2016

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Accepted Word File. Article In Press (Jun 30, 2016) for publication in: *Bulletin of Environmental Contamination and Toxicology*.

Quantitative biomonitoring in the Detroit River using *Elliptio complanata*: Verification of steady state correction factors and temporal trends of PCBs in water between 1998-2015.

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Abstract
Quantitative biomonitoring methods were applied to determine PCB concentrations in water from the Detroit River over a 17 year period. During 2014, mussels were deployed for and extended duration (21-364 d) and time dependent PCB concentrations were fit to a bioaccumulation model to estimate elimination coefficients (k_{tot}) and provide site specific calibration of mussel toxicokinetics. The site specific calibration and different k_{tot} versus K_{OW} relationships from the literature were used to correct for steady state. $\sum$PCB concentrations in water were not significantly dependent on the k_{tot} values used indicating that individual variation exceeds error contributed by steady state correction factors. The model was then applied to estimate $\sum$PCB concentrations in water using the long term (1998-2015) data. $\sum$PCBs concentrations in water exhibited a significant decreasing trend with a half life of 9.12 years resulting in a drop in yearly geometric mean residues from 198.1 pg/L to 43.6 pg/L.
Keywords: Biomonitors, toxicokinetics, polychlorinated biphenyls, persistent organic pollutants, water concentrations

Introduction

Mussels are considered ideal biomonitors of bioaccumulative pollutants in water as a result of their sessile nature, slow growth, low metabolic biotransformation capabilities and low trophic position (O'Connor, 2002). Mussel biomontiors have been widely used to monitor polychlorinated biphenyl (PCB) and other organic contaminants in the Huron-Erie Corridor of the Laurentian Great Lakes since the 1980's (Kauss and Hamdy, 1985). A biomonitoring program that continues to operate is the long running City of Windsor Mussel Biomonitoring Program which commenced in 1996 (Drouillard et al., 2013). The program was developed to perform upstream and downstream sampling across the city's two wastewater treatment plant plumes in order to determine if the city's wastewater utilities and storm water overflows were contributing to elevated loads of persistent organic pollutants (POPs) to the Detroit River. The program uses a well calibrated freshwater mussel species, (*Elliptio complanata*), which are collected from a reference location and caged at each of the program's fixed monitoring sites over a set of defined deployment periods in each year. The study design makes the data amenable to quantitative biomonitoring approaches and as a result has made the program one of the most valuable data sets available for monitoring temporal trends of PCBs in water of the Detroit River.

Quantitative biomonitoring requires the interpretation of biomonitoring data using bioaccumulation models with the goal of converting accumulated chemical residues in the biomonitor to a time integrated estimate of water concentrations for the target contaminants (Gewurtz et al., 2003; Drouillard et al., 2013). This conversion requires three operational steps: 1) perform control correction of reference site accumulated residues; 2) convert time-dependent chemical concentrations in the biomonitor to a steady state residue and 3) translate steady state biomonitor residues into a water concentration estimate. Steps 1 and 2 rely on accurate information about chemical and animal specific toxicokinetics, most notably the whole body elimination rate coefficient (k_{tot}) for the biomonitor species being used. Step 3 requires knowledge of the steady state animal/water bioconcentration factor (BCF). Past studies have been performed using *E. complanata* to characterize organic contaminant k_{tot} values under laboratory (Russell and Gobas, 1989; Gewurtz et al., 2002; O'Rourke et al., 2004; Drouillard et al., 2007) and field conditions (O'Rourke et al., 2004; Raeside et al., 2009). Raeside et al. (2009) performed *in situ* calibration of mussels deployed throughout the Huron-Erie corridor by pre-dosing caged animals with non-environmental PCBs and tracking the rate at which dosed PCB were lost from caged animals at each deployment site. The latter study demonstrated that field deployed mussels eliminated PCBs faster compared to laboratory held mussels as well as considerable variation in chemical toxicokinetics across different deployment locations indicating that mussel filtration rates were dependent on site specific conditions. Error in the magnitude of k_{tot} used in steps (1) and (2) of the quantitative biomonitoring model process leads to incorrect water concentration estimates. It is therefore critical to understand what level of error is introduced as a result of steady state correction factors and how to minimize this error when interpreting biomonitoring extrapolated water concentrations.

