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1 # A Comparison of the Toxicokinetics and

2 Bioaccumulation Potential of Mercury and

3 Polychlorinated Biphenyls in Goldfish (*Carassius*

4 *auratus*)

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11 Assimilation Efficiency, Elimination, POPs, Bioaccumulation, Biomagnification

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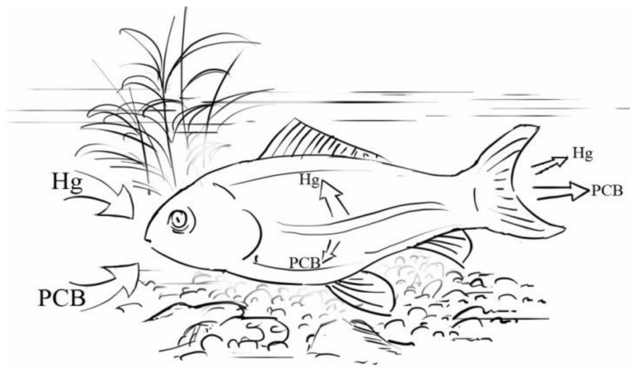


13 ABSTRACT

14 Both mercury (Hg) and polychlorinated biphenyls (PCBs) demonstrate food web

15 biomagnification in aquatic ecosystems, yet their toxicokinetics have not been simultaneously

16 contrasted within a common fish species. This study quantified uptake and elimination rates of

17 Hg and PCBs in goldfish. Fish were exposed to contaminated food containing PCBs and Hg to

18 determine dietary chemical assimilation efficiencies (AE) and elimination coefficients (k_{tot}). To

19 test first order kinetics, three exposure regimes were established by varying the proportion of

20 contaminated fish incorporated into the food. Dietary AEs were 98±10%, 75±12%, and 40±9 %

21 for MeHg, THg and PCBs, respectively. The k_{tot} were 0.010±0.003 d^{-1} and 0.010±0.002 d^{-1} for

22 THg and MeHg. No significant differences were found in k_{tot} among the dosing levels for either

23 THg or MeHg, confirming that Hg elimination was a first order process. For PCB, k_{tot} ranged

24 from 0.007 to 0.022 d^{-1} and decreased with increasing hydrophobicity. This study revealed that

25 Hg had a higher AE compared to PCBs while the k_{tot} of Hg was similar to those measured for the

26 most hydrophobic PCBs. It is concluded that Hg has a 118% higher bioaccumulation potential in

27 goldfish than the highest PCB BMF observed for congeners with a log K_{OW} > 7.

28

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

51 content of the animal (*19*). Hg, however, exhibits strong associations with sulphur-rich proteins
52 and is poorly associated with tissue lipids (*20*). Although the mechanism of biomagnification of
53 organochlorines has been largely resolved (*21, 22*), there are yet to be clear mechanisms
54 describing how Hg is taken up from food, sequestered within tissues and eliminated by fish.
55 Furthermore, no studies have co-examined chemical toxicokinetics for organochlorines and Hg
56 within the same organism. It is important to understand how the magnitude of key toxicokinetic
57 parameters differs in order to directly compare their bioaccumulation potentials.

58 For Hg and PCBs, dietary uptake is considered a major pathway for exposure in fish (*23, 24*).
59 The dietary assimilation efficiency (AE) is a key toxicokinetic parameter that regulates the
60 relative importance of dietary uptake (*14, 25*). For Hg, the AE in fish differs markedly between
61 inorganic Hg and methyl mercury (MeHg) ranging from 4-51% and 65-99% for inorganic and
62 organic forms, respectively (*26, 27*). For PCBs, AE shows very high variation among studies and
63 is weakly related to congener hydrophobicity (*25*) as well as diet composition (*28*). As a result of
64 the high variation in AEs reported by different studies using different experimental conditions,
65 fish sizes and fish diets, it is very difficult to directly compare AEs for Hg and PCBs using the
66 current literature.

67 The main toxicokinetic parameter specifying the time it takes for an organism to reach steady
68 state is the whole body elimination coefficient (k_{tot}). The k_{tot} incorporates the sum of individual
69 elimination coefficients across different chemical loss pathways that include (among others):
70 elimination of chemical across respiratory surfaces, via urine, feces, mucous, protein/lipid
71 excretion (e.g. via reproductive output, sloughing of cells, scales or excretions), metabolic
72 biotransformation and pseudo-elimination as a function of growth dilution (*14, 29*). PCBs are
73 lost predominantly through gills (*30*) although modeling studies suggest that, for more

74 hydrophobic chemicals, feces play a an increasingly more important role (*31*). For PCBs, losses
75 through gills and growth dilution are considered the major elimination pathways (*30*). For Hg,
76 however, less information is available about which of the above routes dominate k_{tot} of fish.
77 Most bioaccumulation models of PCBs and Hg in fish have assumed that chemical elimination
78 occurs by first-order processes (*32*, *33*), whereby elimination rate coefficients are independent of
79 the chemical concentration in the organism. This assumption has been tested and met for PCBs
80 (*29; 30*) but is conflicting for Hg (*32*, *34*). Thus, there remains a need to verify the first order
81 assumption of elimination kinetics of Hg.

