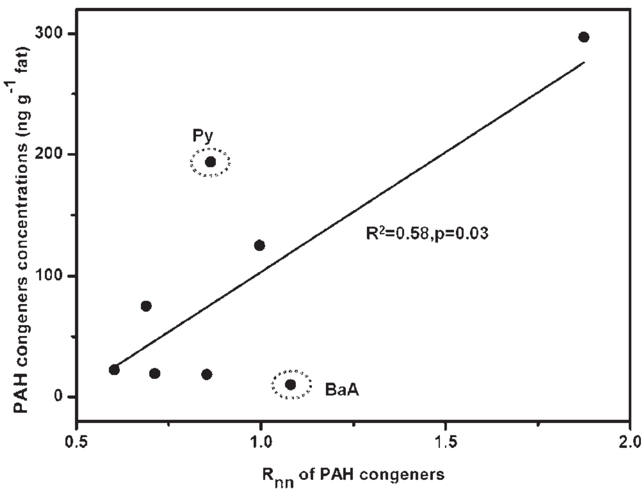

Figure 5. Correlation between R_{nn} of PAH congeners and the concentration of individual PAHs in subcutaneous fat of Hong Kong residents.

Although the data on bioaccessibility values generated from the present study were based on raw fish samples, many other factors such as cooking method, doneness level, and food processing could also affect the final PAH concentrations in the consumed fish. Nevertheless, the correlations obtained in the present study between relative accumulation ratios (R_{nn}) and concentrations of PAH congeners in the human body revealed that fish digestion would be a major route for PAH accumulation in Hong Kong residents.

In summary, 20 species of commonly consumed fish purchased from a Hong Kong market were investigated to evaluate the bioaccessibility of PAHs and potential human health risks. The $\sum$PAHs concentrations in fish muscle ranged from 7.41 to 58.0 ng g^{-1} (mean of 22.4 ng g^{-1}) with LMW PAHs as the predominant PAH congeners. The bioaccessibility of total PAHs in intestinal condition (mean of 31.1%) was significantly higher than that in gastric condition (mean of 24.3%). The bioaccessibilities of PAH congeners were significantly ($p < 0.05$) correlated with their physicochemical parameters such as MW, water solubility, K_{OW}, and K_{OA}, whereas no significant correlation with lipid contents was obtained. On the basis of total PAH data, the PEC values of 30% of samples of freshwater species and 86.7% of samples of marine species exceeded the screening value set by the EPA (0.67 ng g^{-1}, ww). However, when bioaccessibility was taken into consideration, the digestible PEC values accounted 3.4−71.6% (mean of 35.0%) for the original values with all samples below the guideline concentration. The relative accumulation ratios (R_{nn}) of PAH congeners were significantly correlated with their physicochemical parameters and the PAH concentrations in adipose tissue of Hong Kong residents, suggesting that R_{nn} values could effectively reflect PAH accumulation in the human body. The intake of foods such as fish seems to be a major pathway contributing to PAH loadings of Hong Kong residents.

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LITERATURE CITED

(1) Yunker, M. B.; Backus, S. M.; Graf Pannatier, E.; Jeffries, D. S.; Macdonald, R. W. Sources and significance of alkane and PAH hydrocarbons in Canadian arctic rivers. *Estuar. Coast. Shelf Sci.* **2002**, *55* (1), 1−31.

(2) Manoli, E.; Samara, C.; Konstantinou, I.; Albanis, T. Polycyclic aromatic hydrocarbons in the bulk precipitation and surface waters of northern Greece. *Chemosphere* **2000**, *41* (12), 1845−1855.

(3) 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* (5), 301−333.

(4) Dickman, M. D.; Leung, K. M. C. Mercury and organochlorine exposure from fish consumption in Hong Kong. *Chemosphere* **1998**, *37* (5), 991−1015.

(5) Fang, J. K. H.; Wu, R. S. S.; Zheng, G. J.; Au, D. W. T.; Lam, P. K. S.; Shin, P. K. S. The use of muscle burden in rabbitfish *Siganus oramin* for monitoring polycyclic aromatic hydrocarbons and polychlorinated biphenyls in Victoria Harbour, Hong Kong and potential human health risk. *Sci. Total Environ.* **2009**, *407* (14), 4327−4332.