During the 2014 implementation of the City of Windsor's Mussel Biomonitoring Program, the mussel deployment was extended from the normal 182 deployment period to a 364 d deployment period. This provided a unique opportunity to apply non-linear regression methods to fit the time-dependent mussel data to a generalized bioaccumulation model allowing an alternative estimate of in situ k_{tot} values for individual PCB congeners and to contrast this against previous calibration studies. Different k_{tot} values from across the various calibration studies were then applied to estimate PCB concentrations in water (C_W) to determine the level of error (or bias) that may occur when using non-site specific k_{tot} estimates derived from the literature within the model. Finally, the fitted in-situ k_{tot} values were extended to the full 17 year biomonitoring data base of mussel data from this field location to provide temporal trends of PCB concentrations in water of the Detroit River over the 1998 to 2015 study period.

Methods

The Detroit River is designated as an International Joint Commission Great Lakes Area of Concern owing to assessed degraded status of a series of beneficial uses, many of which are tied to organic and metal pollutants present in sediments and water (Drouillard et al., 2006). The City of Windsor Mussel Biomonitor Program routinely cages mussels at 9 locations that include effluent holding tanks of one of its wastewater treatment plants, two smaller tributaries of the Detroit River (Little River and Turkey Creek) and four locations within the Canadian waters of the Detroit River. For purposes of this study, the focus was on the Riverside Marina location (42°20'27.93"N; 82°55'56.04"W) given this was the site where a 364 d deployment was

collected. This station is located in the upstream portion of the Detroit River just downstream of the mixing zone between Little River and the Detroit River. The site is likely jointly influenced by both the Little River and Detroit River inflow from Lake St. Clair. Mussels are collected each year from Balsam Lake, Lindsey, Ontario (typically in late April to early May) and retained at a local aquaculture farm until deployment. Mussels are placed in wire minnow traps, with the ends crushed and suspended in the water column 1 m below the surface. Triplicate mussels are typically collected from each location after 21, 63, 126 and 182 days of deployment and stored frozen until chemical analysis. The exception was for 2014 where the 182 d time point became a 364 d time point.

Shucked mussels were analyzed for PCBs, a suite of 18 organochlorine pesticides and PAHs according to the procedures described by Lazar et al. (1992). PCBs, the focus of this study, were determined by gas chromatography electron capture detector following liquid-liquid extraction, florisil clean-up and instrumental conditions as specified in Lazar et al. (1992). The commonly detected PCB congeners were IUPAC #'s (28/31, 52, 49, 44, 74, 66/95, 101, 99, 87, 110, 151, 149, 118, 153, 105, 138, 158, 182/187, 183, 171, 180, 170/190, 201, 195, 194 and 206). Lipid content was determined gravimetrically as was moisture content following drying at 110^{o}C for 12 h. Instrument detection limits for individual PCBs were determined using a signal to noise ratio of 10 for the equivalent peak width of the baseline signal determined for 7 blank samples. Method detection limits were verified by spiking a set of PCB congeners into synthetic triolein at the Instrument Detection limit for 5 samples and verifying that the spiked concentration was not significantly different from the spiked value. Detection limits ranged from 0.01 to 0.06 ng/g wet weight for individual PCBs. Quality control measures include running a method blank and in-house reference homogenate (Detroit River Carp) along with each batch of six samples extracted. All reference homogenates PCB concentrations must be in compliance within the control chart values used by the quality control officer before release of the data, otherwise corrective actions are performed and the batch is re-run for analysis until QA compliance is achieved. Each sample was also spiked with a recovery standard to determine chemical recovery. The recovery standard changed over the years of the program, originally it was 1,3,5-tribromobenzene and was later switched to PCB 34. The mean±standard error of recoveries for the data set at Riverside Marina was 88.9±0.07%. Data were not recovery corrected.

