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October 29, 2007

Chris Riddle
Environmental Specialist
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One Maritime Plaza, 4th Floor
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Dear Chris:

Please find enclosed the Final Report for our project, SG 287-06, entitled "Solid-phase microextraction fibers as models to predict contaminant bioaccumulation in aquatic invertebrates". This report includes our initial analysis of the data from this study and Attachment 1 with supporting data. Ms. Sarah Sellman will be completing her MS thesis based upon this research with a thesis defense scheduled for December. At that time, I would like to submit the thesis as an additional attachment for the project. Paper copies of this report will be mailed today. Our fiscal report will be submitted by 11/9/2007 as outlined in your letter of August 31.

Thank you for your patience with this project and please don't hesitate to call if you have any questions.

Sincerely,

Roman P. Lanno
Associate Professor

ABSTRACT

The objective of this research was to investigate the capacity of solid-phase microextraction (SPME) fibres to mimic the bioconcentration of polychlorinated biphenyls (PCBs) by aquatic invertebrates, using freshwater mussels (*Dreissena bugensis*) and oligochaetes (*Tubifex tubifex*) as models. Test systems were developed for the exposure of quagga mussels and *Tubifex* to waterborne PCBs for determining PCB uptake. Partitioning of PCBs to SPME (30 µm polydimethylsiloxane (PDMS)) was compared by the bioconcentration of PCBs by mussels and *Tubifex*. The differential partitioning of PCBs to the SPME fiber and organisms could be explained by the relationship between PCB chlorination and water solubility, with moderately chlorinated PCB congeners partitioning to the highest levels. Differences in the absolute amount of PCBs accumulated by mussels and *Tubifex* may be explained by the actual organism exposure scenarios. *Tubifex* were exposed continuously for 14 days while mussel exposure was intermittent since the mussel shells were not always open and the siphoning of water was not continuous over the 8-day exposure period. Thus, *Tubifex* accumulated more PCB during their exposure period. To be used as a tool for predicting the bioconcentration of PCBs in aquatic invertebrates, additional concentrations of PCBs should be tested using the methods described in this study.

INTRODUCTION AND GENERAL BACKGROUND INFORMATION

Polychlorinated biphenyls (PCBs) are one group of persistent, bioaccumulative, and toxic (PBT) chemicals that readily accumulate in biota but do not undergo rapid degradation in the environment. There are a variety of aquatic organisms in the Great Lakes region that are impacted by environmental contaminants such as PCBs. Biota can accumulate PCBs in their lipids and subsequently transfer them up the food web. Dreissenid mussels (*Dreissena bugensis*) and Tubificid worms (*Tubifex tubifex*) are two aquatic organisms at the lower end of the food web that readily bioaccumulate PCBs and transfer them to bioaccumulate in the higher levels of the food web. Thus, it is critical to continually monitor and attempt to remedy the level of PCBs in the Great Lakes in order to understand the bioaccumulation potential and overall environmental impacts PCBs are having on the region.

Estimating the bioaccumulation potential of chemicals and assessing the potentially bioavailable fraction in the environment typically involves exposing live organisms to known concentrations of the test chemical in water or sediment and measuring the uptake of the chemical over time. However, such tests can be expensive, time consuming and highly variable (*i.e.*, dependent upon organism size, age, weight, health, and feeding habits). Recently, chemical uptake by passive sampling devices (PSDs) has been correlated with the uptake of chemicals by organisms. PSDs may have some advantages over directly measuring chemical uptake by organisms including ease of deployment, lower variability, low production costs, and increased sample size due to the ease of replication since the amount of time required to complete tests is considerably less than with live organisms.

One type of PSD is the solid-phase microextraction fiber (SPME). The SPME device consists of a fiber coated with a thin polymer phase, such as polydimethylsiloxane (PDMS). This polymer phase is hydrophobic and acts as a sink to which hydrophobic molecules will partition from water. Thus, SPMEs can be used for sampling dissolved hydrophobic contaminants and the polarity and thickness of the polymer coating can be varied to optimize the uptake of contaminants with varying polarities. After the sampling period, SPME fibers can be conveniently introduced directly into a gas chromatograph system for PCB analysis. Chemical analysis using SPMEs requires no solvent extraction,

is less time-consuming, and does not require the use large amounts of solvents as conventional methods do.

Passive sampling devices such as SPMEs have been used for environmental monitoring and chemical analysis for the last few decades. However, recently, a “biomimetic” application of SPMEs has been suggested. For chemicals that are not metabolized to any great degree, SPMEs are capable of mimicking chemical accumulation in biota based upon physicochemical partitioning of hydrophobic compounds between aqueous and hydrophobic phases. This application of SPME technology has tremendous potential benefits for environmental monitoring and ecological risk assessment of hydrophobic chemicals such as PCBs.

OBJECTIVE

For the reasons outlined briefly above, this project was implemented with the goal of calibrating the bioaccumulation of PCBs by *Dreissena bugensis* (quagga mussels) and *Tubifex tubifex* (*Tubifex* worms) with sorption by SPMEs in order to develop a baseline model that can be used to predict the uptake of PCBs by mussels and *Tubifex* worms without conducting tests with live organisms. Since SPMEs mimic the bioconcentration process of simple partitioning, dietary uptake and chemical metabolism are not accounted for when comparing SPME uptake of chemicals to bioaccumulation in an organism. However, quagga mussels and *Tubifex* worms are fairly tolerant of environmental contaminants, readily accumulate hydrophobic compounds, and are relatively poor at metabolizing them, making them good animal models for examining PCB bioconcentration.