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

86 **Materials and methods**

87 **Fish Husbandry and Dosing.** A total of 332 goldfish (*Carassius auratus*) (2.32±0.68g) were
88 obtained from Leadley Environmental Inc., Essex, Ontario, Canada. Goldfish were selected as
89 the study organism because they are hardy under experimental conditions, have flexible feeding
90 habits and are commonly used as a fish species in toxicokinetic studies for different pollutants
91 including PCBs and Hg (*35*, *36*). Goldfish are also a common invasive species in North
92 American waters, including the Great Lakes. Although not commonly consumed as a food fish,
93 goldifish bear similarities in feeding ecology and habitat to common carp (*Cyprinus carpio*)
94 which frequently achieve high levels of fish consumption restrictions due to their degree of
95 contamination. Fish were maintained in holding tanks (400 L capacity) at the University of

96 Windsor's Great Lakes Institute for Environmental Research (GLIER) at a constant temperature
97 20.9±1.4ºC. The stock density was 5 L water per fish. A recirculating water system and filters
98 were used to maintain water quality, and half of the water from the tank was replaced by fresh
99 dechlorinated water every week. Water pH was 7.4±0.1 and the dissolved oxygen level was
100 5.7±1.3 $mg \cdot L^{-1}$. Fecal matter at the bottom of the tank was removed every three days to
101 minimize re-uptake of chemicals by fish. Fish were inspected daily and observed to be in good
102 health, and mortality during the experiment was below 10%.

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

112 To circumvent experimental artifacts associated with the dosing method and establish a more
113 natural exposure for each pollutant, the contaminated food fed to experimental fish was
114 generated by incorporating fish meal derived from feral fish collected from contaminated
115 systems. This approach ensured that fish were exposed to chemicals in a manner that replicates
116 normal environmental exposures. Fish food preparation is described in the supporting
117 information. Three dosing treatments using three contamination levels of fish food were
118 generated to achieve low, medium, and high contamination levels in experimental fish. In order

119 to obtain a significant difference in tissue residues between the low Hg and high Hg treatment
120 fish for the elimination studies, the dosing duration was adjusted such that low treatment fish had
121 their contaminated diet discontinued after 15 days whereas high dose fish were fed for 42 days.
122 Sample collection during the uptake phase of the study took place at days 0, 7, and 15 (n=3, 5
123 and 5) for low dosed treatment fish, at days 0, 7, 14, 21, and 28 (n=3, 5, 5, 5 and 5) for medium
124 dosed treatment fish, and days 0, 7, 14, 21, and 42 (n=3, 5, 5, 5 and 5) for the high dosed group.

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

136 **Chemical Analysis.** Total Hg and PCB analyses were conducted at the GLIER analytical
137 laboratory, University of Windsor, using accredited standard operating procedures (accredited
138 through the Canadian Association for Laboratory Accreditation; CALA). Total Hg
139 concentrations were measured using a Direct Mercury Analyzer, DMA-80 (Milestone Inc.). The
140 DMA-80 was calibrated using a 10 point calibration curve from a certified liquid Hg standard
141 (High-Purity Standards, Charleston, USA). Approximately 0.15 g whole fish homogenate was

142 weighed on a clean nickel boat, and then placed on the autosampler of the instrument. QA/QC
143 procedures included incorporation of blanks (empty nickel boats), duplication of a random
144 sample for every six samples analyzed and certified reference tissues (Dorm-3 and Dolt-4,
145 National Research Council Canada; BT-Cnt2L and W-CntVG, in house standards) randomly
146 placed into the autosampler wells to represent 20% of samples being analyzed within a given
147 batch. Moisture content of samples was determined by gravimetric means to establish dry weight
148 Hg concentrations.

149 MeHg analysis was performed on a subset of samples at the Biotron's Analytical Services
150 laboratory at the University of Western Ontario (ISO 17025). Concentrations were analyzed by
151 the Tekran 2700 MeHg auto-analyzer using US EPA method 1630. Approximately 0.15 g whole
152 fish homogenate were weighed into a 60 mL Savillex digestion vessel, with 10 mL of 5 M nitric
153 acid solution added. Samples were vortexed for 10 seconds at 3000 RPM and allowed to sit
154 overnight, followed by digestion in an oven at 80°C for eight hours. 10 mL of ultrapure
155 deionized water was added to each vessel after the samples were completely cool and then
156 vortexed for 10 seconds at 3000 RPM. Approximately 0.1 g digestion product was transferred
157 from the sample vessel to the instrument vial, and ultrapure deionized water was added until a
158 final weight of 28.5-29.5 g was achieved. 1 mL of acetate buffer was added to adjust the pH to 4-
159 4.5, followed by ethylation with 30 µL 1% $NaBEt_4$. Sample vials were then rapidly shaken three
160 times after capping and placed onto the instrument autosampler. The instrument was calibrated
161 using an 8 point calibration curve from 1 ppm MeHg stock solution (Brooks Rand). QA/QC
162 procedures included measurement of replicates, method blanks and certified reference samples
163 (Human Hair IAEA-086, International Atomic Energy Agency; Tort-3, National Research
164 Council Canada) analyzed for approximately every 10th samples.

