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In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via consumption of market fishes

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HIGHLIGHTS

- Concentration and bioaccessibility of tHg and MeHg were tested in Hong Kong market fish.
- Risk assessments of MeHg should take bioaccessibility into account.
- 9% of children in Hong Kong had EDI_{bio} for MeHg that would exceed the RfD.

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ABSTRACT

In order to evaluate effects of exposure to mercury (tHg) and methylmercury (MeHg) of Hong Kong residents via consumption of fish, total and bioaccessible concentrations of tHg and MeHg were measured in 10 freshwater and 10 marine fishes collected from markets in Hong Kong. Concentrations of tHg and MeHg in fishes ranged from 27.2 to 311 ng g^{-1} (median 88.9 ng g^{-1}) and ND to 116 ng g^{-1} (median 45.0 ng g^{-1}), respectively. Concentrations of MeHg in marine fishes (64.4 ± 28.5 ng g^{-1}) were significantly greater than those in freshwater fishes (40.3 ± 26.0 ng g^{-1}). Bioaccessibility tHg and MeHg was predicted for edible flesh of twenty fishes by use of an *in vitro* gastrointestinal assay. Bioaccessibilities of tHg and MeHg ranged from 21.4 to 51.7% (mean 37.4%) and 19.5 to 59.2% (mean 43.7%), respectively. Based on total concentrations, diets of 36% of adults and 51% of children exceeded the reference dose (RfD, 100 ng kg^{-1} body mass (bm) d^{-1}) for MeHg, but when bioaccessibility was considered, consumption of local market fish would not result in an EDI_{bio} exceeded the RfD of MeHg for Hong Kong adults. These contradictory results suggested that risk assessments based on total concentrations would overestimate exposure because not all of contaminants consumed are bioaccessible. Furthermore, 9% of children had EDI_{bio} for MeHg that exceeded the RfD, which suggests that more attention should be paid to consumption of local fish on health and development of children in Hong Kong.

1. Introduction

Mercury (Hg) is a persistent and hazardous environmental pollutant and has been known for thousands of years, but due to increased mobilization into the lithosphere and atmosphere over the past few decades, it has become a global issue [1]. Hg has a residence time of 1–2 years in the atmosphere, which allows it to be transported long distances via oceanic and atmospheric processes [2]. In the environment, ionic Hg can be transformed into one of the most toxic forms, methylmercury (MeHg), by both abiotic and biotic processes [3]. MeHg is a well-documented neurotoxin that can induce irreversible damage to the central nervous system [4]. Several specific effects on health and development of children included subtle neurodevelopmental abnormalities, such as visuospatial errors [5], and deficits in motor speed, and attention have been reported [6]. Therefore, for protection of public health, the World Health Organization (WHO) has developed

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provisional tolerable weekly intake guidelines for adults and women of child-bearing age [7]. These guidelines are based on observable effects of MeHg on the central nervous system.

Numerous studies have revealed that consumption of seafood including fish products is the primary pathway for human exposure to total mercury (tHg) and MeHg [8]. Concentrations of Hg in tissues of people who consumed greater amounts of fish were significantly greater than those who consumed lesser amounts of fish [9]. This was particularly true for persons who consumed larger, predatory fishes and marine mammals, due to biomagnification by these organisms that feed at higher levels of the aquatic food chains. MeHg, a persistent organic compound, is subject to change in fugacity that result in trophic magnification [10,11]. The National Health and Nutrition Examination Survey in the United States (NHANES) found that concentrations of tHg in hair were three-fold greater for women and two-fold greater for children who frequently consumed fish compared with persons who consumed no fish [12]. Residents of Hong Kong consume fish or shellfish four times per week, with an average consumption of 164.4 g d^{-1} per person [13]. This rate of consumption is greater than that of 142.2 g d^{-1} set for subsistence consumers by USEPA [14]. A recent study showed that concentrations of tHg and MeHg in muscle of fishes collected from the Pearl River Delta (PRD) ranged from 7.43 to 76.7 and from 5.93 to 76.1 ng g^{-1} wet weight (ww), respectively [15]. Furthermore, pregnant women in Hong Kong consume larger amounts of fish than women in other areas of the world. As a result, their offspring have the potential to be exposed to greater concentrations of tHg and MeHg [16]. However, there has been no systematic study about risks posed by Hg to health of Hong Kong people due to exposure to tHg and MeHg via consumption of fish from local markets.

Solvents sometimes used in dosing studies can result in overestimate of the fraction of contaminants that are absorbed through ingestion of foods [17]. Results of previous studies indicated that just 23.1% of dichlorodiphenyltrichloroethane (DDTs) and 55.4% of polycyclic aromatic hydrocarbons (PAHs) are bioaccessible [18,19]. Bioaccessibility of a contaminant in food is defined as the fraction of the contaminant mobilized from food matrices during gastrointestinal digestion [20]. To more realistically assess risks due to dietary intake of tHg from consumption of fish, it is necessary to not only determine concentrations in the diet, but also determine bioaccessibility. There have been few studies of the bioaccessible fractions of tHg or MeHg in foods [21,22]. The study of Torres-Escribano et al. [23] revealed that the bioaccessibility of tHg in frozen swordfish was 64 ± 14%, which was greater than that observed in another study which reported that only 17% of tHg in swordfish was bioaccessible [24]. However, these limited published data only concerned the bioaccessibility of tHg in specific fish species such as swordfish. There was no information about bioaccessibility of tHg in other commonly consumed fishes. Furthermore, bioaccessibility of MeHg, which is more accumulated and has greater toxic potency than Hg, had not been elucidated yet.