For the 2014 data (which included the 364 d deployment), non-linear regression was performed to fit control corrected PCB concentrations in mussels according to the following equation:

$$C_{mcc(t)} = BCF \cdot C_W \cdot (1 - e^{-k_{tot} \cdot t}) \quad (1)$$

where $C_{mcc(t)}$ is the control corrected time dependent mussel concentration (pg/kg lipid equivalent), BCF is the bioconcentration factor associated with animal lipids (kg/L) for a given PCB congener, C_W is water concentration (pg/L), k_{tot} is the whole body elimination rate coefficient (d^{-1}) for a given PCB congener and t is the deployment time (d). Lipid equivalent concentrations are generated by establishing lean dry weight as having a partition capacity of 0.05 of of lipids and adding this to the fraction of lipid in the sample (Drouillard et al. 2013). Control correction was performed according to:

$$C_{mcc(t)} = C_{m(t)} - C_{m(o)} \cdot e^{-k_{tot} \cdot t} \quad (2)$$

Where $C_{m(t)}$ is the time dependent mussel concentration (pg/kg lipid equivalent) and $C_{m(o)}$ is the concentration in the non-deployed control mussels (pg/kg lipid equivalent). BCF is set to the *n*-octanol/water partition coefficient value of each PCB congener as reported by Hawker and Connell (1988). Non-linear regression solutions (and 95% confidence intervals) were generated for C_W and k_{tot} by least squares procedures fit to Eq. 2 using Systat 13 Statistical Software after providing estimates of initial starting C_w and k_{tot} values used in the fitting procedure. Several starting estimates were generated to ensure the best model solution was achieved. Initially, k_{tot} values were assigned based on the Raeside et al. (2009) expression (see below) to establish control correction and solve Eq. 1. Once site specific k_{tot} values were generated from Eq. 1, the process was repeated using the newly solved k_{tot} in Eq. 2 and then the data were re-fit to Eq. 1. Only congeners where the model fit yielded an R^2 greater than 0.25 were accepted as an adequate model fit. Linear regression analysis was subsequently performed on log k_{tot} values against log K_{OW} to generate a predictive relationship for site specific in situ k_{tot} values. Non-linear regression model estimates of C_W were not interpreted but rather estimated by the procedure below.

For the 2014 and the larger temporal data base consisting of 17 years of data generated at Riverside Marina (inclusive of 2014), the below model was used to extrapolate water concentration estimates for each mussel analyzed. The model is given by:

$$C_W = \frac{C_{m(t)} - C_{m(o)} \cdot e^{-k_{tot} \cdot t}}{\left(1 - e^{-k_{tot} \cdot t}\right)} \cdot \frac{1}{BCF} \quad (3)$$

The k_{tot} values used with Eq. 3 were derived from 3 different predictive relationships. The first was based on the site-specific in situ k_{tot} relationship generated for k_{tot} values fitted against chemical hydrophobicity as generated using the approach described in Eq. 1. The second was based on the k_{tot} relationship for PCBs measured in laboratory held *E. complanata* (O'Rourke et al., 2004):

$$k_{tot} = -0.59 \cdot K_{OW} + 2.05 \quad (4)$$

The third was based on the combined in-situ relationship for *E. complanata* deployed in the Huron-Erie corridor reported by Raeside et al. (2009):

$$k_{tot} = -0.34 \cdot K_{OW} + 1.13 \quad (5)$$

C_W was estimated for each individual PCB congener detected in the database. Sum PCBs (∑PCB) are designated as the sum of C_W concentrations across the detected congeners identified in methods. Non-detected values were censored and not included in the ∑PCB estimate. Linear regression analysis was performed on ln C_w (∑PCBs) versus Julian date to examine for temporal trends at the biomonitoring station. Analysis of variance (ANVOA) was used to determine if the above slope was significantly different from zero. The water concentration half life was estimated from the slope of the above relationship, where t1/2 = ln (2)/slope. Analysis of covariance (ANCOVA) was performed to determine if the slope of sum PCB concentration with time differed when temporal trends were determined using the full data set or when the data were truncated to include only the longest deployment time in each year. Two way ANOVA was performed on log-transformed ∑PCB C_w data to test for difference in C_W estimates with deployment time for the 2014 data set and also to determine if significant differences in C_W occurred depending on which k_{tot} versus K_{OW} relationship was used within the bioaccumulation model.

