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A Comparison of the Toxicokinetics and Bioaccumulation Potential of Mercury and Polychlorinated Biphenyls in Goldfish (*Carassius auratus*)

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KEYWORDS

Assimilation Efficiency, Elimination, POPs, Bioaccumulation, Biomagnification

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ABSTRACT

Both mercury (Hg) and polychlorinated biphenyls (PCBs) demonstrate food web biomagnification in aquatic ecosystems, yet their toxicokinetics have not been simultaneously contrasted within a common fish species. This study quantified uptake and elimination rates of Hg and PCBs in goldfish. Fish were exposed to contaminated food containing PCBs and Hg to determine dietary chemical assimilation efficiencies (AE) and elimination coefficients ($k_{\text{tot}}$). To test first order kinetics, three exposure regimes were established by varying the proportion of contaminated fish incorporated into the food. Dietary AEs were 98±10%, 75±12%, and 40±9% for MeHg, THg and PCBs, respectively. The $k_{\text{tot}}$ were 0.010±0.003 d$^{-1}$ and 0.010±0.002 d$^{-1}$ for THg and MeHg. No significant differences were found in $k_{\text{tot}}$ among the dosing levels for either THg or MeHg, confirming that Hg elimination was a first order process. For PCB, $k_{\text{tot}}$ ranged from 0.007 to 0.022 d$^{-1}$ and decreased with increasing hydrophobicity. This study revealed that Hg had a higher AE compared to PCBs while the $k_{\text{tot}}$ of Hg was similar to those measured for the most hydrophobic PCBs. It is concluded that Hg has a 118% higher bioaccumulation potential in goldfish than the highest PCB BMF observed for congeners with a log $K_{\text{OW}} > 7$. 
Introduction

Bioaccumulation of mercury (Hg) and polychlorinated biphenyls (PCBs) by fish is a global issue because the consumption of fish is the most important exposure route of these toxic pollutants to human populations (1, 2). Within North America, Hg is the main driver of fish consumption advisories issued by regulatory agencies (3), followed by organochlorine compounds such as PCBs and polychlorinated dibenzo-\( p \)-dioxins (4). Environmental contamination by Hg and PCB is observed in both marine and freshwater systems (5-7), including systems considered far removed from point sources (8, 9).

One of the major factors contributing to Hg and PCB fish consumption advisories is related to their ability to biomagnify (10, 11). Although biomagnification is commonly defined as a food web process, the actual mechanism of biomagnification occurs at the scale of the individual (12). Net positive bioaccumulation occurs in individuals as a result of kinetic conditions where the chemical uptake rates via food exceed whole body elimination rates (13, 14). Kinetic processes vary with trophic level as a result of the size of organisms and different assimilation efficiencies. Quantifying the factors regulating food web biomagnification in aquatic ecosystems requires a thorough understanding of chemical toxicokinetics at the level of the individual.

Although similarities between Hg and PCB biomagnification patterns are widely reported in food webs, these substances have different mechanisms of accumulation (15-17). The tissue-distributions and retention of PCBs and other organochlorines are regulated by different processes than those of Hg. Organochlorines undergo passive partitioning (i.e. diffusive flux) between aqueous and organic phases (primarily neutral lipid) within the organism and external to the animal as regulated by chemical-physical properties such as hydrophobicity (18) and lipid
Hg, however, exhibits strong associations with sulphur-rich proteins and is poorly associated with tissue lipids. Although the mechanism of biomagnification of organochlorines has been largely resolved, there are yet to be clear mechanisms describing how Hg is taken up from food, sequestered within tissues and eliminated by fish. Furthermore, no studies have co-examined chemical toxicokinetics for organochlorines and Hg within the same organism. It is important to understand how the magnitude of key toxicokinetic parameters differs in order to directly compare their bioaccumulation potentials.

For Hg and PCBs, dietary uptake is considered a major pathway for exposure in fish. The dietary assimilation efficiency (AE) is a key toxicokinetic parameter that regulates the relative importance of dietary uptake. For Hg, the AE in fish differs markedly between inorganic Hg and methyl mercury (MeHg) ranging from 4-51% and 65-99% for inorganic and organic forms, respectively. For PCBs, AE shows very high variation among studies and is weakly related to congener hydrophobicity as well as diet composition. As a result of the high variation in AEs reported by different studies using different experimental conditions, fish sizes and fish diets, it is very difficult to directly compare AEs for Hg and PCBs using the current literature.

The main toxicokinetic parameter specifying the time it takes for an organism to reach steady state is the whole body elimination coefficient ($k_{tot}$). The $k_{tot}$ incorporates the sum of individual elimination coefficients across different chemical loss pathways that include (among others): elimination of chemical across respiratory surfaces, via urine, feces, mucous, protein/lipid excretion (e.g. via reproductive output, sloughing of cells, scales or excretions), metabolic biotransformation and pseudo-elimination as a function of growth dilution. PCBs are lost predominantly through gills although modeling studies suggest that, for more
hydrophobic chemicals, feces play a an increasingly more important role (31). For PCBs, losses through gills and growth dilution are considered the major elimination pathways (30). For Hg, however, less information is available about which of the above routes dominate k_tot of fish. Most bioaccumulation models of PCBs and Hg in fish have assumed that chemical elimination occurs by first-order processes (32, 33), whereby elimination rate coefficients are independent of the chemical concentration in the organism. This assumption has been tested and met for PCBs (29; 30) but is conflicting for Hg (32, 34). Thus, there remains a need to verify the first order assumption of elimination kinetics of Hg.