165 Owing to limitations in sample availability, MeHg analysis was conducted on selected samples.
166 During uptake, MeHg was measured in treatment fish at 0, 7, and 15 days of dosing for low
167 treatment fish (n=1, 5 and 2), at 0, 14, 21, and 28 days of dosing for medium treatment fish (n=1,
168 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
169 5). During the elimination phase, MeHg was tested in treatment fish on days 0 (n=2, 3, and 5 for
170 low, medium, and high treatment, respectively), 28 (n=4, 5, and 4), 56 (n=5, 4, and 3), and 84
171 (n=5, 4, and 1) of elimination. For controls, MeHg was measured in one control fish (n=1) from
172 each sampling date throughout both phases of the study (days 0, 7, 14, 21, 28, and 42 during
173 uptake, days 0, 7, 14, 21, 28, 42, 56, 70, and 84 during elimination) to verify if there was a
174 significant change in the MeHg:THg ratio. MeHg analysis was also performed on fish food from
175 the low (n=3), medium (n=2) and high (n=3) dosing treatments.

176 PCB concentrations were measured using an Agilent 6890 Series Plus gas chromatograph (GC)
177 with a 63Ni-micro electron capture detector (ECD) and an Agilent 7683 autosampler. The PCB
178 extraction method is described in Daley et al. (*37*) and clean-up is described by Lazar et al. (*38*).
179 Approximately 0.5 g whole fish homogenate was added to a glass mortar, and then ground with
180 15 g of activated sodium sulfate by a glass pestle. The mixture was transferred into a micro
181 extraction column containing 25mL of 1:1 v/v dichloromethane: hexane (DCM:HEX) and spiked
182 with 35 ng PCB 34 as a recovery standard. After one hour extraction, the column was eluted,
183 followed by a second elution with 15 mL of DCM:HEX. 10% of the extract was removed for
184 determination of neutral lipids by gravimetric means (*19*). Clean-up of remaining extracts was
185 performed by activated florisil (*38*) and concentrated to a final volume of 1 mL for GC-ECD
186 analysis. QA/QC procedures included monitoring internal standard (PCB34) recoveries, use of a

187 method blank (sodium sulfate) and an in-house tissue reference sample (Detroit River carp) co-
188 extracted for every batch of six samples analyzed. PCB 34 recoveries averaged 73±11%.

189 **Data Analysis.** In order to account for differences in tissue capacity, all Hg concentrations are
190 expressed in units of $\mu g \cdot g^{-1}$ lean dry weight (excluding moisture and lipid content) (*39*). All PCB
191 concentrations are expressed in units of $\mu g \cdot g^{-1}$ lipid weight. PCB congeners included in the
192 analysis consisted of only those congeners where detection occurred in more than 60% of the
193 samples. Congener specific log K_{OW} values for PCBs were obtained from Hawker and Connell
194 (*40*).

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

202 Growth correction for THg and MeHg concentration was based on lean dry body weight pool
203 over time while growth correction for PCBs considered growth on the change of the lipid pool in
204 fish over time. Two different growth models (linear, logarithmic) were evaluated to explain
205 growth during the uptake and elimination phases, and it was found that the fish growth over time
206 in this study was best fitted to linear growth models. For linear growth, growth rate (g, d^{-1})
207 within a given treatment was calculated to be equal to the slope generated from a plot of W_t/W_0

208 versus time (days), where W_0 and W_t refer to the weight (lean dry body weight for Hg or whole

209 body lipid weight for PCB) in the organism at day 0 and day of sampling.

210
$$C_{cg(t)} = C_{c(t)} \cdot (1 + g \cdot t) \quad (1)$$

211 where $C_{cg(t)}$ is the control and growth corrected concentration (µg·g^{-1} lean dry body weight for

212 Hg or µg·g^{-1} lipid weight for PCBs) in the animal at time (t) in days, $C_{c(t)}$ is the control corrected

213 concentration (µg·g^{-1} lean dry body weight for Hg or µg·g^{-1} lipid weight for PCBs), and g is the

214 growth rate (d^{-1}). If growth for a given compartment was non-significant over the uptake or

215 elimination phase of the study, no growth correction was performed.

216 Whole body chemical elimination rate coefficients (k_{tot}, d^{-1}) were calculated for total Hg, MeHg

217 and PCB congeners and set equal to the slope generated from a plot of ln[$C_{cg(t)}$/$C_{cg(0)}$] with time,

218 where $C_{cg(0)}$ is the control and growth corrected concentration (µg·g^{-1} lean dry body weight for

219 Hg or µg·g^{-1} lipid weight for PCBs) in the animal at day 0 of elimination. The chemical half-life

220 ($t_{1/2}$, d) in fish was calculated as

221
$$t_{1/2} = \frac{\ln(2)}{k_{tot}} \quad (2)$$

222 ~~Chemical uptake coefficients (k_d) were calculated as the slopes of the least squares regression~~

223 ~~between the fish-to-food chemical concentration ratio and dosing time. Assuming all fish~~

224 ~~consumed food equally, the feeding rates (I) were measured as the percentage of total feeding~~

225 ~~amount to total body mass of fish in the tank~~Dietary chemical AEs were calculated for each

226 treatment fish sacrificed during the uptake phase according to:

227
$$AE = \frac{X_f + X_{ex}}{X_c} = \frac{C_{c(t)} \cdot W_t + [C_{cg(t)} \cdot (1 - e^{-k_{tot} \cdot t}) \cdot W_t]}{I \cdot \cancel{W_t} \cdot C_{diet} \cdot t} \quad (3)$$