MATERIALS

A mixture of 21 PCB congeners (AccuStandard, Inc.) was used in all experiments. Congeners in the mixture ranged in chlorination levels from dichlorinated up to decachlorinated, and varied in octanol-water partitioning coefficients (Log Kow) and water solubility. Each congener was present in the mixture at 100 ug/mL. A summary of the congeners included in the mixture as well as their median water solubility and Log Kow values is provided in Table 1. Median water solubilities and Log

Kow values represent the median value from all of the values listed in Mackay et al. (1992). “NA” indicates a numerical value was not available in the Mackay handbook. In addition, a single congener (PCB 30 or 2,4,6-trichlorobiphenyl, AccuStandard, Inc) was used as an internal standard since it was not present in the congener mixture.

Table 1: Summary of water solubility and log Kow of PCB congeners used in this study (estimated from Mackay et al. 1992).

BZ #	PCB Congener	Median Water Solubility (mg/L)	Median Log Kow
8	2,4'-Dichlorobiphenyl	0.682	5.14
18	2,2',5'-Trichlorobiphenyl	0.211	5.6
28	2,4,4'-Trichlorobiphenyl	0.153	5.62
44	2,2',3,5'-Tetrachlorobiphenyl	0.1	5.81
52	2,2',5,5'-Tetrachlorobiphenyl	0.0296	5.91
66	2,3',4,4'-Tetrachlorobiphenyl	0.04	5.96
77	3,3',4,4'-Tetrachlorobiphenyl	0.00301	6.17
101	2,2',4,5,5'-Pentachlorobiphenyl	0.0103	6.4
105	2,3,3',4,4'-Pentachlorobiphenyl	NA	NA
118	2,3',4,4',5'-Pentachlorobiphenyl	NA	NA
126	3,3',4,4',5'-Pentachlorobiphenyl	NA	NA
128	2,2',3,3',4,4'-Hexachlorobiphenyl	0.001155	6.98
138	2,2',3,4,4',5'-Hexachlorobiphenyl	0.00181	6.72
153	2,2',4,4',5,5'-Hexachlorobiphenyl	0.0012	6.9
170	2,2',3,3',4,4',5'-Heptachlorobiphenyl	0.000512	7.08
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	0.00053	7.2
187	2,2',3,4',5,5',6'-Heptachlorobiphenyl	0.00249	7.17
195	2,2',3,3',4,4',5,6'-Octachlorobiphenyl	NA	NA
201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl	NA	NA
206	2,2',3,3',4,4',5,5',6'-Nonachlorobiphenyl	0.0000784	7.94
209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl	0.000018	8.27

Quagga mussels were collected from Lake Erie near The Ohio State University Stone Laboratory. *Tubifex* worms were obtained from the Army Corps of Engineers Waterways Experiment Station, Vicksburg, Mississippi. Live cultures of each organism were maintained in the laboratory until use in experiments.

SPME fibers were purchased from Sigma-Aldrich, Inc. All fibers were manufactured by Supelco, Inc. SPME fibers used in all experiments consisted of a 30- μm polydimethylsiloxane (PDMS) coating. Fibers were 1 cm in length and designed for use with manual SPME fiber holders.

METHODS

SPME Fiber Experiments

SPME fibers were exposed to an aqueous mixture of PCBs by suspending the individual manual holders in a 1-L Erlenmeyer flask. Fibers were exposed in triplicate to two different concentrations (30 ng/L and 60 ng/L) of PCBs for 1, 2, 4, 8, 16, 32, 64, 128, and 256 hours in order to establish PCB uptake kinetics for the fibers. Each flask (with a single SPME fiber) was filled with 600 mL of the PCB solution. Exposure water was prepared with de-ionized water and either 0.3 or 0.6 mL of a 60 $\mu\text{g/L}$ PCB solution to achieve the appropriate exposure concentration (30 ng/L or 60 ng/L, respectively). Flasks were then placed on a stir plate with a glass stir bar for the specific exposure duration. Glass stir bars were selected to minimize loss of high Log Kow PCBs to the experimental system. Each flask was stirred at the same, constant rate throughout the exposure duration and each flask was covered with a piece of foil to minimize evaporation of the PCB solution.

Data from these experiments was used to establish correlations with PCB uptake by mussels and *Tubifex* worms (methods described below). SPME fibers were exposed to the PCB mixture in different Erlenmeyer flasks than the biota in order to provide a more precise comparison measure of PCB exposure. Care was taken to execute the SPME fiber uptake experiments as identically as possible to the organism accumulation experiments; however, they could not be completed simultaneously due to space and equipment constraints. SPME measurements of PCBs were also conducted in flasks in which the organisms had been exposed to determine the level of PCB depletion due to organism exposure.