The main objectives of this study were to simultaneously determine AEs and k_tot for PCBs and Hg in order to directly quantify their bioaccumulation potentials in a model organism, the goldfish (Carassius auratus). Furthermore, this study design resulted in differences in the initial Hg levels among treatments enabling testing of first order elimination kinetics.

Materials and methods

Fish Husbandry and Dosing. A total of 332 goldfish (Carassius auratus) (2.32±0.68g) were obtained from Leadley Environmental Inc., Essex, Ontario, Canada. Goldfish were selected as the study organism because they are hardy under experimental conditions, have flexible feeding habits and are commonly used as a fish species in toxicokinetic studies for different pollutants including PCBs and Hg (35, 36). Goldfish are also a common invasive species in North American waters, including the Great Lakes. Although not commonly consumed as a food fish, goldfish bear similarities in feeding ecology and habitat to common carp (Cyprinus carpio) which frequently achieve high levels of fish consumption restrictions due to their degree of contamination. Fish were maintained in holding tanks (400 L capacity) at the University of
Windsor’s Great Lakes Institute for Environmental Research (GLIER) at a constant temperature 20.9±1.4°C. The stock density was 5 L water per fish. A recirculating water system and filters were used to maintain water quality, and half of the water from the tank was replaced by fresh dechlorinated water every week. Water pH was 7.4±0.1 and the dissolved oxygen level was 5.7±1.3 mg·L⁻¹. Fecal matter at the bottom of the tank was removed every three days to minimize re-uptake of chemicals by fish. Fish were inspected daily and observed to be in good health, and mortality during the experiment was below 10%.

The study consisted of three experimental tanks and one internal control tank, all sharing the same water supply. During the dosing period, fish from three experimental tanks were fed with fish pellets of three contamination levels every day to maximize chemical uptake. During the elimination period, the fish were fed with commercial fish flakes (Cobalt Aquatics, South Carolina, USA) once every two days to minimize growth. Commercial fish flakes were also used to feed the control fish during the uptake portion of the study. Fish food was weighed before feeding, and excess food was removed three hours later from each tank. The total amount of food consumed was determined by subtracting the dry weight of unconsumed food from the pre-weighed amount (dry weight).

To circumvent experimental artifacts associated with the dosing method and establish a more natural exposure for each pollutant, the contaminated food fed to experimental fish was generated by incorporating fish meal derived from feral fish collected from contaminated systems. This approach ensured that fish were exposed to chemicals in a manner that replicates normal environmental exposures. Fish food preparation is described in the supporting information. Three dosing treatments using three contamination levels of fish food were generated to achieve low, medium, and high contamination levels in experimental fish. In order
to obtain a significant difference in tissue residues between the low Hg and high Hg treatment fish for the elimination studies, the dosing duration was adjusted such that low treatment fish had their contaminated diet discontinued after 15 days whereas high dose fish were fed for 42 days.

Sample collection during the uptake phase of the study took place at days 0, 7, and 15 \((n=3, 5\text{ and } 5)\) for low dosed treatment fish, at days 0, 7, 14, 21, and 28 \((n=3, 5, 5, 5\text{ and } 5)\) for medium dosed treatment fish, and days 0, 7, 14, 21, and 42 \((n=3, 5, 5, 5\text{ and } 5)\) for the high dosed group.

During the elimination phase, fish were placed back on to the control diet and collected from each tank on days 0, 7, 14, 21, 28, 42, 56, 72, and 84 \((n=5, 5, 5, 5, 5, 5, 5\text{ and } 5\text{ for the low dosed tank, }n=5, 5, 5, 4, 4, 4, 4\text{ and } 5\text{ for the medium dosed tank, and }n=5, 5, 5, 4, 4, 4, 5\text{ and } 5\text{ for the high dosed tank, respectively})\), with day 0 representing the final day of the uptake study for each respective dose treatment. Five fish were taken from each of the three experimental and control tanks on each sampling date. Fish were immediately euthanized using a concentrated clove oil solution, and body length and weight were measured in the lab. The University of Windsor’s Animal Care Committee Guidelines were strictly followed throughout the duration of the experiment. Following euthanasia and morphometric measurements, whole fish was homogenized into a fine paste after removing the gut content. Samples were then frozen until analysis of Hg and PCBs.

**Chemical Analysis.** Total Hg and PCB analyses were conducted at the GLIER analytical laboratory, University of Windsor, using accredited standard operating procedures (accredited through the Canadian Association for Laboratory Accreditation; CALA). Total Hg concentrations were measured using a Direct Mercury Analyzer, DMA-80 (Milestone Inc.). The DMA-80 was calibrated using a 10 point calibration curve from a certified liquid Hg standard (High-Purity Standards, Charleston, USA). Approximately 0.15 g whole fish homogenate was
weighed on a clean nickel boat, and then placed on the autosampler of the instrument. QA/QC procedures included incorporation of blanks (empty nickel boats), duplication of a random sample for every six samples analyzed and certified reference tissues (Dorm-3 and Dolt-4, National Research Council Canada; BT-Cnt2L and W-CntVG, in house standards) randomly placed into the autosampler wells to represent 20% of samples being analyzed within a given batch. Moisture content of samples was determined by gravimetric means to establish dry weight Hg concentrations.