The objectives of the present study were to: (1) quantify concentrations of tHg and MeHg and assess bioaccumulation of these compounds in twenty edible fishes available in Hong Kong markets; (2) develop an *in vitro* method to simulate digestion and determine bioaccessibilities of tHg and MeHg in muscle of fishes; and (3) evaluate dietary intake of tHg and MeHg and predict potential risks to Hong Kong residents based on the total and bioaccessible concentrations.

2. Materials and methods

2.1. Sample collection and treatment

Twenty fishes commonly consumed in Hong Kong were purchased from six markets located in different areas of Hong Kong from May to November 2009 [25]. Detailed information on the species collected, including common English and Latin names, length, weight, origins (production areas), and feeding habits are listed (Table 1). Generally, at least six individuals for each species were obtained except Mandarin fish, which due to their large size (1.4–1.6 kg per fish) only three individuals were collected. Altogether 279 samples of fishes were obtained. All samples were a composite of fish of different sizes. These represented mature individuals because their lengths were longer than their corresponding known size at maturation. The edible muscle of fish (including axial and ventral muscle) was dissected, freeze-dried, and homogenized by grounding into powder prior to chemical analysis.

2.2. Digestible fraction

A slightly modified version of the *in vitro* digestion that has been described previously [18,19] was applied. The enzyme (pepsin, pancreatin and bile salt) concentrations were adjusted in the present study. Briefly, to simulate anaerobic conditions of the stomach, the entire digestion process was performed in capped Teflon centrifuge tubes (50 mL) in the dark. Freeze-dried samples (2 g) were placed in 20 mL of synthetic gastric juice (2.0 g L^{-1} pepsin, 0.5 g L^{-1} malate and 0.5 g L^{-1} citrate in 0.15 M NaCl, acidified with HCl to pH 1.8) and shaken at 100 rpm for 2 h at 37 °C. The mixture was then centrifuged (15 min, 37 °C, 1500 rpm) and a subsample of suspensions of food in gastric juices were transferred to centrifuge tubes for purification, separation and quantification of tHg and MeHg. The remainder of suspension of fish muscle and gastric juice was added to artificial intestinal juice (30 mL, 2.0 g L^{-1} pancreatin, 5.0 g L^{-1} α-amylase, 7 g L^{-1} lipase and 5 g L^{-1} bile salts, in 0.15 M NaCl, pH 6.8) to determinate intestinal bioaccessibility. The mixture was resuspended and shaken at 30 rpm for 6 h at 37 °C. After incubation, tubes were centrifuged at 1500 rpm at 37 °C for 15 min to separate supernatant and solid. Supernatants of subsamples of suspensions of both gastric and intestinal juices were filtered through 0.45 μM Teflon Millipore filters (Bedford, MA, USA). Concentrations of tHg and MeHg were determined in the soluble fraction.

2.3. Quantification and QA/QC

Samples of fish muscle were digested with concentrated nitric acid in glass vials. Digestion for quantification of tHg was conducted as follows: dorsal muscle of fishes (0.5 g) was digested with nitric acid (5 mL) at 100 °C for 4 h with the tube shaken every 30 min. After cooling, the solution was diluted with Milli-Q (18.2 MΩ cm at 25 °C, Millipore Milli-Q Synthesis System) water to 25 mL, homogenized and centrifuged at 3000 rpm for 10 min. Concentrations of tHg were subsequently determined by use of cold vapor atomic absorption spectrometry (Varian model VGA 77 coupled to a Varian AAS model 220FS).

MeHg was quantified by use of a modification of previously described methods [15]. Approximately 0.1–0.15 g (dry weight) of each sample of dorsal muscle was digested in 3 mL 25% KOH/MeOH solution for 8 h at 65 °C. After cooling, the solution was diluted to 40 mL with Milli-Q water (Milli-pore, Bedford, MS). The solution (30 μL) was added to 40 mL vials with Teflon lined septa caps. Samples were adjusted to pH 4.9 with NaOH (300 μL), ethylated with the addition of NaBEt4 (40 μL), and made up to volume with Milli-Q water, capped, shaken and loaded into the auto sampler of the MeHg analyzer. It was ensured that there was no air in the vials. Concentrations of MeHg were determined by use of aqueous ethylation, purge and trap, and GC-CVAFS detection (Books Rand, MERX), following USEPA method 1630 [26].

For each batch of 15 field samples, a method blank (solvent), a spiked blank (standards spiked into solvent), a sample duplicate, and certified reference materials (CRMs) obtained from the

Table 1
Information on fishes obtained from local markets of Hong Kong during May to November 2009.