Quagga Mussel Uptake Experiments

Prior to the experimental period, the bottom of an aquarium was lined with glass microscope slides and individual healthy quagga mussels (*i.e.*, with open siphons) were removed from the main culture and placed on the glass slides. Mussels that had attached by their byssal threads to the glass slides after a 48-hr period were considered healthy and suitable for use in experiments. Organisms were not fed during this period.

Dreissenid mussel bioassays were conducted in quadruplicate to account for the variation in filtration rates of individual mussels by placing one mussel (pre-attached to a glass microscope slide) in a 1-L beaker, similar to the SPME experimental design. Beakers were filled with 600 mL of dechlorinated tap water. Then 0.6 mL of a 60 µg/L PCB solution in acetone was added to achieve a final concentration of 60 ng/L. Mussels were exposed to 60 ng/L in quadruplicate, for 1, 2, 3, 4, 5, 6, 7, and 8 days. A dechlorinated water control and acetone control (in quadruplicate) were also set up for the longest exposure duration.

In order to maintain a constant exposure concentration of 60 ng/L and ensure adequate dissolved oxygen levels, the water in the exposure chambers was renewed daily. New beakers were prepared with the 60 ng/L PCB mixture, and quagga mussels were carefully removed from their exposure chambers by lifting up the glass slides with a pair of tongs and gently placing the mussel attached to the slide into a new beaker. The same procedure was used to renew water daily for both the control and acetone control as a measure to monitor stress to the organisms.

Following the exposure period, mussels were shucked. Shell length and wet tissue weight were recorded, taking special care not to use any plastic tools or surfaces to minimize sorption loss of the highly hydrophobic PCB congeners. Tissues were placed into 16-mL amber vials with 2 mL of HPLC-grade methanol and stored at 0°C overnight until extraction the following day. The tissue extraction procedure is detailed further below.

In addition, a few SPME measurements of the exposure water were taken for the first 3 days of exposure. Rather than discarding the water that was changed out each day, the exposure water was retained in the 1-L beaker after the mussels were removed. A glass stir bar was placed in the beaker, and an individual SPME was placed into each of

the four replicates on a stir plate. The SPME remained in the water for a period of 16 hours, and was then removed and analyzed in order to determine the amount of PCBs remaining in the water exposure to the organisms.

Tubifex Uptake Experiments

Prior to the experimental period, *Tubifex* were removed from the culture boxes by carefully picking them up with a dissection probe that was bent to form a small hook. *Tubifex* were placed into a small, tared weighing dish that. *Tubifex* were added to the dish until approximately 305 ± 2 mg of tissue was present. Groups of *Tubifex* were then placed into 1-L beakers containing 600 mL of deionized water and 0.3 mL of a 60 $\mu\text{g/L}$ PCB mixture to achieve a final exposure concentration of 30 ng/L. *Tubifex* were suspended in the mixture in triplicate for 1, 2, 4, 7, 8, 9, and 14 days. A deionized water control and acetone control were also set up in triplicate for the longest exposure duration.

During the experimental period, water was renewed daily in order to maintain a constant exposure concentration. Water from each beaker was carefully poured off until no more than 10 mL of water was remaining in each beaker. The beaker was then renewed with 600 mL of a new 30 ng/L PCB solution (or water or acetone control) by carefully pouring the water slowly down the side of the beaker, minimizing disturbance to the *Tubifex*.

Following the exposure period, *Tubifex* were removed from the exposure water and wet tissue weight was recorded, being careful not to use any plastic tools or surfaces to minimize sorption loss of the highly hydrophobic PCB congeners. *Tubifex* were then placed in 16-mL amber vials with 2 mL of HPLC-grade methanol and stored at 0°C overnight until the tissue was to be extracted the following day. The tissue extraction procedure is described further below.

In addition, a number of SPME measurements of the exposure water were taken at various points throughout the exposure duration. On five of the days when *Tubifex* tissues were removed for PCB analysis (*i.e.*, for Day 1, Day 2, Day 4, Day 7, and Day 8), the exposure water was retained in the 1-L beaker after the *Tubifex* were removed. A glass stir bar was placed in the beaker, and an individual SPME was placed into each of

the three replicates on a stir plate. The SPME remained in the water for a period of 16 hours, and was then removed and analyzed in order to determine the amount of PCBs remaining in the water following exposure to the organism.

Tissue Extraction Methodology

Mussel Tissue Extraction

Tissue from individual mussels was placed in a 16-mL amber vial with 2 mL of HPLC-grade methanol overnight. The next day, the sample was placed in an ultrasonicator for approximately 5 minutes. Following sonication, 0.2 g of clean sand was added to the vial containing the tissue. Tissues were then ground with a tissue homogenizer for approximately 1 minute. Then 3 mL of hexane was added to the vial, and the tissue was ground for another minute. An additional 2 mL of hexane was used to rinse the homogenizer and the rinsate was collected in the 16-mL vial.

The sample was then vortexed for approximately 30 seconds. The sample was centrifuged for 10 minutes at 3,000 RPM and 21°C. The top layer (*i.e.*, hexane layer) was pipetted off with a disposable glass pipet and placed into a prepared, conditioned glass solid phase extraction (SPE) column.