MeHg analysis was performed on a subset of samples at the Biotron’s Analytical Services laboratory at the University of Western Ontario (ISO 17025). Concentrations were analyzed by the Tekran 2700 MeHg auto-analyzer using US EPA method 1630. Approximately 0.15 g whole fish homogenate were weighed into a 60 mL Savillex digestion vessel, with 10 mL of 5 M nitric acid solution added. Samples were vortexed for 10 seconds at 3000 RPM and allowed to sit overnight, followed by digestion in an oven at 80°C for eight hours. 10 mL of ultrapure deionized water was added to each vessel after the samples were completely cool and then vortexed for 10 seconds at 3000 RPM. Approximately 0.1 g digestion product was transferred from the sample vessel to the instrument vial, and ultrapure deionized water was added until a final weight of 28.5-29.5 g was achieved. 1 mL of acetate buffer was added to adjust the pH to 4-4.5, followed by ethylation with 30 μL 1% NaBEt₄. Sample vials were then rapidly shaken three times after capping and placed onto the instrument autosampler. The instrument was calibrated using an 8 point calibration curve from 1 ppm MeHg stock solution (Brooks Rand). QA/QC procedures included measurement of replicates, method blanks and certified reference samples (Human Hair IAEA-086, International Atomic Energy Agency; Tort-3, National Research Council Canada) analyzed for approximately every 10th samples.
Owing to limitations in sample availability, MeHg analysis was conducted on selected samples. During uptake, MeHg was measured in treatment fish at 0, 7, and 15 days of dosing for low treatment fish (n=1, 5 and 2), at 0, 14, 21, and 28 days of dosing for medium treatment fish (n=1, 5, 4, and 3), and at days 0, 7, 14, 21, and 42 of dosing for the high dosing group (n=1, 4, 4, 5, and 5). During the elimination phase, MeHg was tested in treatment fish on days 0 (n=2, 3, and 5 for low, medium, and high treatment, respectively), 28 (n=4, 5, and 4), 56 (n=5, 4, and 3), and 84 (n=5, 4, and 1) of elimination. For controls, MeHg was measured in one control fish (n=1) from each sampling date throughout both phases of the study (days 0, 7, 14, 21, 28, and 42 during uptake, days 0, 7, 14, 21, 28, 42, 56, 70, and 84 during elimination) to verify if there was a significant change in the MeHg:THg ratio. MeHg analysis was also performed on fish food from the low (n=3), medium (n=2) and high (n=3) dosing treatments.

PCB concentrations were measured using an Agilent 6890 Series Plus gas chromatograph (GC) with a 63Ni-micro electron capture detector (ECD) and an Agilent 7683 autosampler. The PCB extraction method is described in Daley et al. (37) and clean-up is described by Lazar et al. (38). Approximately 0.5 g whole fish homogenate was added to a glass mortar, and then ground with 15 g of activated sodium sulfate by a glass pestle. The mixture was transferred into a micro extraction column containing 25mL of 1:1 v/v dichloromethane: hexane (DCM:HEX) and spiked with 35 ng PCB 34 as a recovery standard. After one hour extraction, the column was eluted, followed by a second elution with 15 mL of DCM:HEX. 10% of the extract was removed for determination of neutral lipids by gravimetric means (19). Clean-up of remaining extracts was performed by activated florisil (38) and concentrated to a final volume of 1 mL for GC-ECD analysis. QA/QC procedures included monitoring internal standard (PCB34) recoveries, use of a
method blank (sodium sulfate) and an in-house tissue reference sample (Detroit River carp) co-extracted for every batch of six samples analyzed. PCB 34 recoveries averaged $73 \pm 11\%$.

**Data Analysis.** In order to account for differences in tissue capacity, all Hg concentrations are expressed in units of $\mu g \cdot g^{-1}$ lean dry weight *(excluding moisture and lipid content)* (39). All PCB concentrations are expressed in units of $\mu g \cdot g^{-1}$ lipid weight. PCB congeners included in the analysis consisted of only those congeners where detection occurred in more than 60% of the samples. Congener specific log $K_{OW}$ values for PCBs were obtained from Hawker and Connell (40).

All concentrations were corrected for control contamination prior to calculating toxicokinetic parameters. Control correction was performed by subtracting the mean contaminant concentration in control fish from the contaminant concentrations measured in each treatment fish at the equivalent sampling point. There were no significant changes in control fish (ANOVA, $p>0.05$) in the MeHg:THg ratio during the experiment. Thus, MeHg concentrations in each control fish were estimated using the product of the mean MeHg:THg ratios in control fish and respective THg concentrations.