	Common English name	Scientific name	*n*	Length (cm)	Weight (g)	WC (%)	LC (%)	Feeding Habits	Origin
Freshwater fish									
1	Tilapia	*Oreochromis mossambicus*	10	27 ± 2.1	432 ± 8.4	77 ± 1.3	3.3 ± 0.72	Omnivore	Shunde, PRD
2	Spotted snakehead	*Channa maculate*	10	32 ± 0.4	449 ± 25	72 ± 0.5	6.2 ± 0.78	Carnivore	Shunde, PRD
3	Snakehead	*Channa asiatiea*	12	29 ± 0.1	254 ± 13	75 ± 1.3	6.2 ± 1.6	Carnivore	Shunde, PRD
4	Rice field eel	*Monopterus albus*	14	59 ± 3.7	279 ± 17	78 ± 0.1	1.4 ± 0.54	Carnivore	Shunde, PRD
5	Mud carp	*Cirrhina molitorella*	15	28 ± 1.4	414 ± 56	79 ± 0.8	2.3 ± 0.73	Omnivore	New Territories, HK
6	Mandarin fish	*Siniperca kneri*	3	43 ± 2.1	1519 ± 119	78 ± 1.6	4.1 ± 0.57	Carnivore	Shunde, PRD
7	Grey mullet	*Mulgil cephalus*	18	31 ± 0.9	379 ± 10	75 ± 0.3	4.6 ± 0.91	Carnivore	Shunde, PRD
8	Grass carp	*Ctenopharyngodon idellus*	6	42 ± 1.4	1338 ± 106	77 ± 0.5	4.1 ± 0.86	Herbivore	New Territories, HK
9	Catfish	*Clarias fuscus*	21	31 ± 1.0	316 ± 22	72 ± 3.0	6.3 ± 0.88	Carnivore	New Territories, HK
10	Bighead carp	*Aristichthys nobilis*	6	36 ± 1.0	875 ± 39	79 ± 0.5	1.1 ± 0.01	Omnivore	New Territories, HK
Marine fish									
11	Yellow seafin	*Acanthopagrus latus*	9	26 ± 0.2	417 ± 9.4	69 ± 1.1	7.1 ± 1.1	Omnivore	South China Sea
12	Yellow croaker	*Pseudosciaena crocea*	15	31 ± 1.7	353 ± 55	70 ± 1.6	13 ± 2.8	Carnivore	South China Sea
13	Tongue sole	*Cynoglossus robustus*	18	32 ± 0.8	193 ± 20	75 ± 1.7	1.1 ± 0.41	Carnivore	South China Sea
14	Snubnose pompano	*Trachinotus blochii*	9	27 ± 0.6	409 ± 36	66 ± 1.2	15 ± 2.2	Carnivore	Sai Kung, HK
15	Orange-spotted grouper	*Epinephelus coioides*	9	30 ± 0.3	432 ± 7.2	73 ± 1.3	5.2 ± 0.61	Carnivore	South China Sea
16	Golden threadfin bream	*Nemipterus virgatus*	15	22 ± 2.3	181 ± 58	69 ± 5.3	5.1 ± 0.85	Carnivore	South China Sea
17	Goldspotted rabbitfish	*Siganus punctatus*	36	20 ± 1.2	122 ± 16	79 ± 0.9	2.5 ± 0.52	Herbivore	South China Sea
18	Bleeker's grouper	*Epinephelus bleekeri*	10	31 ± 1.8	364 ± 58	79 ± 0.9	1.7 ± 0.55	Carnivore	Hainan Province
19	Bigeye	*Priacanthus macracanthus*	33	24 ± 2.2	201 ± 54	80 ± 0.4	1.9 ± 0.27	Carnivore	Hainan Province
20	Bartail flathead	*Platycephalus indicus*	10	40 ± 16	489 ± 388	71 ± 3.7	1.3 ± 0.32	Carnivore	South China Sea

WC: water content in fish muscle; LC: lipid content in fish muscle (%, ww).

International Atomic Energy Agency National Research Council Canada (i.e., TORT-2 [lobster hepatopancreas reference material for trace metals]) were also analyzed throughout the complete procedures. For instrumental quantification of concentrations of tHg and MeHg in fish muscle, recoveries of tHg and MeHg from the standard reference materials were within the certified values with recovery rates of 102 ± 4.08% and 97.2 ± 4.64%, for tHg and MeHg, respectively. The average coefficient of variation (SD/mean) for duplicate samples was <10%.