Results and Discussion

Satisfactory fit to Eq. 1 was obtained for 21 of 26 congeners measured using the 2014 dataset where the R^2 of model fit to the empirical mussel data exceeded the criteria (R^2>0.25) used to specify adequate model prediction. The R^2 values of successfully fitted congeners ranged from 0.27 to 0.76. Congeners excluded due to lack of satisfactory model fit (R^2<0.25) included PCB 31/28, 52, 66/95, 158 and 183. The bioaccumulation pattern and model fit for four selected PCB congeners (PCBs 49, 110, 138 and 180) to the 2014 data are presented in Figure 1. Goodness of fit tests (observed vs predicted linear regression relationship) were significant for PCB 110 ($p<0.05$) and highly significant for PCBs 138 and 180 ($p<0.01$). The goodness of fit test was not significant for PCB 49 given that range of concentrations was low across time points owing to rapid achievement of steady state for low K_{OW} compounds. Most congeners exhibited linear uptake over the first 30 days and approached steady state by 122 days. Model estimated k_{tot} values for individual PCBs demonstrated a highly significant decreasing trend with increasing hydrophobicity described by:

$$\log k_{tot} = -0.31 \pm 0.05 \cdot \log K_{OW} + 0.54 \pm 0.034;\ R^2 = 0.66; p < 0.001 \qquad (6)$$

Figure 2 presents the model fitted congener specific k_{tot} values as well as k_{tot} versus K_{OW} relationships described by Eqs. 4, 5 and 6. Model fitted k_{tot} values were generally bracketed by k_{tot} predictions generated by Eqs. 4 and 5. The slope from Eq. 6 was most consistent (± 1 standard error) for the slope reported by Eq. 5, although the intercept was lower. This is likely due to the fact that both Raeside's k_{tot} relationship (Eq. 5) and those generated from the present research reflect in-situ calibrated measures whereas O'Rourke's study (Eq. 4) was conducted under laboratory conditions. Mussel filtration rates are known to be affected by site specific characteristics including temperature, dissolved oxygen, food quality and quantity characteristics (Björk and Gilek, 1997; Heinonen et al., 1996). Across the various biomonitor calibration studies, field deployed mussels consistently appeared to exhibit higher filtration rates and chemical toxicokinetics compared to laboratory held animals (O'Rourke et al., 2001; Raeside et al., 2009). The lower intercept associated with Eq. 6 relative Eq. 5 is probably related to overwintering temperatures which would have slowed mussel filtration rates during this period.

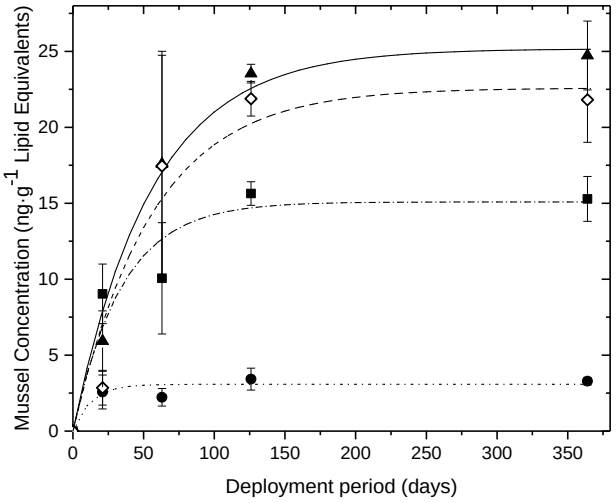

Figure 1. Bioaccumulation rates of PCBs 49 (●), 110 (■), 138 (▲) and 180 (◊) in mussels biomonitors during 2014. Lines represent model fit to equation 1.