Growth correction for THg and MeHg concentration was based on lean dry body weight pool over time while growth correction for PCBs considered growth on the change of the lipid pool in fish over time. Two different growth models (linear, logarithmic) were evaluated to explain growth during the uptake and elimination phases, and it was found that the fish growth over time in this study was best fitted to linear growth models. For linear growth, growth rate ($g, d^{-1}$) within a given treatment was calculated to be equal to the slope generated from a plot of $W_t/W_0$.
versus time (days), where $W_0$ and $W_t$ refer to the weight (lean dry body weight for Hg or whole body lipid weight for PCB) in the organism at day 0 and day of sampling.

$$C_{cg(t)} = C_c(t) \cdot (1 + g \cdot t) \quad (1)$$

where $C_{cg(t)}$ is the control and growth corrected concentration ($\mu g \cdot g^{-1}$ lean dry body weight for Hg or $\mu g \cdot g^{-1}$ lipid weight for PCBs) in the animal at time (t) in days, $C_c(t)$ is the control corrected concentration ($\mu g \cdot g^{-1}$ lean dry body weight for Hg or $\mu g \cdot g^{-1}$ lipid weight for PCBs), and $g$ is the growth rate ($d^{-1}$). If growth for a given compartment was non-significant over the uptake or elimination phase of the study, no growth correction was performed.

Whole body chemical elimination rate coefficients ($k_{tot}, d^{-1}$) were calculated for total Hg, MeHg and PCB congeners and set equal to the slope generated from a plot of $\ln\left[\frac{C_{cg(t)}}{C_{cg(0)}}\right]$ with time, where $C_{cg(0)}$ is the control and growth corrected concentration ($\mu g \cdot g^{-1}$ lean dry body weight for Hg or $\mu g \cdot g^{-1}$ lipid weight for PCBs) in the animal at day 0 of elimination. The chemical half-life ($t_{1/2}, d$) in fish was calculated as

$$t_{1/2} = \frac{\ln(2)}{k_{tot}} \quad (2)$$

Chemical uptake coefficients ($k_d$) were calculated as the slopes of the least squares regression between the fish-to-food chemical concentration ratio and dosing time. Assuming all fish consumed food equally, the feeding rates ($I$) were measured as the percentage of total feeding amount to total body mass of fish in the tank. Dietary chemical AEs were calculated for each treatment fish sacrificed during the uptake phase according to:

$$AE = \frac{X_{ef} + X_{ex}}{X_c} = \frac{C_c(t) \cdot W_t + [C_{cg(t)} \cdot (1 - e^{-k_{tot} \cdot t}) \cdot W_t]}{I \cdot W_{c} \cdot C_{diet} \cdot t} \quad (3)$$
where \(X_f\) refers to the total mass of chemical (\(\mu g\)) in the fish at sacrifice, \(X_{ex}\) represents mass of chemical (\(\mu g\)) lost by elimination during the uptake period and \(X_c\) is the total mass of chemical (\(\mu g\)) ingested over the uptake period. \(C_{ct}(t)\) is the control-corrected (no growth correction) concentration (\(\mu g \cdot g^{-1}\) lean dry body weight for Hg or \(\mu g \cdot g^{-1}\) lipid weight for PCBs) in the fish at sacrifice, \(W_t\) is the tissue weight (lean dry weight (g) for Hg or whole body lipid weight (g) for PCBs), \(C_{cg}(t)\) is the control-growth-corrected chemical concentration (no growth correction) in fish at sacrifice (\(\mu g \cdot g^{-1}\) lean dry body weight for Hg or \(\mu g \cdot g^{-1}\) lipid weight for PCBs), assuming all fish consumed food equally, the food ingestion rates (I) were measured as dry weight fish food consumed by each fish per day (g dry food \cdot d^{-1}), \(I\) is the food ingestion rate \(C_{diet}\) is the dry weight concentration of chemical in food (\(\mu g \cdot g^{-1}\) dry wt) and \(t\) is time of the uptake period (days).

The biomagnification factor (BMF) was calculated as per \((41, 42)\) according to:

\[
\text{BMF} = \frac{I_b \cdot AE}{k_{tot}} \tag{4}
\]

Where \(I_b\) is the body weight adjusted food ingestion rate. For consistency with the literature, BMFs for Hg are reported in g dry food \cdot g^{-1} dry wt organism by using an \(I_b\) with units of g dry food \cdot g^{-1} dry wt organism \cdot d^{-1}. For PCBs, the BMF is most commonly reported in units of g lipid wt food \cdot g^{-1} lipid wt organism \((41, 46)\) and is calculated using an \(I_b\) with units of g lipid food \cdot g^{-1} lipid organism \cdot d^{-1}. To facilitate comparisons of the magnitude of BMF between Hg and PCBs, BMFs of PCB congeners were also expressed in a common unit of dry food \cdot g^{-1} dry wt organism.

Analysis of variance (ANOVA) was used to test for differences in Hg or PCBs in different food samples (control and treatments), and differences in contaminant levels in fish at the end of the
uptake period (control and treatments). Linear regression analysis was performed to compute growth rates, determine $k_{tot}$ values and describe the relationship between BMF of PCB congeners and log $K_{OW}$. ANOVA was used with linear regression to test whether slopes were significantly different from a value of zero. Analysis of Covariance (ANCOVA) was used with linear regression to test for significant differences in the slope of contaminant concentrations with time across dosing treatments. Furthermore, ANOVA and nonlinear regression were used to describe the relationship between BMFs and log $K_{OW}$. Prior to using parametric tests, data were evaluated for normality and homogenous variance between treatments using the Kolmogorov-Smirnov and Levene’s tests. A non-parametric Kruskal-Wallis test was used to evaluate the difference in ingestion rates among treatments when data failed normality assumptions even after a ln transformation. All statistical analyses were conducted using IBM SPSS version 20.