2.4. Estimation of daily intake and assessment of risk

In the present study, bioaccessibility was defined as the fraction of tHg or MeHg mobilized into the digestive juices from fish muscle, which would be available for absorption. For the bioaccessibility of tHg or MeHg, recoveries were determined by calculating the ratio of the sum of tHg or MeHg in digestible and undigestible fractions to the concentration of tHg or MeHg in certified reference materials. Recoveries of tHg and MeHg in bioaccessibility studies ranged from 85 to 96%. Bioaccessibility (%BA) of tHg (MeHg) was calculated as the ratio of the amount of tHg (MeHg) in the liquid phase to the total amount in fish muscle (Eq. (1)) [20]. The total Hg in the liquid phase of the intestinal digestion was considered as equal to the total Hg in samples of fish muscle minus the Hg in gastric juice.

$$\%\mathrm{BA} = \left(\frac{\text{BA extracted Hg}}{\text{total Hg}}\right) \times 100 \quad (1)$$

Daily intake of MeHg by Hong Kong residents via fish consumption was estimated by Eq. (2) [27];

$$\mathrm{EDI} = \frac{\left(\dfrac{\sum_{j=1}^{n}(\mathrm{C}mj \times \mathrm{IR}j)}{\sum_{j=1}^{n}\mathrm{IR}j}\right)\left(\sum_{j=1}^{n}\mathrm{IR}j\right)}{\mathrm{BW}} = \frac{\sum_{j=1}^{n}(\mathrm{C}mj \times \mathrm{IR}j)}{\mathrm{BW}} \quad (2)$$

where EDI = estimated dietary intake of MeHg from consumption of market fish, expressed as ng kg^{-1} bw d^{-1}; C_m = Concentrations of MeHg in market fish, expressed as a wet weight basis (ng g^{-1}, ww); IR = rate of ingestion of fish by residents of Hong Kong, which was estimated to be 93 and 50 g d^{-1} for adults and children, respectively [28]; BW = body weight for adults and children, which were defined as 60 [19] and 21.8 kg [28], respectively. The reference dose (RfD) value reported by the United States Environmental Protection Agency (US EPA) is 100 ng kg^{-1} bw d^{-1} [29]. The acceptable daily intake (ADI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 229 ng kg^{-1} bw d^{-1} for MeHg [30]. The EDI for adults and children was calculated using the total and bioaccessible concentrations of MeHg obtained in this study.

2.5. Data analysis

Statistical analysis was performed by use of SPSS 17.0 for Windows. Since recoveries were adequate, concentrations of tHg and MeHg reported as mean ± SD ng g^{-1}, wet weight (ww), were not adjusted for recovery. Assumptions of normality and homogeneity of variance for application of parametric statistical procedures were confirmed by use of the Kolmogorov–Smirnov test and Levine's test, respectively. Student *t*-test was used to compare the daily intakes of tHg and MeHg by consumption of marine and freshwater fishes. One-way ANOVA was used to compare the daily intakes of tHg and MeHg among fishes. Pearson correlation analysis was used to investigate relationship between concentrations of tHg and MeHg in fishes. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Concentrations of tHg and MeHg in fishes from markets in Hong Kong

Concentrations of tHg and MeHg ranged from 27.2 to 311 ng g^{-1} (mean 100 ng g^{-1}, median 88.9 ng g^{-1}, ww) and ND (not detected) to 116 ng g^{-1} (mean 52.3 ng g^{-1}, median 45.0 ng g^{-1}, ww), respectively (Table 2). The greatest concentrations of tHg and MeHg were observed in Snubnose pompano and Goldspotted rabbitfish, which had concentrations of 210 ± 117 and 95.1 ± 12.2 ng g^{-1}, ww, respectively. The Snubnose pompano is a large predatory fish in the marine food web and can live up to 10 years. In previous studies, Snubnose pompano collected from Hong Kong was found to be contaminated by persistent organic pollutants such as PAHs, organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) [18,19,25]. The least concentrations of tHg and MeHg were observed in mud carp and catfish, which were 37.3 ± 8.73 and 7.81 ± 6.88 ng g^{-1}, ww, respectively. There

Table 2
Concentrations of tHg and MeHg in fish muscle from markets in Hong Kong (ng g^{-1}, ww).