228 where X_f refers to the total mass of chemical (µg) in the fish at sacrifice, X_{ex} represents mass of
229 chemical (µg) lost by elimination during the uptake period and X_c is the total mass of chemical
230 (µg) ingested over the uptake period. $C_{c(t)}$ is the control-corrected (no growth correction)
231 concentration (µg·g^{-1} lean dry body weight for Hg or µg·g^{-1} lipid weight for PCBs) in the fish at
232 sacrifice, W_t is the tissue weight (lean dry weight (g) for Hg or whole body lipid weight (g) for
233 PCBs), $C_{cg(t)}$ is the ~~control-growth-corrected~~ chemical concentration (no growth correction) in
234 fish at sacrifice (µg·g^{-1} lean dry body weight for Hg or µg·g^{-1} lipid weight for PCBs), assuming
235 all fish consumed food equally, the food ingestion rates (I) were measured as dry weight fish
236 food consumed by each fish per day (g dry food ·d^{-1}), ~~I is the food ingestion rate~~ C_{diet} is the dry
237 weight concentration of chemical in food (µg·g^{-1} dry wt) and t is time of the uptake period
238 (days).

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

240 $$\mathrm{BMF} = \frac{I_b \cdot AE}{k_{tot}} \qquad (4)$$

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

247 Analysis of variance (ANOVA) was used to test for differences in Hg or PCBs in different food
248 samples (control and treatments), and differences in contaminant levels in fish at the end of the

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

260 **Results**

261 The chemical concentrations in fish and their diet in both control and dosing groups are
262 summarized in Table 1. By the end of the uptake phase, THg concentrations (mean±SD) were
263 0.45±0.12, 1.35±0.21 and 3.13±0.37 $\mu g \cdot g^{-1}$ lean dry wt for low, medium and high dosed fish,
264 which were significantly higher than the mean THg concentrations from their corresponding
265 control fish (ANOVA, $p<0.01$). MeHg concentrations (mean±SD) were 0.48±0.05, 1.30±0.25,
266 and 3.58±0.47 $\mu g \cdot g^{-1}$ lean dry wt for three dosing treatments, which were also significantly
267 higher than the mean MeHg concentrations from their corresponding control fish (ANOVA,
268 $p<0.05$). Significant differences were confirmed among the three treatment groups in both THg
269 and MeHg concentrations using ANVOA ($p<0.01$). The mean MeHg:THg ratios were 96±16%,
270 84±2%, 90±16%, and 114±13% for control, low, medium and high dosed fish, respectively. No
271 significant differences were observed in MeHg:THg ratios among treatments (ANOVA, $p>0.05$).

272 A total of 34 PCB congeners (17/18, 28/31, 33, 44, 49, 52, 70, 74, 87, 95, 99, 101, 110, 128,
273 151/82, 149, 118, 153, 105/132, 138, 158, 156/171, 170, 177, 180, 183, 187, 191, 194, 199,
274 195/208, 205, 206, and 209) were detected in the fish samples, with 8 congeners (17/18, 28/31,
275 33, 44, 52, 70, 74, and 205) being excluded because less than 60% of the samples were above the
276 detection limit. Sum PCB concentrations (mean±SD) were 0.31±0.13, 0.83±0.29, and 4.66±0.53
277 $\mu g \cdot g^{-1}$ lipid for the three dosing groups. Only the high dose group showed a significantly higher
278 sum PCB level ($p<0.01$; ANOVA) in tissue residues compared to the control. Thus toxicokinetic
279 parameters generated for PCBs were measured only for the high dose group.

280 **Chemical Elimination**

281 During the elimination phase of the study, the ~~feeding~~ body weight adjusted food ingestion rates
282 (mean±SD) were 0.040±0.007, 0.040±0.005, 0.040±0.007, and 0.040±0.006 g dry wt food $\cdot g^{-1}$
283 dry wt fish$\cdot d^{-1}$ for low, medium, high treatment, and control fish, respectively. No significant
284 differences were found in feeding rates across the treatments (ANOVA, $p>0.05$). No significant
285 growth was observed in either lipid weight or lean dry weight during the elimination phase
286 (ANOVA, $p>0.05$). No significant growth was observed in either lipid weight or lean dry weight
287 during the elimination phase (ANOVA, $p>0.05$). Thus, growth correction was not required for
288 the Hg nor PCB data in the elimination study.

289 Elimination rate coefficients were determined for all chemicals that revealed significant
290 elimination during the study period. For THg and MeHg significant elimination (ANOVA,
291 $p<0.05$) was observed for each dosing treatment. The mean MeHg:THg ratios (mean±SD)
292 during elimination were 86±10%, 104±13%, and 103±13% for low, medium, and high dosed
293 fish, respectively. Within each treatment, the MeHg: THg ratio did not change significantly

294 throughout the elimination phase of the study (ANOVA, $p>0.05$). For sum PCBs, the high dose
295 treatment samples showed significant elimination (ANOVA, $p<0.05$) over time. On a congener
296 specific basis, 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82,
297 153, 158, 170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) were observed to
298 demonstrate significant elimination during the elimination phase.