PCB concentrations in water were subsequently estimated for the 2014 data using Eq. 2 and predictive k_{tot} relationships generated from either Eqs. 4, 5 or 6. Results are presented in Figure 3 as mean±standard error concentrations at each deployment period and as yearly average water concentration estimates denoted by horizontal lines. All three methods generated the same water concentration for the 364 d deployment because each of the k_{tot} relationships predict that

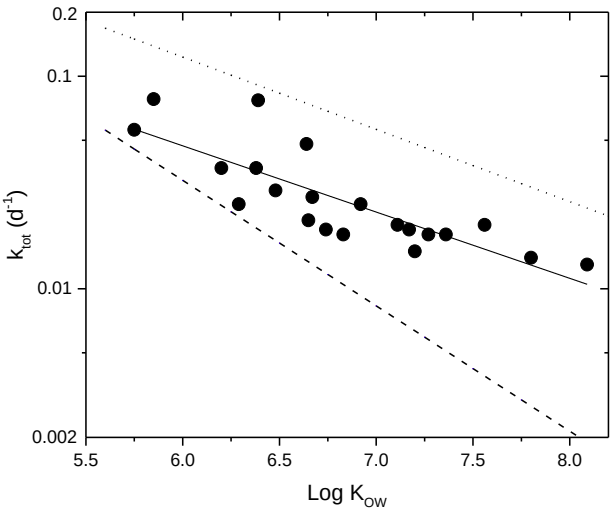

Figure 2. Whole body elimination coefficients of PCBs from in mussels biomonitors as a function of chemical hydrophobicity. Symbols (●) represent fitted model estimates of k_{tot}. Solid line is the linear regression fit to the data (Eq. 6; $p<0.001$), dashed lines are predicted k_{tot} values based on Eqs. 4 and 5.

mussels fully achieve steady state over this time frame. Water concentrations for the 21 d deployment were the most variable across k_{tot} algorithms because shorter deployments necessitate the largest steady state correction factors. For example, the Eq. 4 k_{tot} expression generated a mean 21 d ∑PCB water concentration that was 20.3% higher than that that derived from the k_{tot} relationship using Eq 6. Use of Eq. 5. yielded a mean concentration estimate 13% lower compared to that using Eq. 6. By day 63, Eq. 5 produced a mean ∑PCB water concentration that was within 3.3% of that generated by the site specific expression (Eq. 6), while Eq. 4 yielded a water concentration within 17.2% of the Eq. 6 generated value. The yearly mean ∑PCB concentrations from Eq. 4 and 5 were within 2.8 and 8.4% of the yearly mean generated from Eq. 6, respectively.

An ANOVA was performed to determine the effect of deployment time and estimation procedure (k_{tot} relationship utilized) on ∑PCB water concentrations generated for the 2014 data. PCB concentrations in water were significantly different across the deployment periods

($F_{1,41}$=5.11; p<0.05) but not significantly dependent ($F_{2,41}$=0.16; p>0.8) on the k_{tot} relationship utilized. This implies that between replicate variability of mussel accumulated residues was greater than the error introduced by using different correction factors associated with different k_{tot} relationships.

The k_{tot} relationship from Eq 6. was subsequently applied to estimate congener specific and ∑PCB water concentrations for full temporal data set generated at the Riverview Marina (1998-2015; n = 209 observations). The geometric mean ∑PCB concentration in water across years was 108.4 pg/L and ranged from 6.4 to 826.2 pg/L across individual measurements. Figure 4 presents mean sum PCB concentrations as a function of time. There was a highly significant

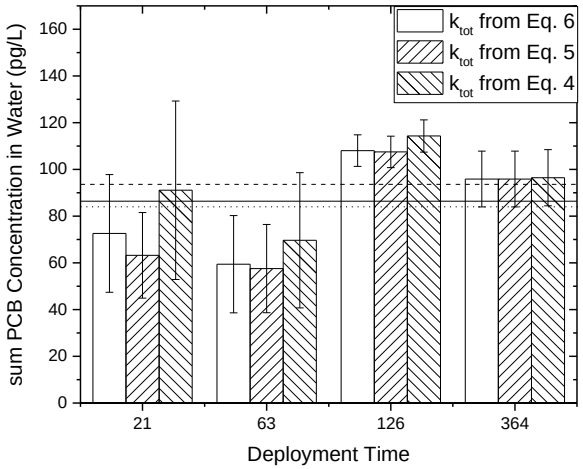

Figure 3. Biomonitor estimated sum PCB water concentrations at the Riverside Marina site during 2014 over different deployment periods

($F_{1,207}$ = 86.78; p<0.001) decreasing trend in ln PCB concentrations in water across years described by:

$$\ln C_{wat} = -0.076 \pm 0.008 \cdot Year + 156.6 \pm 16.3; \quad R^2 = 0.29 \qquad (7).$$

Based on Eq. 7, the half life of ∑PCBs in water at this location in the Detroit River is 9.12 years. As a cross check against the temporal trend outlined in Eq. 7, a second regression was performed omitting the early time points and limiting the data to only the longest deployment time in each year. This was performed in order to reduce potential error associated with steady state correction factors. The second regression yielded a slope of -0.057±0.012 that was not significantly different (ANCOVA; $F_{1,262}$; p>0.2) from the regression generated using the full data set. On the truncated data set, the estimated half life was somewhat slower at 12.1 years.