(6) Kong, K. Y.; Cheung, K. C.; Wong, C. K. C.; Wong, M. H. Residues of DDTs, PAHs and some heavy metals in fish (tilapia) collected from Hong Kong and Mainland China. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2005**, *40* (11), 2105−2115.

(7) Cheung, K. C.; Leung, H. M.; Kong, K. Y.; Wong, M. H. Residual levels of DDTs and PAHs in freshwater and marine fish from Hong Kong markets and their health risk assessment. *Chemosphere* **2007**, *66* (3), 460−468.

(8) Laurent, C.; Feidt, C.; Grova, N.; Mpassi, D.; Lichtfouse, E.; Laurent, F.; Rychen, G. Portal absorption of C-14 after ingestion of spiked milk with C-14-phenanthrene, C-14-benzo[*a*]pyrene or C-14-TCDD in growing pigs. *Chemosphere* **2002**, *48* (8), 843−848.

(9) Grova, N.; Feidt, C.; Laurent, C.; Rychen, G. [C-14] Milk, urine and faeces excretion kinetics in lactating goats after an oral administration of [C-14] polycyclic aromatic hydrocarbons. *Int. Dairy J.* **2002**, *12* (12), 1025−1031.

(10) Harrad, S.; Wang, Y.; Sandaradura, S.; Leeds, A. Human dietary intake and excretion of dioxin-like compounds. *J. Environ. Monit.* **2003**, *5* (2), 224−228.

(11) Cabanero, A. I.; Madrid, Y.; Camara, C. Selenium and mercury bioaccessibility in fish samples: an in vitro digestion method. *Anal. Chim. Acta* **2004**, *526* (1), 51−61.

(12) 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* (3), 1218−1226.

(13) Oomen, A. G.; Sips, A.; Groten, J. P.; Sijm, D.; 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* (2), 297−303.

(14) Holman, H. Y. N.; Goth-Goldstein, R.; Aston, D.; Yun, M.; Kengsoontra, J. Evaluation of gastrointestinal solubilization of petroleum hydrocarbon residues in soil using an in vitro physiologically based model. *Environ. Sci. Technol.* **2002**, *36* (6), 1281−1286.

(15) Van de Wiele, T. R.; Verstraete, W.; Siciliano, S. D. Polycyclic aromatic hydrocarbon release from a soil matrix in the in vitro gastrointestinal tract. *J. Environ. Qual.* **2004**, *33* (4), 1343−1353.

(16) Tang, X. Y.; Tang, L.; Zhu, Y. G.; Xing, B. S.; Duan, J.; Zheng, M. H. Assessment of the bioaccessibility of polycyclic aromatic hydrocarbons in soils from Beijing using an in vitro test. *Environ. Pollut.* **2006**, *140* (2), 279−285.

(17) U.S. EPA. Method 3620B: Florisil Cleanup; Washington, DC, **1996**.

(18) U.S. EPA. Method 3540C: Soxhlet Extraction; Washington, DC, **1996**.

(19) U.S. PEA. Method 3640A: Gel-Permeation Cleanup; Washington, DC, **1994**.

(20) U.S. EPA. Method 8270C: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS); Washington, DC, **1996**.

(21) U.S. EPA. Workshop report on developmental neurotoxic effects associated with exposure to PCBs, Sept 14−15, 1992, Research Triangle Park, NC; Risk Assessment Forum, Washington, DC, **1993**.

(22) U.S. EPA. Provisional Guidance for Quantitative Risk Assessment of PAH, EPA/600/R-93/089; Washington, DC, **1993**.

(23) Nisbet, I. C. T.; LaGoy, P. K. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regul. Toxicol. Pharmacol.* **1992**, *16* (3), 290−300.

(24) U.S. EPA. *Guidance for Assessing Chemical Contaminant, Data for Use in Fish Advisories, Vol. 1: Fish Sampling and Analysis*, 3rd ed.; EPA 823-R-95-007; Office of Water: Washington, DC, 2000.

(25) Fairey, R.; Taberski, K.; Lamerdin, S.; Johnson, E.; Clark, R. P.; Downing, J. W.; Newman, J.; Petreas, M. Organochlorines and other environmental contaminants in muscle tissues of sportfish collected from San Francisco Bay. *Mar. Pollut. Bull.* **1997**, *34* (12), 1058−1071.