**Results**

The chemical concentrations in fish and their diet in both control and dosing groups are summarized in Table 1. By the end of the uptake phase, THg concentrations (mean±SD) were 0.45±0.12, 1.35±0.21 and 3.13±0.37 µg·g$^{-1}$ lean dry wt for low, medium and high dosed fish, which were significantly higher than the mean THg concentrations from their corresponding control fish (ANOVA, p<0.01). MeHg concentrations (mean±SD) were 0.48±0.05, 1.30±0.25, and 3.58±0.47 µg·g$^{-1}$ lean dry wt for three dosing treatments, which were also significantly higher than the mean MeHg concentrations from their corresponding control fish (ANOVA, p<0.05). Significant differences were confirmed among the three treatment groups in both THg and MeHg concentrations using ANOVA (p<0.01). The mean MeHg:THg ratios were 96±16%, 84±2%, 90±16%, and 114±13% for control, low, medium and high dosed fish, respectively. No significant differences were observed in MeHg:THg ratios among treatments (ANOVA, p>0.05).
A total of 34 PCB congeners (17/18, 28/31, 33, 44, 49, 52, 70, 74, 87, 95, 99, 101, 110, 128, 151/82, 149, 118, 153, 105/132, 138, 158, 156/171, 170, 177, 180, 183, 187, 191, 194, 199, 195/208, 205, 206, and 209) were detected in the fish samples, with 8 congeners (17/18, 28/31, 33, 44, 52, 70, 74, and 205) being excluded because less than 60% of the samples were above the detection limit. Sum PCB concentrations (mean±SD) were 0.31±0.13, 0.83±0.29, and 4.66±0.53 µg·g⁻¹ lipid for the three dosing groups. Only the high dose group showed a significantly higher sum PCB level (p<0.01; ANOVA) in tissue residues compared to the control. Thus toxicokinetic parameters generated for PCBs were measured only for the high dose group.

**Chemical Elimination**

During the elimination phase of the study, the feeding-body weight adjusted food ingestion rates (mean±SD) were 0.040±0.007, 0.040±0.005, 0.040±0.007, and 0.040±0.006 g dry wt food ·g⁻¹ dry wt fish·d⁻¹ for low, medium, high treatment, and control fish, respectively. No significant differences were found in feeding rates across the treatments (ANOVA, p>0.05). No significant growth was observed in either lipid weight or lean dry weight during the elimination phase (ANOVA, p>0.05). No significant growth was observed in either lipid weight or lean dry weight during the elimination phase (ANOVA, p>0.05). Thus, growth correction was not required for the Hg nor PCB data in the elimination study.

Elimination rate coefficients were determined for all chemicals that revealed significant elimination during the study period. For THg and MeHg significant elimination (ANOVA, p<0.05) was observed for each dosing treatment. The mean MeHg:THg ratios (mean±SD) during elimination were 86±10%, 104±13%, and 103±13% for low, medium, and high dosed fish, respectively. Within each treatment, the MeHg: THg ratio did not change significantly.
throughout the elimination phase of the study (ANOVA, p>0.05). For sum PCBs, the high dose
treatment samples showed significant elimination (ANOVA, p<0.05) over time. On a congener
specific basis, 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82,
153, 158, 170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) were observed to
demonstrate significant elimination during the elimination phase.

Figure 1 presents elimination rates of control corrected THg and MeHg concentrations in fish
through time. The mean $k_{tot}$ of THg were 0.012, 0.011 and 0.007 d$^{-1}$ in low, medium and high
dose groups (linear regression, p<0.01). ANCOVA revealed no significant difference in THg
elimination rates across the three dosing groups (p>0.05). For MeHg, $k_{tot}$ in low, medium and
high dose groups were 0.017, 0.010 and 0.011 d$^{-1}$(linear regression, p<0.05). MeHg also showed
no significant difference in elimination among the treatments (ANCOVA, p>0.05). Given the
lack of differences in $k_{tot}$ between dosing groups, data were combined across doses to yield
overall mean ($\pm$SD) $k_{tot}$ values of 0.010 ±0.003 d$^{-1}$ and 0.010 ±0.005 d$^{-1}$for THg and MeHg,
respectively, corresponding to a half-life of 69 days.

The $k_{tot}$ values for individual PCB congeners ranged from 0.007 to 0.022 d$^{-1}$. There was a strong
negative relationship (linear regression, p<0.01) between PCB $k_{tot}$ values and congener log $K_{OW}$
(Figure 2). Half-lives for PCBs ranged from 28 to 100 days across congeners and were positively
associated (linear regression, p<0.01) with chemical log $K_{OW}$ according to the equation:

$$t_{1/2(\text{PCBs})} = 20.38 \pm 4.72 \cdot \log K_{OW} - 77.25 \pm 32.89; \quad R^2 = 0.45 \quad (5)$$

Given the measured Hg half-life of 69 days in these same fish, Eq. 5 can be used to demonstrate
that Hg is eliminated at the same rate as a PCB having a log $K_{OW}$ value of 7.2 (e.g. PCB183).
Assimilation

Body weight adjusted food ingestion Feeding rates (mean±SD) during the uptake study were 0.060±0.005, 0.060±0.004, 0.060±0.004, and 0.070±0.01 g dry wt food·g⁻¹ dry wt·d⁻¹ fish for low, medium, high treatment, and control fish, respectively. No significant differences in feeding rates were measured among treatments (ANOVA, p>0.05). There were significant linear relationships between lean dry weight and days of dosing from high dosed fish, and between lipid weight and days of dosing for both medium and high dosed fish (linear regression, p<0.05). Such relationships were also found to be significant when using the logarithmic growth model (linear regression, p<0.05), but with a lower R value, indicating the linear model provided a better prediction of fish growth. Thus, growth correction was performed for Hg concentrations in high dosed fish and for PCB concentrations in medium and high dosed fish during the uptake portion of this study.