Glass SPE columns were prepared by first packing them with a glass fiber filter, 1.5 g of silica gel, and 1 g of sodium sulfate. Columns were then conditioned with 3 mL of hexane three times. Solvent layers were poured through the conditioned SPE columns and collected in clean 100-mL volumetric flasks covered with a piece of foil and pierced with a small hole for placing the extraction column in. Each 100-mL volumetric flask was pre-washed and then used to collect the rinsate used in conditioning of the columns for an extra rinse.

Another 3 mL of hexane was added to the 16-mL amber vial. The sample was vortexed, centrifuged, and the top layer was pipetted off, as described above. This was repeated one more time for a total of 3 x 3 mL hexane extractions. Finally, an additional 5 mL of hexane was added directly to the glass extraction column to collect any PCBs that may have sorbed to the glass column.

Each extract was blown down under a gentle stream of nitrogen until no more solution remained. The sample was then re-dissolved in the 100-mL volumetric flask with 1 mL of the internal standard, PCB 30. A portion of each sample was transferred to

2-mL gas chromatography (GC) vials with 300 μ L inserts for analysis, and the remainder of the sample was placed in a separate vial for potential future analysis. One blank sample was processed for every five tissue samples to ensure method efficiency and make certain that no sample contamination occurred.

Tubifex Tissue Extraction

The same basic procedure described above for the mussel tissue extraction was used for extracting *Tubifex* tissues with a few minor modifications. *Tubifex* tissues were not ground with the tissue homogenizer, and sand was not added to the sample. Rather, 1 mL of HPLC-grade water was added to the 16-mL vial and then the sample was extracted with hexane as described above.

SPME Fiber Extraction

No solvent extraction was required for SPME samples since the fibers were exposed in manual holders, as described above. This sampling technique allows the fiber holder to be manually inserted directly into the GC injection port for desorption, as described later.

Sample Analysis

All samples were analyzed using a Varian CP-3800 gas chromatograph equipped with a CP-8400 autosampler, and electron capture detector (ECD). The PCB mixture was analyzed by GC-ECD using a DB-5 60-m column (i.d. 0.25 mm, film thickness 0.1 μ m) with helium as a carrier gas and nitrogen as a make-up gas. All samples were analyzed with a 100:1 split on the detector.

Quality assurance / quality control (QA/QC) was included in the analysis for each of the three types of experiments (SPME fiber, quagga mussel, and *Tubifex* worm). For every ten samples, one blank, one duplicate sample, and one spike recovery test were performed. A detection limit was determined by ten repetitions of the lowest concentration of a five-point calibration curve for the PCB mixture.

RESULTS & DISCUSSION

The PCB congeners within the mixture tested tend to differ in terms of uptake and bioavailability depending primarily upon the level of chlorination and level of

hydrophobicity of the particular congener. In general, the uptake kinetics of the various PCB congeners was similar, differing only in the magnitude and time of uptake. Due to the large amount of data generated with the mixture of 21 PCB congeners, the results and discussion for this report will focus on only three of the 21 congeners. The remainder of the data will be presented in the Attachment 1. The three congeners chosen (PCB 66, PCB 105, and PCB 128) represent the mid-range of chlorination level (*i.e.*, tetra-, penta-, and hexachlorinated), and in general provide a mid-range comparison of uptake. That is, the “extreme” chlorination levels in the mixture (*i.e.*, dichloro- and decachloro-) behave differently due to their specific chemical and physical characteristics and will not be discussed at length in this report.

SPME Results

SPME uptake experiments were designed to determine the time at which the fiber reaches steady state conditions within the test system. It was reasonably anticipated, based upon previous work from pilot studies, that initially the concentration on the fiber would increase rapidly and then level off to a plateau (*i.e.*, reach steady state) after approximately 8 hours. To confirm this, the exposure duration was extended to 16, 32, 128, and 256 hours. The SPME uptake curves over time for PCB 66, PCB 105, and PCB 128 are presented below (Figures 1-3). Each point on the graph represents an average of the three replicates for each of the nine exposure durations (1, 2, 4, 8, 16, 32, 64, 128, and 256 hours). Data for a 60 ng/L 256 hr exposure is not included in the graphs below. Error bars presented represent one standard deviation of the mean fiber concentrations (mmol PCB).