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

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

312 $$t_{1/2(PCBs)} = 20.38 \pm 4.72 \cdot \log K_{OW} - 77.25 \pm 32.89;\ \ R^2 = 0.45 \qquad (5)$$

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

315 **Assimilation**

316 Body weight adjusted food ingestion ~~Feeding~~ rates (mean±SD) during the uptake study were
317 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
318 low, medium, high treatment, and control fish, respectively. No significant differences in feeding
319 rates were measured among treatments (ANOVA, $p>0.05$). There were significant linear
320 relationships between lean dry weight and days of dosing from high dosed fish, and between
321 lipid weight and days of dosing for both medium and high dosed fish (linear regression, $p<0.05$).
322 Such relationships were also found to be significant when using the logarithmic growth model
323 (linear regression, $p<0.05$), but with a lower R value, indicating the linear model provided a
324 better prediction of fish growth. Thus, growth correction was performed for Hg concentrations in
325 high dosed fish and for PCB concentrations in medium and high dosed fish during the uptake
326 portion of this study.

327 ~~Uptake rate coefficients were calculated for THg and MeHg for each treatment, as well as for~~
328 ~~each of 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82, 153, 158,~~
329 ~~170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) from high dosed fish. The~~
330 ~~mean (±SD) k_d values for THg and MeHg were 0.04±0.003 and 0.05±0.01 d^{-1}. There were no~~
331 ~~significant differences in k_d values among three dosing levels for either THg or MeHg~~
332 ~~(ANCOVA, $p>0.05$). For PCB congeners, k_d ranged from 0.02 to 0.04 d^{-1}, and no significant~~
333 ~~relationship was observed between k_d and log K_{OW} (linear regression, $p>0.05$).~~

334 Chemical AEs were calculated for THg and MeHg from each treatment sample, as well as for
335 each of 24 PCB congeners (87, 95, 99, 101, 110, 118, 128, 138, 105/132, 149, 151/82, 153, 158,
336 170, 156/171, 177, 180, 183, 187, 194, 199, 195/208, 206, and 209) from high dosed fish. The

337 AE for PCB congeners ranged from 23% to 63%, however, no significant relationship was
338 observed between AE and log K_{OW} (linear regression, $p>0.05$). Because no significant difference
339 was found in AE among congeners (ANOVA, $p>0.05$), the AE of one of the most common PCB
340 (PCB180, log K_{OW}= 7.4) was used for comparison with the AE of Hg. Figure 3 shows mean
341 (±SD) AE values for THg and MeHg, and PCB180, which were 75±12%, 98±10%, and 44±16%,
342 respectively. The mean AE values for THg, MeHg, and PCBs differed significantly from each
343 other (ANOVA, $p<0.05$).

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

353 **Discussion**

354 This study is the first to simultaneously compare dietary assimilation efficiencies and elimination
355 rate coefficients of Hg and PCB in a freshwater fish species. The results showed that mean
356 dietary AEs for MeHg were higher than those observed for all PCB congeners. The AE was
357 98±12% for MeHg, and 40±9% for PCBs, which are both comparable to those reported in
358 previous studies (*42*, *43*). Leaner and Mason (*44*) reported that MeHg dietary AEs ranged from

359 90% to 92% in sheepshead minows (*Cyprinodon variegatus*), while Pickhardt et al (*27*) found
360 AE values between 90% and 94% in mosquitofish (*Gambusia affinis*), and 85% to 91% in redear
361 sunfish (*Lepomis microlophus*). For PCBs, AE had varied from 23% to 101% (*25, 31, 45*).
362 Buckman et al (*46*) found that AE for 92 PCB congeners ranged from 40% to 50%, and Liu et al.
363 (*45*) concluded that AE values for 47 PCBs were under 60% when using a similar fish diet (high-
364 fat pellet), all of which are consistent with our values. There are many factors that regulate
365 chemical AEs for Hg and PCBs in fish, such as composition of the dietary matrices, digestibility
366 of the food, ingestion rates, fish physiology, and water chemistry and temperature (*47, 48*).

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

373 The k_{tot} for MeHg in our study is comparable with past laboratory studies on goldfish (*36*). De
374 Freitas and colleagues reported Hg elimination rate coefficients of 0.02 and 0.008 d^{-1} in goldfish
375 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
376 20.9±1.4 ºC falls within the above range (*36*). The consistency of k_{tot} across dosing treatments as
377 determined in this study supports the conclusion that Hg elimination in fish is a first order
378 process. The k_{tot} values for THg and MeHg were not significantly different from each other
379 because MeHg was the dominant Hg species in the fish. Thus, the first order kinetics of Hg
380 elimination observed from this study is driven by the elimination kinetics of MeHg. The data for
381 inorganic Hg were insufficient to characterize its elimination kinetics.

382 The evidence supporting first order kinetics for Hg in the literature is conflicting. A negative
383 relationship between the dosage level in fish and half-life of MeHg was reported by Ruohtula
384 and Miettinen (*34*) for rainbow trout (*Salmo gairdneri*). However, their fish were dosed with 3.0,
385 0.4 and 0.1 $\mu g \cdot g^{-1}$ MeHg, and only the fish of the highest dosage revealed a significantly faster
386 excretion. The fish from the two lower dosage groups did not show significantly different
387 elimination, suggesting their results were inconclusive. In contrast, Trudel and Rasmusen (*32*)
388 demonstrated no correlation between initial Hg concentration and the Hg elimination rate
389 coefficient based on 41 previous case studies, which supports our findings.