	Common Name	LC (%)	tHg					MeHg				
			Range	Median	Mean ± STD	Digestible	BA (%)	Range	Median	Mean ± STD	Digestible	BA (%)
Freshwater fish												
1	Tilapia	3.3 ± 0.72	36.2–102	97.2	78.5 ± 36.7	33.0	42.1	9.31–42.4	19.2	23.6 ± 17.0	13.1	55.5
2	Spotted snakehead	6.2 ± 0.78	63.5–76.8	76.5	72.2 ± 7.59	26.4	36.6	36.1–41.9	37.0	38.3 ± 3.10	19.0	49.5
3	Snakehead	6.2 ± 1.6	36.2–177	40.9	84.6 ± 79.8	27.7	32.7	9.84–61.6	20.2	30.5 ± 27.4	13.1	42.8
4	Rice field eel	1.4 ± 0.54	146–155	147	149 ± 4.59	58.5	39.2	71.3–96.7	88.5	85.5 ± 13.0	32.9	38.4
5	Mud carp	2.3 ± 0.73	27.2–42.5	42.1	37.3 ± 8.73	12.7	34.1	21.5–38.5	26.4	28.8 ± 8.72	12.2	42.4
6	Mandarin fish	4.1 ± 0.57	121–173	156	150 ± 26.0	60.4	40.3	49.1–88.1	71.4	69.5 ± 19.6	34.9	50.1
7	Grey mullet	4.6 ± 0.91	39.4–42.7	41.7	41.3 ± 1.68	16.9	40.8	27.2–34.6	31.4	31.1 ± 3.69	14.6	47.0
8	Grass carp	4.1 ± 0.86	121–144	137	134 ± 11.8	49.4	37.0	21.0–78.1	25.2	41.4 ± 31.8	19.9	48.0
9	Catfish	6.3 ± 0.88	30.6–158	122	103 ± 65.4	50.2	48.7	ND–13.0	10.4	7.81 ± 6.88	4.4	56.1
10	Bighead carp	1.1 ± 0.01	75.5–103	99.3	92.5 ± 14.9	32.6	35.2	31.0–55.1	51.8	45.9 ± 13.1	16.4	35.6
Marine fish												
11	Yellow seafin	7.1 ± 1.1	75.6–171	85.0	111 ± 52.8	23.6	21.4	68.6–77.8	74.3	73.5 ± 4.65	21.6	29.3
12	Yellow croaker	13 ± 2.8	82.0–94.9	87.8	88.2 ± 6.49	19.5	22.1	61.3–71.6	62.1	65.0 ± 5.70	12.7	19.5
13	Tongue sole	1.1 ± 0.41	97.1–151	107	118 ± 28.5	29.7	25.1	47.6–81.2	80.0	69.6 ± 19.1	18.3	26.3
14	Snubnose pompano	15 ± 2.2	81.7–311	236	210 ± 117	77.3	36.9	41.3–103	49.3	64.5 ± 33.5	25.1	38.8
15	Orange-spotted grouper	5.2 ± 0.61	48.5–79.4	54.2	60.7 ± 16.4	31.4	51.7	24.5–27.3	26.8	26.2 ± 1.49	15.2	57.9
16	Golden threadfin bream	5.1 ± 0.85	36.8–68.3	46.0	50.4 ± 16.2	22.0	43.6	31.4–52.4	36.8	40.2 ± 10.9	23.8	59.2
17	Goldspotted rabbitfish	2.5 ± 0.52	102–149	119	123 ± 24.1	41.4	33.6	82.0–106	96.9	95.1 ± 12.2	33.3	35.0
18	Bleeker's grouper	1.7 ± 0.55	83.0–149	144	125 ± 36.7	50.8	40.6	39.4–116	115	90.2 ± 44.0	48.0	53.2
19	Bigeye	1.9 ± 0.27	39.8–57.2	46.2	47.7 ± 8.83	19.0	39.8	31.9–41.4	38.2	37.2 ± 4.84	16.1	43.2
20	Bartail flathead	1.3 ± 0.32	82.3–210	90.0	128 ± 71.8	60.3	47.2	54.5–109	83.5	82.4 ± 27.4	38.3	46.5

was no significant ($p > 0.05$) difference between concentrations of tHg in freshwater fishes (94.3 ± 49.2 ng g^{-1}, ww) and marine fishes (106 ± 62.4 ng g^{-1}, ww). However, the concentrations of MeHg in marine fishes (64.4 ± 28.5 ng g^{-1}, ww) were significantly ($p < 0.05$) greater than that in freshwater fishes (40.3 ± 26.0 ng g^{-1}, ww). The reason is further discussed below. The concentrations of Hg in none of the individual fish exceeded the Codex guideline of 1 mg tHg kg^{-1} (ww) [31] and USFDA guideline for MeHg of 1 mg kg^{-1} (ww) [32].

Concentrations of tHg observed in the present study (27.2–311 ng g^{-1}, mean 100 ng g^{-1}, median 88.9 ng g^{-1}, ww) were comparable with those reported for 8–9 fishes collected from Hong Kong in 2007 (3–1370 ng g^{-1}, median 63 ng g^{-1}, ww) [33], 26 marine fishes from South Korea (4.89–1008 ng g^{-1}, mean 100 ng g^{-1}, ww) [34], 23 marine fishes and four species of shellfish from the North Sea (39–610 ng g^{-1}, ww) [35], and three freshwater fishes and six marine fishes from Cambodia (<10–960 ng g^{-1}, ww) [36]. Concentrations of tHg in fishes from Hong Kong markets in the present study were less than those in predatory fishes such as swordfish (0.77 ± 0.83 μg g^{-1}, ww) and shark (0.73 ± 0.54 μg g^{-1}, ww) from Taiwan [37] and predatory whales (1.64–46.9 μg g^{-1}, ww) from Japan [38]. This result might be due to the fact that accumulation of tHg is the result of bioaccumulation through food chains, which results in greater concentrations of tHg in predatory fishes [39].

Concentrations of MeHg in fishes collected from Hong Kong markets in the present study (ND to 116 ng g^{-1}, mean 52.3 ng g^{-1}, median 45.0 ng g^{-1}, ww) were comparable to those in fishes collected from Hong Kong markets in a previous study (3–1010 ng g^{-1}, median 48 ng g^{-1}, ww) [33] and South Korea (1.02–780 ng g^{-1}, median 55.6 ng g^{-1}, ww) [34], greater than those in fishes collected from the Pearl River Delta (5.93–76.1 ng g^{-1}, ww) [15], but lesser than those in fishes from the North Sea (45–330 ng g^{-1}, ww) [35]. Generally, concentrations of MeHg in fishes from Hong Kong markets were greater than those of fishes collected from areas surrounding Hong Kong.