Uptake rate coefficients were calculated for THg and MeHg for each treatment, as well as for each of 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82, 153, 158, 170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) from high dosed fish. The mean (±SD) $k_d$ values for THg and MeHg were 0.04±0.003 and 0.05±0.01 d⁻¹. There were no significant differences in $k_d$ values among three dosing levels for either THg or MeHg (ANCOVA, p>0.05). For PCB congeners, $k_d$ ranged from 0.02 to 0.04 d⁻¹, and no significant relationship was observed between $k_d$ and log $K_{OW}$ (linear regression, p>0.05).

Chemical AEs were calculated for THg and MeHg from each treatment sample, as well as for each of 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82, 153, 158, 170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) from high dosed fish. The
AE for PCB congeners ranged from 23% to 63%, however, no significant relationship was observed between AE and log $K_{OW}$ (linear regression, $p>0.05$). Because no significant difference was found in AE among congeners (ANOVA, $p>0.05$), the AE of one of the most common PCB (PCB180, log $K_{OW}=7.4$) was used for comparison with the AE of Hg. Figure 3 shows mean (+SD) AE values for THg and MeHg, and PCB180, which were 75±12%, 98±10%, and 44±16%, respectively. The mean AE values for THg, MeHg, and PCBs differed significantly from each other (ANOVA, $p<0.05$).

Biomagnification factors were calculated for MeHg and THg using mean AE, I and $k_{tot}$ values derived from each treatment, and for PCB congeners using the PCB data from high dosed fish. The mean BMF values for MeHg and THg were 6.1 and 4.5 g dry wt food·g$^{-1}$ dry wt fish. The BMF value for PCB congeners ranged from 1.4 to 7.4 g lipid food·g$^{-1}$ lipid fish. Figure 4 showed BMF for PCB calculated on a dry weight food to dry weight fish basis, and it ranged from 0.72 to 3.8 g dry wt food·g$^{-1}$ dry wt fish. There was a significantly linear relationship (BMF = 0.90 log $K_{OW} – 4.07$, $R^2 = 0.41$, $p<0.01$) and curvilinear relationship (BMF = -0.66log $K_{OW}$ $^2$ + 10.20 log $K_{OW}$ – 36.59, $R^2 = 0.52$, $p<0.01$) observed between BMF and log $K_{OW}$. The curvilinear model had a larger R value, and predicted the maximum BMF to be 2.8.

Discussion

This study is the first to simultaneously compare dietary assimilation efficiencies and elimination rate coefficients of Hg and PCB in a freshwater fish species. The results showed that mean dietary AEs for MeHg were higher than those observed for all PCB congeners. The AE was 98±12% for MeHg, and 40±9% for PCBs, which are both comparable to those reported in previous studies (42, 43). Leaner and Mason (44) reported that MeHg dietary AEs ranged from
90% to 92% in sheepshead minows (*Cyprinodon variegatus*), while Pickhardt et al (27) found AE values between 90% and 94% in mosquitofish (*Gambusia affinis*), and 85% to 91% in redear sunfish (*Lepomis microlophus*). For PCBs, AE had varied from 23% to 101% (25, 31, 45). Buckman et al (46) found that AE for 92 PCB congeners ranged from 40% to 50%, and Liu et al. (45) concluded that AE values for 47 PCBs were under 60% when using a similar fish diet (high-fat pellet), all of which are consistent with our values. There are many factors that regulate chemical AEs for Hg and PCBs in fish, such as composition of the dietary matrices, digestibility of the food, ingestion rates, fish physiology, and water chemistry and temperature (47, 48).

The elimination rate coefficient for MeHg in our study falls within the lower end of the range of elimination rate coefficients measured for PCB congeners. Also, the half-life of Hg was equivalent to that estimated for highly chlorinated PCBs with log $K_{OW} = 7.2$. Considering the elevated dietary AE of Hg coupled with it having an elimination rate coefficient equivalent to the most hydrophobic PCB congeners indicates that Hg has a higher bioaccumulation potential than most of the PCB congers.