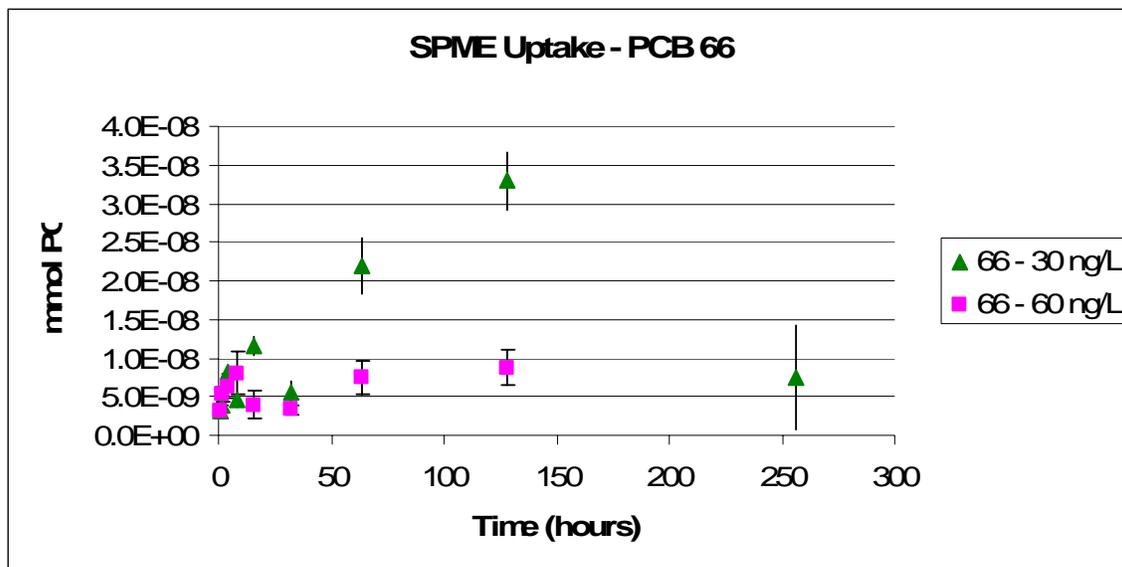


Figure 1: Uptake kinetics of PCB 66 by 30 µm SPME fiber.

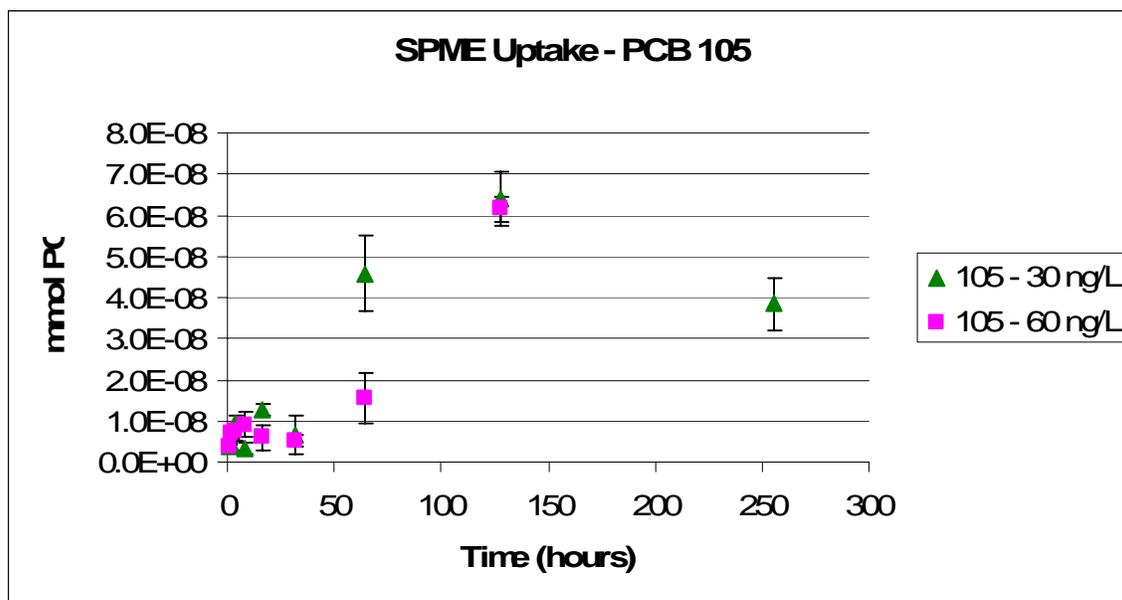


Figure 2: Uptake kinetics of PCB 105 by 30 µm SPME fiber.

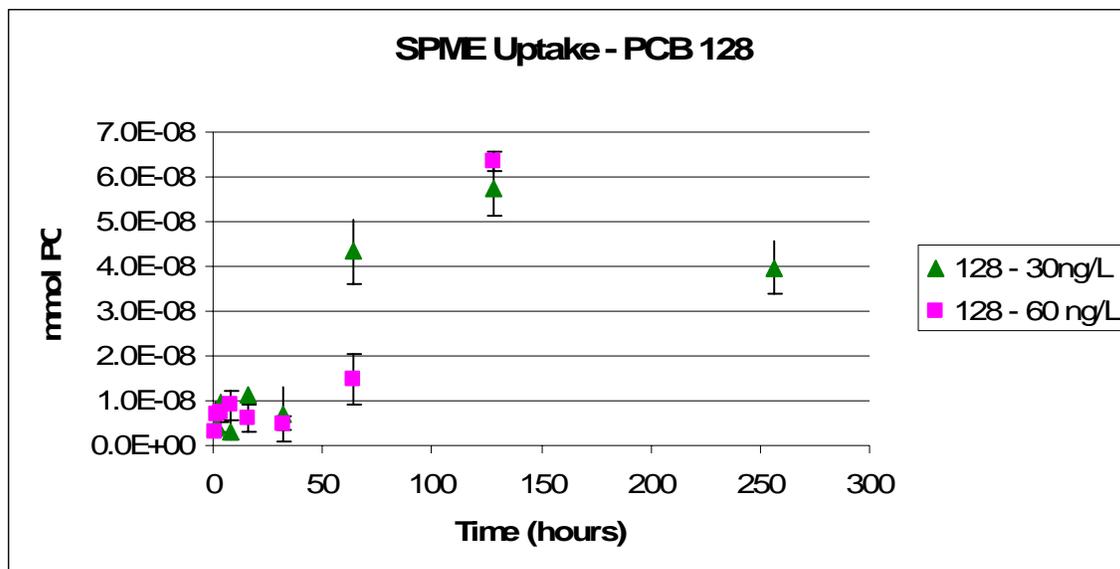


Figure 3: Uptake kinetics of PCB 128 by 30 µm SPME fiber.