The ratios of concentrations of MeHg to tHg of all fishes ranged from undefined to 98.3%, with a median of 57.7%. The difference of %MeHg was likely due to differences in diet, habitat and metabolism among different fishes. The variation of %MeHg in the present study was consistent with those of previous studies of fishes from Hong Kong [33], Czech Republic [40] and Spain [41]. The percent of MeHg in Hong Kong market fish in the present study was less than that of fishes collected from the North Sea (%MeHg: 95 ± 2.5%) [35] and carnivorous marine fishes from Brazil (98%) [42]. Freshwater fish species in the present study were all grown under aquaculture. Therefore, the lesser %MeHg was due to the fact that fishes in the present study had shorter aquatic food chains and lower trophic levels compared to wild carnivorous fishes caught from the North Sea [35] and Brazil [42].

3.2. Contributing factors affecting tHg and MeHg in fishes

Several factors can affect concentrations of tHg and MeHg in fishes. Results of previous studies revealed that trophic level is one of the predominant factors determining accumulation of Hg by fishes [39,43]. However, the results of the present study indicated there was no significant (one-way ANOVA, $p > 0.05$) difference in concentrations of tHg or MeHg among fishes of different trophic levels, i.e., omnivore, carnivore and herbivore, which suggests that feeding habit was not the major factor determining concentrations of tHg and MeHg in fishes from Hong Kong markets. Furthermore, concentrations of both tHg and MeHg were not significantly ($p > 0.05$) correlated with lipid contents of fish muscle. However, fishes from Hainan Province (Bleeker's grouper and Bigeye) had significantly (one-way ANOVA, $p < 0.05$) greater concentrations of MeHg when compared with fishes from other producing areas. This observation might be due to the fact that these two fishes were collected from the wild coast of Hainan, an area which has been previously reported to have greater concentrations of Hg than those in lacustrine wetlands [44]. The result suggests that location might be one determinant of MeHg concentrations in fishes from Hong Kong markets. Other factors such as species, gender, age and the surrounding environment, including sea water and freshwater, might affect accumulation of tHg and MeHg in muscle of fishes. However, it is difficult to evaluate the relative effects of these factors on accumulation of Hg among the twenty different fish species with different sources in the present study.

There was a significant correlation ($r = 0.476$, $p < 0.001$) between concentrations of MeHg and those of tHg. Similar results have been reported for fishes, mussels and aquatic mammals collected from Korea [34] or Canada [45]. In the present study, the %MeHg of freshwater fishes (46.9 ± 24.5%) was significantly ($p < 0.05$) lower

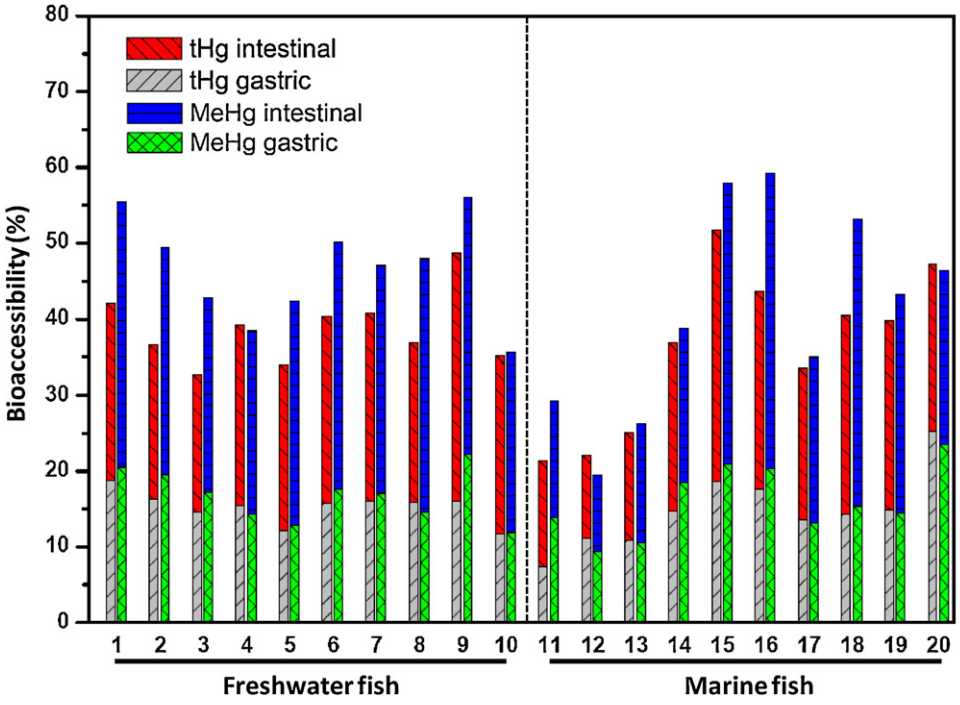

Fig. 1. Bioaccessibilities of tHg and MeHg under *in vitro* gastric and intestinal conditions for 20 fishes.