This research demonstrates that chemical toxicokinetics in deployed biomonitors will vary spatially and are also likely to vary at the same location in different years due to changing in situ conditions. However, the impact of this variation on PCB water concentration estimates will likely be small except in cases where mussels are deployed for only short periods of time (e.g. 21 days or less). When mussels are deployed for 60 d or longer, the potential error in PCB water concentration estimates using an assumed k_{tot} relationship will not likely to exceed 20%, even

when laboratory as opposed to in-situ calibration of the biomonitor is performed. Additional sources of error not considered in this research may be generated as a result of error in the BCF expression used within the model. For examples, setting the BCF = K_{OW} in Eq. 1 and 3 assumes that mussel lipids achieve equilibrium with dissolved water concentrations. However, mussels may also accumulate and potentially biomagnify PCBs from ingested seston along with exchange of water contaminants across their gills. *E. complanata* is a size selective filter feeder which consumes only small algal particles (<10 µm in size; Mueller et al., 2004). Small phytoplankton are typically assumed to be in equilibrium with the water, but can also achieve much lower chemical fugacity relative to water during high growth (Swackhamer and Scoglund, 1993). Thus the depression of ingested phytoplankton fugacity during growth would offset biomagnification taking place by the mussels owing to their exposure to this diluted food source. However, further research is necessary to verify this is the case for the study species.

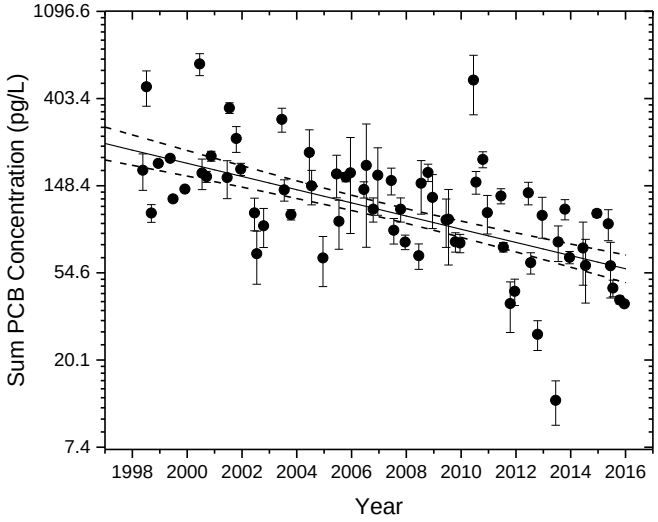

Figure 4. Biomonitor estimated sum PCB water concentrations at Riverside Marina as a function of time. Solid line represents linear regression fit (Eq. 6). Dashed lines are the upper and lower confidence intervals of the regression fit.

The present research also demonstrated that PCB concentrations in water at one location in the Detroit River are undergoing significant declines with time and the estimated half life of PCBs was approximately 9 years. Over the period of 1998 to 2015, the seasonal geometric mean PCB concentration declined from 198.1 pg/L to 43.6 pg/L representing a 454% decrease in measured residues. Based on Eq. 7, geometric mean residues at the biomonitoring station were predicted to have dropped 362% over this period of time. Thus, the Canadian waters of the Detroit River are exhibiting long term improvements with respect to PCB contamination in water.

Acknowledgement

This research was funded by the Public Works Pollution Control Department, City of Windsor with additional funding provided through Ontario Ministry of Environment and Climate

Change's – Canada/Ontario Agreement Funds. Rodica Lazar, Nargis Ismail and David Qiu contributed to laboratory analysis of mussel samples over the program duration.

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