The concentration of PCBs detected on the fiber generally increased at a linear rate during the range of exposures from 1 hour to 128 hours (*i.e.*, 1, 2, 4, 8, 16, 32, 64, 128 hours). However, at the 256-hour exposure when we expected to demonstrate that the SPME fiber had reached steady state conditions, we see a slight decrease in the concentration measured on the SPME fiber. This is seen most dramatically by PCB 66, where the SPME concentration decreases from 3.3×10^{-8} mmol PCB to 7.5×10^{-9} mmol PCB, which is slightly greater than one order of magnitude difference.

It is uncertain at this time what the cause of this phenomenon is. One hypothesis would be that there is a loss of the PCBs from the exposure water, either to the system itself (adsorption to glassware) or volatilization. However, most of the PCB congeners exhibit average Log Kow values of 5.9 - 6.9, and in general, chemicals within this range of Log Kow are not considered to be volatile. Furthermore, all of the flasks were covered during the exposure period to minimize volatilization. The same intermediate stock solution was used to spike the water in each of the flasks. The error bars, as seen on the graphs above, do not indicate a significant variability between replicates.

Due to the uncertainty associated with determining the time required for the SPME fiber to reach steady state, all comparisons of SPME data will be made with PCB

levels measured at 128 hours, which generally represents the maximum detected concentration measured on the SPME fiber over the range of exposure times investigated.

Quagga Mussel Results

Similar to the SPME experimental design, the quagga mussel experiments were designed to examine the uptake of PCBs into the mussel tissue over time. Typically, bioaccumulation tests with live organisms consist of a 28-day exposure duration. Quagga mussel experiments were originally designed to last for 32 days; however, during initial pilot tests, quagga mussels did not survive for longer than eight days in the test systems. Therefore, uptake of PCBs by quagga mussels was examined each day over an 8-day period. Quagga mussel uptake curves for PCB 66, PCB 105, and PCB 128 are presented below (Figures 4-6). Each point on the graph represents an average of four replicates for each of the exposure durations (1, 2, 3, 4, 5, 6, 7, and 8 days). Error bars represent one standard deviation of the average tissue concentration (mmol PCB/mg lipid). The remainder of the uptake curves for the other 18 PCB congeners is included in Attachment 1.

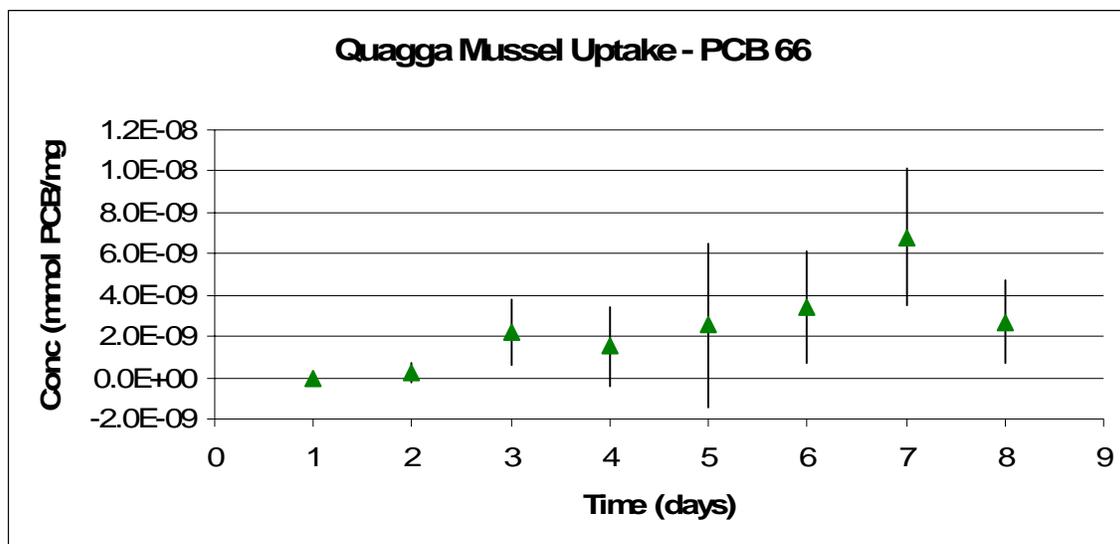


Figure 4: Uptake kinetics of PCB 66 by quagga mussels over an 8-day period.