than that of marine fishes (67.2 ± 20.8%). It has been suggested that lower ratios of MeHg to tHg are characteristic of more contaminated locations, where due to direct uptake of tHg from the environment occurs in addition to uptake through food chains [46]. Furthermore, the %MeHg in axial muscle of fishes has been reported to be greater at higher trophic levels [47]. This might be due to the fact that the freshwater fishes investigated in the present study were all grown under conditions of aquaculture, which has truncated and artificial trophic levels while marine fishes such as Yellow croaker and Goldspotted rabbitfish were caught from the wild in the South China Sea. In the present study, the greater %MeHg observed at higher trophic levels might also be the reason why concentrations of MeHg in marine fishes were significantly ($p < 0.05$) greater than that in freshwater fishes, although the body weight of fishes was significantly ($p < 0.05$) larger for freshwater fishes than for marine fishes and no significant ($p > 0.05$) difference between concentrations of tHg in marine and freshwater fishes was observed.

3.3. Bioaccessibility of tHg and MeHg in fish muscle

Bioaccessibilities of tHg under *in vitro* gastric conditions ranged from 7.45 to 25.2%, with a mean of 15.1%, were significantly ($p < 0.05$) less than that under *in vitro* intestinal conditions, which had a range of 10.9–33.0%, with a mean of 22.4% (Fig. 1). Similarly, bioaccessibilities of MeHg under *in vitro* gastric conditions, which ranged from 9.37 to 23.5%, with a mean of 16.4%, were significantly ($p < 0.05$) less than that under *in vitro* intestinal conditions, which ranged from 10.2 to 38.8%, with a mean of 27.4%. The greater bioaccessibility under *in vitro* intestinal condition is consistent with results of previous studies of Hg [23] and trace organic pollutants, such as PAHs [48], and OCPs [49]. The reason for this result might be formation of aqueous suspensions of micelles by constituents of bile [50]. No significant ($p > 0.05$) difference in bioaccessibility of either tHg or MeHg was observed between marine and freshwater fishes under *in vitro* gastric conditions. However, bioaccessibilities of MeHg in marine fishes under *in vitro* intestinal conditions were significantly ($p < 0.05$) lower than those in freshwater fishes. There was no significant correlation ($p > 0.05$) between bioaccessibility and lipid contents or concentrations of tHg or MeHg under either *in vitro* gastric or intestinal condition, which suggests that lipid content and contaminant abundance in muscle of fishes are not significant determinants of bioaccessibility.

There are few published reports of bioaccessibility of Hg in fishes to which the results of this study could be compared. The mean bioaccessible fraction of tHg was 64 ± 14% in frozen swordfish collected from Spain [23]. The lower bioaccessibility of tHg observed in the present study might be due to several factors as follows: (1) differences in methodology for determining the digestible fraction. Compared with the study of Torres-Escribano et al. [23], the lower concentrations of pepsin and pancreatin in digestion juice, and filtration through the 0.45 μM filter used in the present study would all tend to result in less estimates of bioaccessibility of tHg; (2) differences among fishes. The results of a previous study revealed that the bioaccessibility of tHg in swordfish was significantly greater than that in sardine [24]; (3) conditions of storage of samples of fishes. In the study of Torres-Escribano et al. [23], swordfish had been frozen before analysis, while in the present study fish were immediately analyzed after being bought from local markets. Bioaccessibility of tHg or MeHg might be influenced by freezing rates, thawing conditions and storage temperature leading to protein denaturation.

Bioaccessibility of MeHg in the present study (43.7 ± 10.8%), particularly in freshwater fishes (46.5 ± 6.75%), was comparable with that observed in previous studies in freshwater fishes collected from Zhengjiang Province, China (48.5 ± 7.24%) [51] and nine species of marine fishes from Hong Kong (16.0–67.7%) [52]. Lower values of tHg bioaccessibility in three kinds of seafood products were observed in swordfish, sardine and tuna, which had bioaccessible fractions of 17%, 13%, and 9%, respectively [24]. This could be due to the ability of enzymes used in the *in vitro* method to release Hg complexed with selenium (Se) [22]. Considering the fact that physicochemical properties target compounds, matrices of samples, and constituents of digestive juices can significantly affect the results, a number of operating parameters, such as temperature, pH, and fluid/solid ratios, should be considered when applying *in vitro*

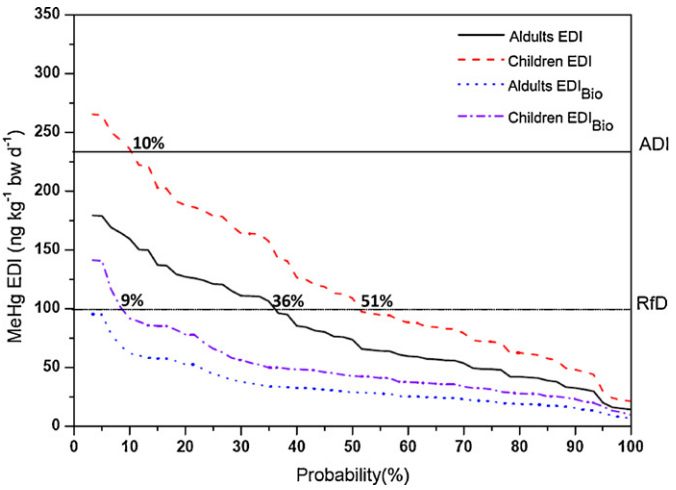

Fig. 2. Probability density distribution of estimated daily intakes of total (EDI) and bioaccessible (EDI_{Bio}) MeHg through consumption of fishes from markets in Hong Kong by adults and children in Hong Kong. ADI = acceptable daily intake = 229 ng kg^{-1} bw d^{-1} [30]; RfD = reference dose = 100 ng kg^{-1} bw d^{-1} [27].