The $k_{tot}$ for MeHg in our study is comparable with past laboratory studies on goldfish (36). De Freitas and colleagues reported Hg elimination rate coefficients of 0.02 and 0.008 d$^{-1}$ in goldfish weighing 1 and 7.4 g respectively, at 22 ºC. Our $k_{tot}$ was 0.01 d$^{-1}$ for fish of 2.32±0.68 g at 20.9±1.4 ºC falls within the above range (36). The consistency of $k_{tot}$ across dosing treatments as determined in this study supports the conclusion that Hg elimination in fish is a first order process. The $k_{tot}$ values for THg and MeHg were not significantly different from each other because MeHg was the dominant Hg species in the fish. Thus, the first order kinetics of Hg elimination observed from this study is driven by the elimination kinetics of MeHg. The data for inorganic Hg were insufficient to characterize its elimination kinetics.
The evidence supporting first order kinetics for Hg in the literature is conflicting. A negative relationship between the dosage level in fish and half-life of MeHg was reported by Ruohutula and Miettinen (34) for rainbow trout (Salmo gairdneri). However, their fish were dosed with 3.0, 0.4 and 0.1 µg·g⁻¹ MeHg, and only the fish of the highest dosage revealed a significantly faster excretion. The fish from the two lower dosage groups did not show significantly different elimination, suggesting their results were inconclusive. In contrast, Trudel and Rasmusen (32) demonstrated no correlation between initial Hg concentration and the Hg elimination rate coefficient based on 41 previous case studies, which supports our findings.

Several studies on Hg elimination in freshwater and marine fish species (26, 34) reported a pattern of biphasic elimination, where there was a rapid loss of chemical immediately after dosing, followed by a slower loss process. Biphasic elimination kinetics was not observed in our study, which might be a result of the differences in the dosing method between this study and the earlier cited studies. Lags associated with the inter-tissue transport kinetics post assimilation often result in higher blood concentrations of the chemical relative to other tissues following initial chemical exposure (31). Given the role of blood as a central compartment and its stronger association with chemical elimination, an elevated blood concentration favors a higher initial elimination of chemicals (49). In this study, fish were exposed to a naturally contaminated diet for several weeks prior to initiation of elimination, whereas fish in other studies were typically given a single oral dose, an intramuscular injection or a short term (from several hours to days) aqueous exposure. The dosing methodology of our study is more representative of natural exposure and uptake dynamics given that the Hg was ingested in a form (i.e. protein-associated) more consistent with Hg exposures taking place in aquatic ecosystems.
PCB elimination has been consistently shown to follow first order kinetics (29, 30). PCB elimination has been studied in a wide range of fish species, but few investigations are available for goldfish. The $k_{\text{tot}}$ value for PCBs ranged from 0.007 to 0.022 d$^{-1}$ in our study, while Hattula and Carlog (35) reported the half-life for the sum PCB at 21 d in goldfish, which corresponds to an elimination rate of 0.03 d$^{-1}$. Even though a similar body size of 1.8 g and water temperature 21-23 °C were used in their study, comparisons of $k_{\text{tot}}$ could not be made for individual congeners due to analytical limitations. Paterson et al. (50) found $k_{\text{tot}}$ values ranging from 0.004 to 0.02 d$^{-1}$, using yellow perch (*Perca flavescens*) of 8.3 g under summer water temperature (23 °C). Van Geest et al. (51) reported that PCB elimination rate coefficients ranged from 0.009 to 0.037 d$^{-1}$, using fathead minnows (*Pimephales promelas*) of less than 1 g and at 23 °C. Our $k_{\text{tot}}$ values were comparable with both studies on the scale of individual congeners.

PCBs elimination by aquatic species is mechanistically understood to result from diffusive fluxes across respiratory surfaces and through fecal egestion, driven by the chemical fugacity gradients between the animal and the elimination media (21, 30, 43). The physiological mechanism of Hg elimination, however, remains largely unknown for fish. It might involve demethylation biotransformation reactions as described to occur in some species of birds and mammals with subsequent loss of inorganic Hg by kidneys (52) or as a result of protein turnover during routine metabolism. Madenjian et al. (53) suggested that sex-based differences in Hg elimination from fish might be hormonally controlled.

BMFs estimated for MeHg approached a value of 6.1 g dry wt food·g$^{-1}$ dry wt fish in goldfish, and ranged from 1.4 to 7.4 g lipid food·g$^{-1}$ lipid fish for PCB congeners, the BMF/K$\text{OW}$ model predicts an upper limit of 2.8 g dry wt food·g$^{-1}$ dry wt fish. Previous studies reported BMFs for MeHg in fish ranging between 1 and 10 g dry wt food·g$^{-1}$ dry wt fish based on laboratory and
field data (15, 26), while estimates of BMFs reported for PCB congeners have ranged from 0.7 to
9.0 g lipid food g\(^{-1}\) lipid fish, depending on chemical hydrophobicity (41, 46). Figure 4
summarizes PCB BMFs (g dry wt food g\(^{-1}\) dry wt fish) as a function of \(K_{OW}\) with both linear and
curvilinear fits to the obtained data. The curvilinear fit implies a maximum PCB BMF of 2.8 g
dry wt food g\(^{-1}\) dry wt fish, much less than the observed value for Hg. The linear model would
indicate that only PCBs having log \(K_{OW}\) values greater than 11 would exhibit a BMF that
approaches Hg shows a significantly curvilinear relationship between BMF and log \(K_{OW}\), with a
BMF upper limit of 4.8. Although AEs from the present work did not show significant \(K_{OW}\)
dependence as has been observed elsewhere (31, 45, 46), \(k_{tot}\) was observed to exhibit a slope
transition for congeners exceeding a log \(K_{OW}\) of 7.0, consistent with the curvilinear BMF-\(K_{OW}\)
relationship most commonly reported in the literature. Overall, it is concluded that Hg has a
118\% higher biomagnification factor leading to a higher bioaccumulation potential compared
with the highest BMF modeled for PCB congeners based on the curvilinear BMF relationship.
Even under a linear BMF model, Hg BMFs exceeded BMFs for the most common
superhydrophobic PCBs, e.g. PCB 180. This implies Hg has a higher bioaccumulation and food
web biomagnification potential compared to PCBs and is consistent with the observation that Hg
more frequently contributes to fish consumption advisories in in-land lakes that are remote from
point sources, whereas PCBs tend to dominate fish consumption advice in the Laurentian Great
Lakes where industrial sources and legacy contamination of sediments remain acute. Further
research to understand differences in PCB and Hg toxicokinetics in other fish species using
simultaneous chemical exposures would be useful to verify if bioaccumulation potential is
consistent among different species, and whether observed differences are maintained across
different food webs, diet conditions and environmental conditions.
Figure 1. THg and MeHg elimination by fish from three treatments, squares represent low (■), circles represent medium (●), crosses represent high dosing treatment (×); THg was tested for fish at days 0, 7, 14, 21, 28, 42, 56, 72, and 84 (n=5, 5, 5, 5, 5, 5, 5, 5 and 5 for low dosed treatment, n=5, 5, 5, 5, 4, 4 and 5 for medium dosed treatment, and n=5, 5, 5, 4, 4, 5 and 5 for high dosed fish). MeHg was tested in fish on days 0, 28, 56, and 84 (n=2, 4, 5 and 5 for low dosed group, n=3, 5, 4 and 4 for medium dosed group, and n=5, 4, 3 and 1 for high dosed treatment). Vertical bars represent standard error.
Figure 2. Elimination rate coefficients of PCB congeners from high dosed fish.