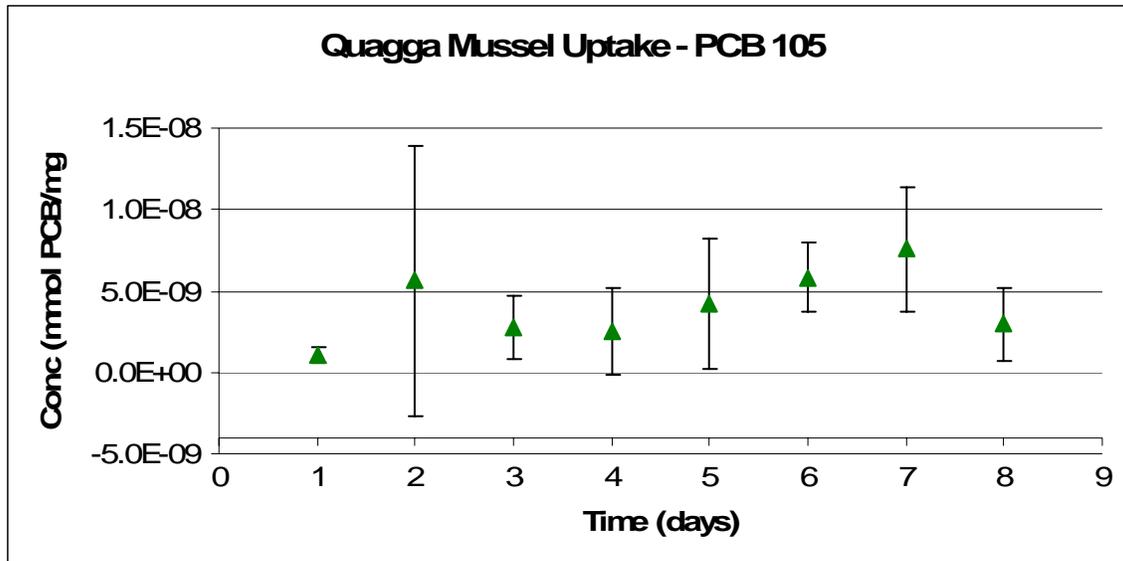


Figure 5: Uptake kinetics of PCB 105 by quagga mussels over an 8-day period.

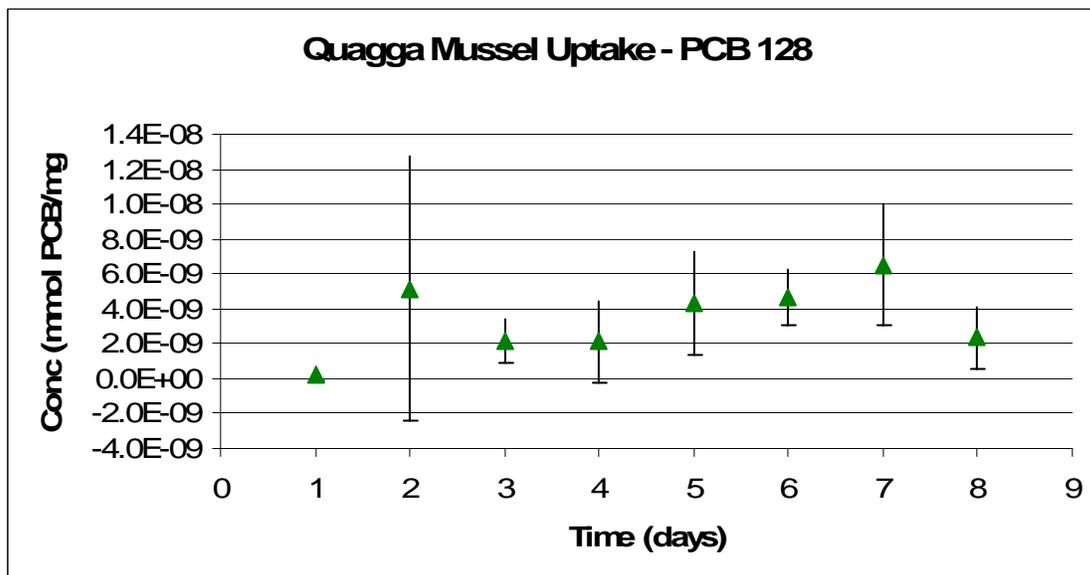


Figure 6: Uptake kinetics of PCB 128 by quagga mussels over an 8-day period.

The concentration of PCBs detected in the quagga mussels generally increased slowly during the first 7 days of exposure and decreased between days 7 and 8.

The experimental design of this study was such that the exposure water was renewed daily in order to account for potentially rapid uptake of PCBs due to the rapid

filtration of water by quagga mussels. Every third day, algal concentrate was placed in the exposure chamber prior to changing the water in order to feed the organisms and keep them healthy. Water in each beaker was spiked with the same intermediate stock solution. Quagga mussel exposures were conducted in quadruplicate in the event that if single mussels were to die or not readily filter the water (*i.e.*, have both siphons visible) during the test, the sample size would still be sufficiently large to provide an estimate of variability. Minor differences in PCB uptake can be noted by examining the raw data. For instance, some congeners are detected in one mussel but not another during the same exposure duration. Nonetheless, as seen from the error bars in the graph, the variability between replicates is relatively constant.

Because it is anticipated that further investigations are required to determine steady state conditions of quagga mussels within the exposure design, steady state values were not modeled for purposes of this final report. Rather, PCB tissue concentrations (mmol PCB/mg lipid) were evaluated based upon the Day 7 exposure tissue concentrations, which in general represents the maximum detected concentration measured in quagga mussel tissue over the range of exposure times investigated.