gastrointestinal methods. This limitation of the *in vitro* methods should be investigated and, hopefully, standardized procedures can be formulated for use in the future. In summary, the present study contributes the greatest amount of data to the published literature about the bioaccessibility of tHg and MeHg among fishes. All of the above mentioned factors may affect the ability of the enzymes used in the *in vitro* digestion method to solubilize tHg and MeHg from proteins and need further study in our near future.

3.4. Estimation of intake and assessment of risk

The probability density distribution of estimated daily intakes of total (EDI) and bioaccessible (EDI_{Bio}) MeHg through consumption of fish from markets in Hong Kong by adults and children was developed (Fig. 2). The results demonstrated that 10% of children would consume an EDI for MeHg exceeding the ADI of 229 ng kg^{-1} bw d^{-1}. It was predicted that 36% of adults and 51% of children would have an EDI for MeHg that would exceed the RfD of 100 ng kg^{-1} bw d^{-1}. This result was similar to results of previous studies that consumption of fish products in Hong Kong would result in an EDI for MeHg exceeded the RfD for both adults and children [15,53]. A daily exposure dose of MeHg exceeding the RfD indicates that people might suffer harmful effects over a lifetime, or may be at risk for deleterious non-cancer effects from chronic exposure through the diet [37], by consuming fishes from Hong Kong markets.

Results of previous studies indicated that total concentrations of pollutants in foodstuffs would overestimate intake through the diet because the entire mass of pollutants are not bioavailable [17]. When exposure was corrected by bioaccessibility, daily intakes of MeHg by adults and children via consumption of fishes from markets in Hong Kong resulted in median EDI_{Bio} values of 28.6 ng kg^{-1} bw d^{-1} and 42.3 ng kg^{-1} bw d^{-1}, respectively. Thus, when corrected for bioaccessible fractions, consumption of fishes from markets in Hong Kong would not result in an EDI_{bio} for MeHg that would exceed the RfD of 100 ng kg^{-1} bw d^{-1} for adults. The results of the assessment of risk based on total and digestible concentrations were contradictory, which was mainly due to the fact that only limited proportion of MeHg in fish muscle was digestible. Considering that a proportion of MeHg was not bioavailable, former studies about human health risk assessments based on total concentrations of MeHg in fish should be adjusted by taking bioaccessibility into account [15,54]. The EDI_{bio} for MeHg exceeded the RfD for 9% of Hong Kong children, which suggests that more attention should be paid to consumption of fish from markets in Hong Kong on the health and development of children.

Previous studies indicated that the *in vitro* digestion model may underestimate the Hg bioavailability from foods [55]. It might be due to a lack of brush border enzymes or Hg absorption mechanisms in the *in vitro* digestion system. Therefore, it will be more credible when the validity of using bioaccessibility assays for predicting bioavailability is ensured prior to use in human health risk assessment. Furthermore, results of the assessment could be influenced by uncertainty factors such as rates of ingestion of fish and further investigations would be necessary to better characterize bioavailability of MeHg in fish to humans. Other pollutants, such as PAHs [18], OCPs [19] and PBDEs [25] were also observed in fishes from markets in Hong Kong. It was assumed that the effects of these pollutants would be additive, further studies would be required before a more concrete conclusion can be drawn. However, the present results provide useful information showing that the consumption of fish might impose health risks to the local population, especially pregnant and nursing mothers who are more likely to be susceptible to MeHg.

4. Conclusions

This study provided a database of concentrations of tHg and MeHg and their bioaccessibility for twenty species of fishes available in Hong Kong markets. Mean concentrations of MeHg in marine fish (64.4 ± 28.5 ng g^{-1}) were significantly greater than those in freshwater fish (40.3 ± 26.0 ng g^{-1}). Concentrations of MeHg and tHg in fishes from markets in Hong Kong were moderate when compared with those found in other countries. There was a significant correlation ($r = 0.476$, $p < 0.001$) between Me–Hg and tHg. Bioaccessibilities of tHg and MeHg ranged from 21.4 to 51.7% (mean 37.4%) and 19.5 to 59.2% (mean 43.7%), respectively. This result is consistent with the results of assessments of risks by use of conventional procedures based on total concentrations overestimating the actual exposure of humans. More attention should be paid on the local fish consumption on the health and development of children because 9% of children still had higher EDI_{bio} of MeHg compared to RfD even when the bioaccessibility was taken into account.

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