390 Several studies on Hg elimination in freshwater and marine fish species (*26, 34*) reported a
391 pattern of biphasic elimination, where there was a rapid loss of chemical immediately after
392 dosing, followed by a slower loss process. Biphasic elimination kinetics was not observed in our
393 study, which might be a result of the differences in the dosing method between this study and the
394 earlier cited studies. Lags associated with the inter-tissue transport kinetics post assimilation
395 often result in higher blood concentrations of the chemical relative to other tissues following
396 initial chemical exposure (*31*). Given the role of blood as a central compartment and its stronger
397 association with chemical elimination, an elevated blood concentration favors a higher initial
398 elimination of chemicals (*49*). In this study, fish were exposed to a naturally contaminated diet
399 for several weeks prior to initiation of elimination, whereas fish in other studies were typically
400 given a single oral dose, an intramuscular injection or a short term (from several hours to days)
401 aqueous exposure. The dosing methodology of our study is more representative of natural
402 exposure and uptake dynamics given that the Hg was ingested in a form (i.e. protein-associated)
403 more consistent with Hg exposures taking place in aquatic ecosystems.

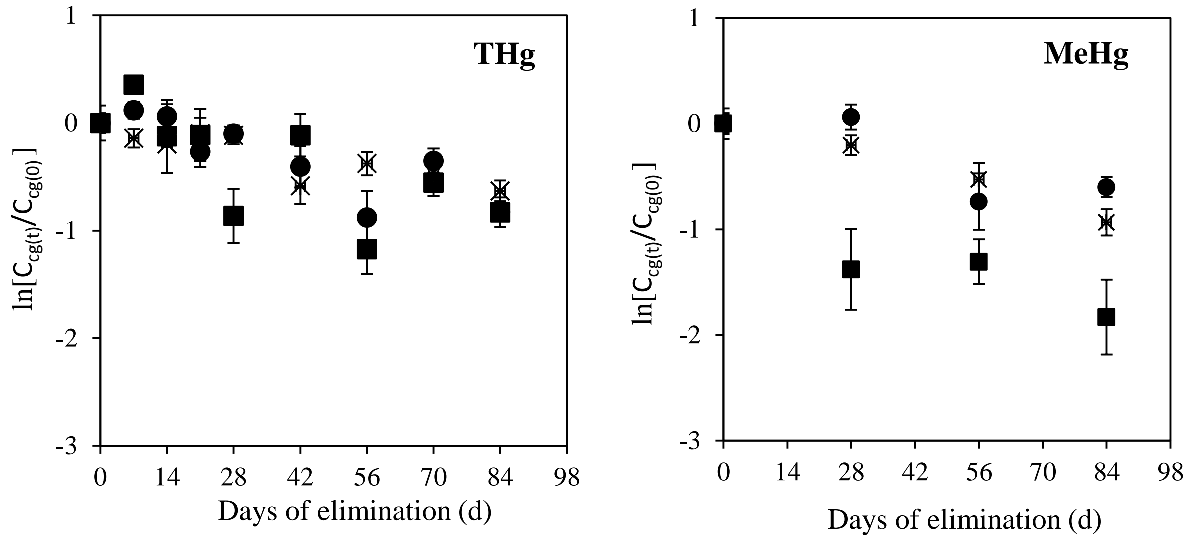
404 PCB elimination has been consistently shown to follow first order kinetics (*29, 30*). PCB
405 elimination has been studied in a wide range of fish species, but few investigations are available
406 for goldfish. The k_{tot} value for PCBs ranged from 0.007 to 0.022 d^{-1} in our study, while Hattula
407 and Carlog (*35*) reported the half-life for the sum PCB at 21 d in goldfish, which corresponds to
408 an elimination rate of 0.03 d^{-1}. Even though a similar body size of 1.8 g and water temperature
409 21-23 °C were used in their study, comparisons of k_{tot} could not be made for individual
410 congeners due to analytical limitations. Paterson et al. (*50*) found k_{tot} values ranging from 0.004
411 to 0.02 d^{-1}, using yellow perch (*Perca flavescens*) of 8.3 g under summer water temperature (23
412 °C). Van Geest et al. (*51*) reported that PCB elimination rate coefficients ranged from 0.009 to
413 0.037 d^{-1}, using fathead minnows (*Pimephales promelas*) of less than 1 g and at 23 °C. Our k_{tot}
414 values were comparable with both studies on the scale of individual congeners.