Figure 3. Assimilation efficiencies of THg, MeHg, and PCB180; Grey bars for THg and MeHg represent the average AE values from each treatment sample. The grey bar for PCB 180 represents the mean AE value from the high dosed fish sample. Vertical bars represent standard error.
Figure 4. Biomagnification factors of PCB congeners in goldfish. The solid line represents the curvilinear model, and dashed line represents the linear model.

Table 1. Mean (± SD) THg, MeHg, and PCB concentrations in control and dosed fish at the end (day 0 for elimination) of dosing, as well as in fish diet.

<table>
<thead>
<tr>
<th></th>
<th>THg (µg·g⁻¹ lean dry wt)</th>
<th>MeHg (µg·g⁻¹ lean dry wt)</th>
<th>Sum PCBs (µg·g⁻¹ lipid)</th>
<th></th>
<th>THg (µg·g⁻¹ lean dry wt)</th>
<th>MeHg (µg·g⁻¹ lean dry wt)</th>
<th>Sum PCBs (µg·g⁻¹ lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish diet</td>
<td></td>
<td></td>
<td>Fish homogenate (day 0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Low control</td>
<td>0.05±0.003</td>
<td>0.03±0.003</td>
<td>0.01</td>
<td>0.11±0.02</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Low dosed</td>
<td>0.63±0.03 † a</td>
<td>0.44±0.02 † a</td>
<td>0.42±0.02 † a</td>
<td>0.45±0.12 † a</td>
<td>0.48±0.05 † a</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>Medium control</td>
<td>0.05±0.003</td>
<td>0.03±0.003</td>
<td>0.01</td>
<td>0.16±0.06</td>
<td>0.12±0.06</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>Medium dosed</td>
<td>1.11±0.06 † b</td>
<td>0.92±0.01 † b</td>
<td>0.80±0.04 † b</td>
<td>1.35±0.21 † b</td>
<td>1.30±0.25 † b</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>High control</td>
<td>0.05±0.003</td>
<td>0.03±0.003</td>
<td>0.01</td>
<td>0.22±0.06</td>
<td>0.20±0.06</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>High dosed</td>
<td>1.91±0.16 † c</td>
<td>1.86±0.34 † c</td>
<td>2.68±0.29 † c</td>
<td>3.13±0.37 † c</td>
<td>3.58±0.47 † c</td>
</tr>
</tbody>
</table>

Note: Different lowercase letters indicate significant differences in mean THg, MeHg, and sum PCB concentrations among low, medium and high dosing treatments (ANOVA). † Indicates
significant difference (p<0.05; ANOVA) between control and treatment fish for THg, MeHg or sum PCBs

ASSOCIATED CONTENT

Supporting Information

Information regarding fish food preparation; moisture, lipid, and lean dry weight content from the fish food; concentrations for each PCB congener in the fish food; moisture, lipid, and lean dry weight content in individual samples and growth rate determination; fish PCB concentration-time profile during elimination; I, AE, k\text{tot}, t_{1/2}, and BMF for Hg and MeHg from all three dosing treatments, as well as these parameters for PCB congeners from high dosed fish. The material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors, but the main contributions were by J.L. and K.G.D. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

Hg, mercury; MeHg, methylmercury; PCB, polychlorinated biphenyl; POPs, persistent organic pollutants; \( K_{ow} \), octanol-water partition coefficient; Hex, hexane; DCM, dichloromethane; wt, weight; AE, assimilation efficiency; \( k_{tot} \), elimination coefficients; \( k_{d,chemical-uptake} \), chemical uptake coefficients; BMF, biomagnification factor; \( t_{1/2} \), half-life.

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