(26) U.S. EPA. *Risk Assessment Guidance for Superfund, Vol. 1. Human Health Evaluation Manual* (*Part A*); Washington, DC, 1989.

(27) Kong, K. Y.; Cheung, K. C.; Wong, C. K. C.; Wong, M. H. The residual dynamic of polycyclic aromatic hydrocarbons and organochlorine pesticides in fishponds of the Pearl River delta, South China. *Water Res.* **2005**, *39* (9), 1831−1843.

(28) Lake, J. L.; McKinney, R.; Lake, C. A.; Osterman, F. A.; Heltshe, J. Comparison of patterns of polychlorinated biphenyl cogeners in water, sediment, and indigenous organisms from New Bedford harbor, Massachusetts. *Arch. Environ. Contam. Toxicol.* **1995**, *29* (2), 207−220.

(29) Wang, H. S.; Cheng, Z.; Liang, P.; Shao, D. D.; Kang, Y.; Wu, S. C.; Wong, C. K. C.; Wong, M. H. Characterization of PAHs in surface sediments of aquaculture farms around the Pearl River delta. *Ecotoxicol. Environ. Saf.* **2010**, *73* (5), 900−906.

(30) 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* (12), 4524−4529.

(31) Bordajandi, L. R.; Gomez, G.; Abad, E.; Rivera, J.; Fernandez-Baston, M. D.; Blasco, J.; Gonzalez, M. J. Survey of persistent organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic in food samples from Huelva (Spain): levels and health implications. *J. Agric. Food Chem.* **2004**, *52* (4), 992−1001.

(32) Weber, L. P.; Lanno, R. P. Effect of bile salts, lipid, and humic acids on absorption of benzo[*a*]pyrene by isolated channel catfish (*Ictalurus punctatus*) intestine segments. *Environ. Toxicol. Chem.* **2001**, *20* (5), 1117−1124.

(33) Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; Van de Wiele, T.; Wragg, J.;

Rompelberg, C. J. M.; Sips, A.; Van Wijnen, J. H. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol.* **2002**, *36* (15), 3326−3334.
(34) Phillips, D. H. Polycyclic aromatic hydrocarbons in the diet. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **1999**, *443* (1−2), 139−147.
(35) Wong, C. K. C.; Leung, K. M.; Poon, B. H. T.; Lan, C. Y.; Wong, M. H. Organochlorine hydrocarbons in human breast milk collected in Hong Kong and Guangzhou. *Arch. Environ. Contam. Toxicol.* **2002**, *43* (3), 364−372.
(36) Dougherty, C. P.; Holtz, S. H.; Reinert, J. C.; Panyacosit, L; Axelrad, D. A.; Wooddurff, T. J. Dietary exposure to food contaminants across the United States. *Environ. Res.* **2000**, *84* (2), 170−185.
(37) Carlson, D. L.; Hites, R. A. Polychlorinated biphenyls in salmon and salmon feed: global differences and bioaccumulation. *Environ. Sci. Technol.* **2005**, *39* (19), 7389−7395.
(38) Cavret, S.; Laurent, C.; Feidt, C.; Laurent, F.; Rychen, G. Intestinal absorption of C-14 from C-14-phenanthrene, C-14-benzo[*a*] pyrene and C-14-tetrachlorodibenzo-*p*-dioxin: approaches with the Caco-2 cell line and with portal absorption measurements in growing pigs. *Reprod. Nutr. Dev.* **2003**, *43* (2), 145−154.
(39) Chung, S. W. C.; Kwong, K. P.; Yau, J. C. W. Dietary exposure to DDT of secondary school students in Hong Kong. *Chemosphere* **2008**, *73* (1), 65−69.
(40) Chen, M. Y. Y.; Tang, A. S. P.; Ho, Y. Y.; Xiao, Y. Dietary exposure of secondary school students in Hong Kong to polybrominated diphenyl ethers from foods of animal origin. *Food Addit. Contam., Part A* **2010**, *27* (4), 521−529.
(41) Qin, Y. Y.; Leung, C. K. M.; Leung, A. O. W.; Wu, S. C.; Zheng, J. S.; Wong, M. H. Persistent organic pollutants and heavy metals in adipose tissues of patients with uterine leiomyomas and the association of these pollutants with seafood diet, BMI, and age. *Environ. Sci. Pollut. Res.* **2010**, *17* (1), 229−240.

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