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

423 BMFs estimated for MeHg approached a value of 6.1 g dry wt food·g^{-1} dry wt fish in goldfish,
424 and ranged from 1.4 to 7.4 g lipid food·g^{-1} lipid fish for PCB congeners~~, the BMF/K_{OW} model~~
425 ~~predicts an upper limit of 2.8 g dry wt food·g^{-1} dry wt fish~~. Previous studies reported BMFs for
426 MeHg in fish ranging between 1 and 10 g dry wt food·g^{-1} dry wt fish based on laboratory and

427 field data (*15, 26*), while estimates of BMFs reported for PCB congeners have ranged from 0.7 to
428 9.0 g lipid food·g^{-1} lipid fish, depending on chemical hydrophobicity (*41, 46*). Figure 4
429 summarizes PCB BMFs (g dry wt food·g^{-1} dry wt fish) as a function of K_{OW} with both linear and
430 curvilinear fits to the obtained data. The curvilinear fit implies a maximum PCB BMF of 2.8 g
431 dry wt food·g^{-1} dry wt fish, much less than the observed value for Hg. The linear model would
432 indicate that only PCBs having log K_{OW} values greater than 11 would exhibit a BMF that
433 approaches Hg. ~~shows a significantly curvilinear relationship between BMF and log K_{OW}, with a~~
434 ~~BMF upper limit of 4.8.~~ Although AEs from the present work did not show significant K_{OW}
435 dependence as has been observed elsewhere (*31, 45, 46*), k_{tot} was observed to exhibit a slope
436 transition for congeners exceeding a log K_{OW} of 7.0, consistent with the curvilinear BMF-K_{OW}
437 relationship most commonly reported in the literature. Overall, it is concluded that Hg has a
438 118% higher biomagnification factor leading to a higher bioaccumulation potential compared
439 with the highest BMF modeled for PCB congeners based on the curvilinear BMF relationship.
440 Even under a linear BMF model, Hg BMFs exceeded BMFs for the most common
441 superhydrophobic PCBs, e.g. PCB 180. This implies Hg has a higher bioaccumulation and food
442 web biomagnification potential compared to PCBs and is consistent with the observation that Hg
443 more frequently contributes to fish consumption advisories in in-land lakes that are remote from
444 point sources, whereas PCBs tend to dominate fish consumption advice in the Laurentian Great
445 Lakes where industrial sources and legacy contamination of sediments remain acute. Further
446 research to understand differences in PCB and Hg toxicokinetics in other fish species using
447 simultaneous chemical exposures would be useful to verify if bioaccumulation potential is
448 consistent among different species, and whether observed differences are maintained across
449 different food webs, diet conditions and environmental conditions.

450 FIGURES



451

452 **Figure 1.** THg and MeHg elimination by fish from three treatments, squares represent low (■),

453 circles represent medium (●), crosses represent high dosing treatment (×); THg was tested for

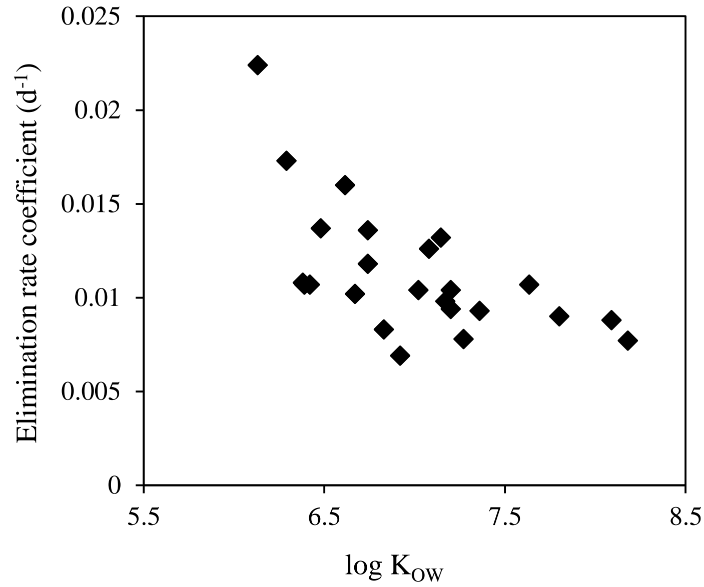
454 fish at days 0, 7, 14, 21, 28, 42, 56, 72, and 84 (n=5, 5, 5, 5, 5, 5, 5, 5 and 5 for low dosed

455 treatment, n=5, 5, 5, 5, 5, 4, 4, 4 and 5 for medium dosed treatment, and n=5, 5, 5, 5, 4, 4, 4, 5

456 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

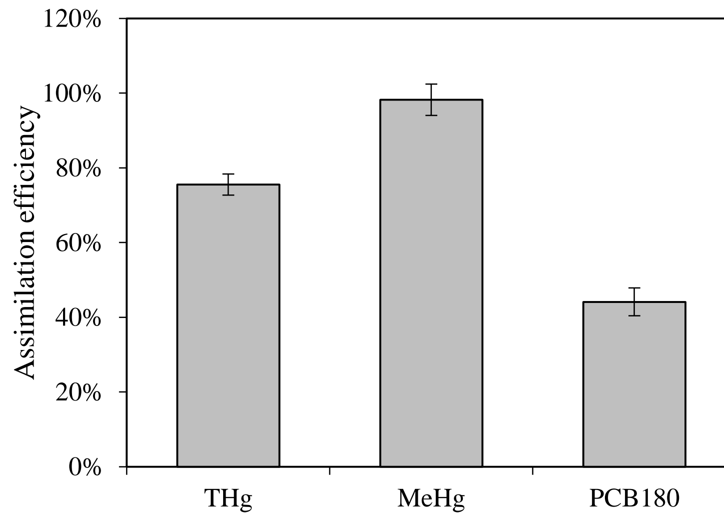
457 low dosed group, n=3, 5, 4 and 4 for medium dosed group, and n=5, 4, 3 and 1 for high dosed

458 treatment). Vertical bars represent standard error.



459

460 **Figure 2.** Elimination rate coefficients of PCB congeners from high dosed fish



461

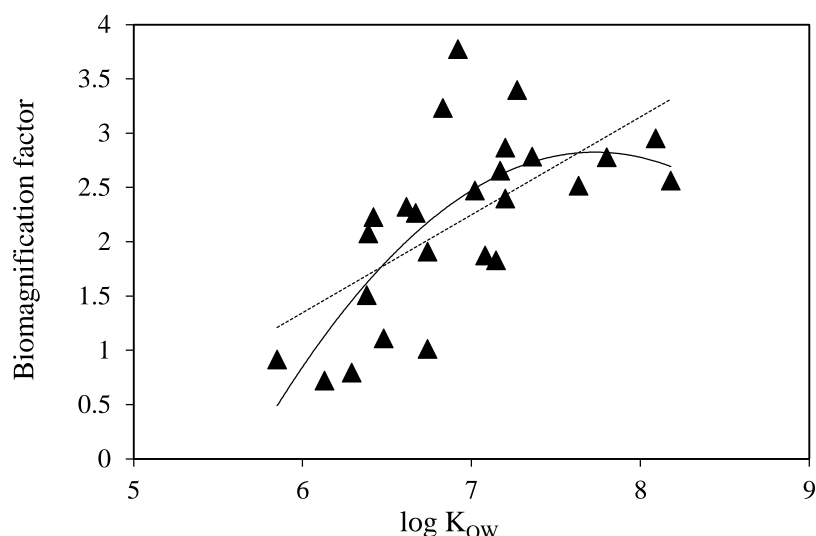
462 **Figure 3.** Assimilation efficiencies of THg, MeHg, and PCB180; Grey bars for THg and MeHg

463 represent the average AE values from each treatment sample. The grey bar for PCB 180

464 represents the mean AE value from the high dosed fish sample. Vertical bars represent standard

465 error.

466



467 **Figure 4.** Biomagnification factors of PCB congeners in goldfish. The solid line represents the

468 curvilinear model, and dashed line represents the linear model.

469 TABLES.

470 **Table 1.** Mean (± SD) THg, MeHg, and PCB concentrations in control and dosed fish at the end

471 (day 0 for elimination) of dosing, as well as in fish diet.

		Fish diet			Fish homogenate (day 0)		
		THg ($\mu g \cdot g^{-1}$ lean dry wt)	MeHg ($\mu g \cdot g^{-1}$ lean dry wt)	Sum PCBs ($\mu g \cdot g^{-1}$ lipid)	THg ($\mu g \cdot g^{-1}$ lean dry wt)	MeHg ($\mu g \cdot g^{-1}$ lean dry wt)	Sum PCBs ($\mu g \cdot g^{-1}$ lipid)
Low	control	0.05±0.003	0.03±0.003	0.01	0.11±0.02	0.07±0.02	0.18±0.12
	dosed	0.63±0.03 † a	0.44±0.02 † a	0.42±0.02 † a	0.45±0.12 † a	0.48±0.05 † a	0.31±0.13 a
Medium	control	0.05±0.003	0.03±0.003	0.01	0.16±0.06	0.12±0.06	0.45±0.27
	dosed	1.11±0.06 † b	0.92±0.01 † b	0.80±0.04 † b	1.35±0.21 † b	1.30±0.25 † b	0.83±0.29 b
High	control	0.05±0.003	0.03±0.003	0.01	0.22±0.06	0.20±0.06	0.61±0.54
	dosed	1.91±0.16 † c	1.86±0.34 † c	2.68±0.29 † c	3.13±0.37 † c	3.58±0.47 † c	4.66±0.53 † c

472 Note: Different lowercase letters indicate significant differences in mean THg, MeHg, and sum

473 PCB concentrations among low, medium and high dosing treatments (ANOVA). † Indicates

474 significant difference ($p<0.05$; ANOVA) between control and treatment fish for THg, MeHg or

475 sum PCBs

476 ASSOCIATED CONTENT

477 **Supporting Information**

478 Information regarding fish food preparation; moisture, lipid, and lean dry weight content from

479 the fish food; concentrations for each PCB congener in the fish food; moisture, lipid, and lean

480 dry weight content in individual samples and growth rate determination; fish PCB concentration-

481 time profile during elimination; I, AE, k_{tot}, $t_{1/2}$, and BMF for Hg and MeHg from all three dosing

482 treatments, as well as these parameters for PCB congeners from high dosed fish. The material is

483 available free of charge via the Internet at http://pubs.acs.org.

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489 **Author Contributions**

490 The manuscript was written through contributions of all authors, but the main contributions were

491 by J.L. and K.G.D. All authors have given approval to the final version of the manuscript.

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500 ABBREVIATIONS

501 Hg, mercury; MeHg, methylmercury; PCB, polychlorinated biphenyl; POPs, persistent organic
502 pollutants; K_{OW}, octanol-water partition coefficient; Hex, hexane; DCM, dichloromethane; wt,
503 weight; AE, assimilation efficiency; k_{tot}, elimination coefficients; ~~k_d, chemical uptake
504 coefficients;~~ BMF, biomagnification factor; $t_{1/2}$, half-life.

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