Tubifex Uptake Results

Tubifex experiments were also designed to investigate the uptake of PCBs in the tissue over time. During pilot studies, it was difficult to keep the *Tubifex* alive in the test system for more than 14 days. Therefore, the longest exposure evaluated was 14 days. *Tubifex* uptake curves for PCB 66, PCB 105, and PCB 128 are presented below (Figures 7-9). Each point on the graph represents an average of three replicates for each of the exposure durations (1, 2, 4, 7, 8, 9 and 14 days), with error bars representing one standard deviation of the average tissue concentration (mmol PCB/mg lipid). The remainder of the uptake curves for the other 18 PCB congeners is included in the Attachment 1.

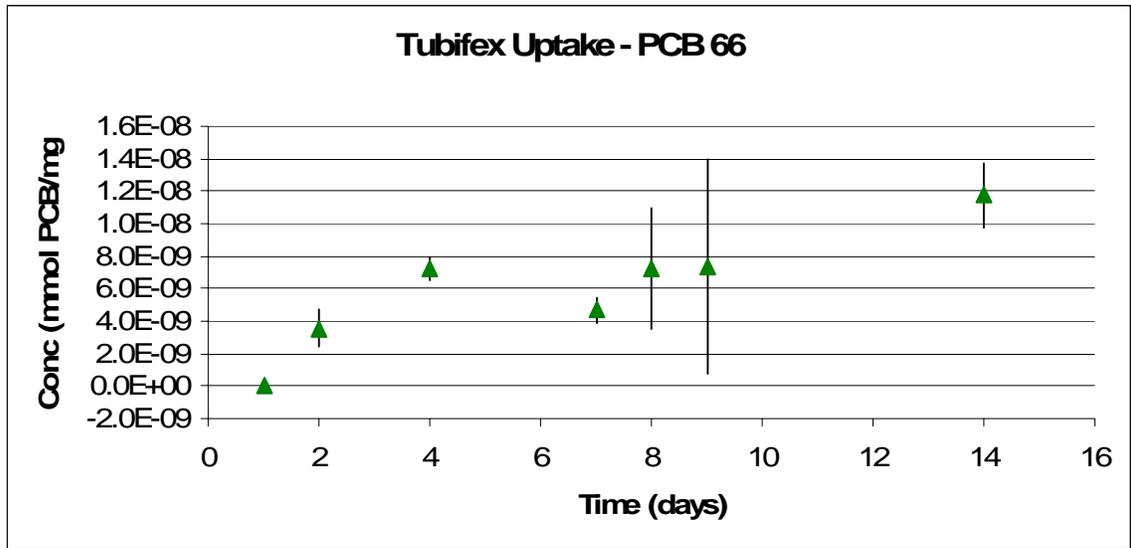


Figure 7: Uptake kinetics of PCB 66 by *Tubifex tubifex* over a 14-day exposure period.

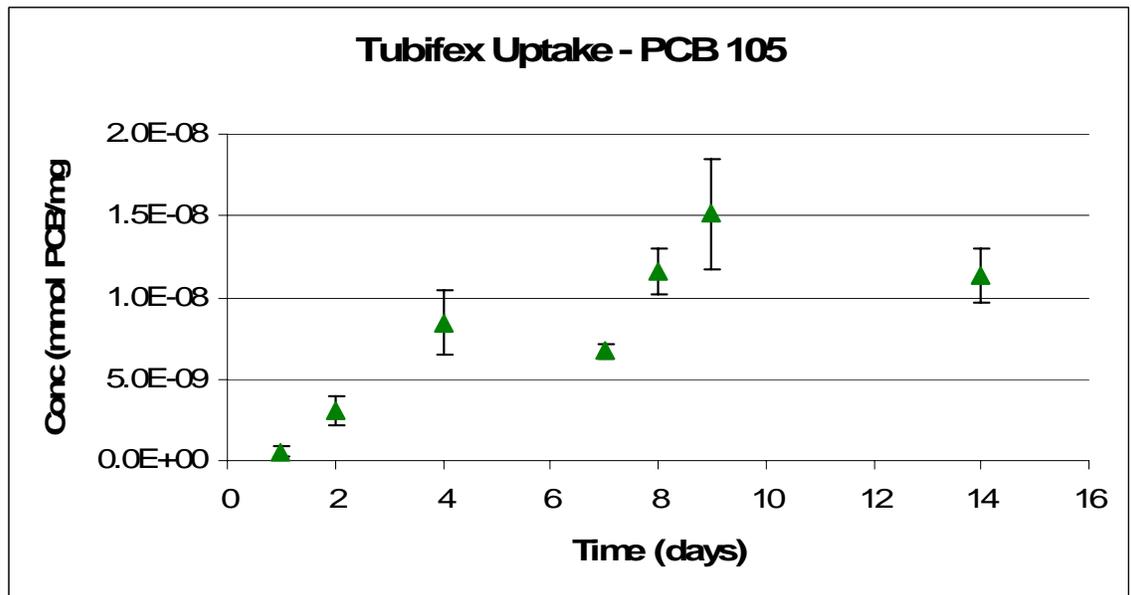


Figure 8: Uptake kinetics of PCB 105 by *Tubifex tubifex* over a 14-day exposure period.

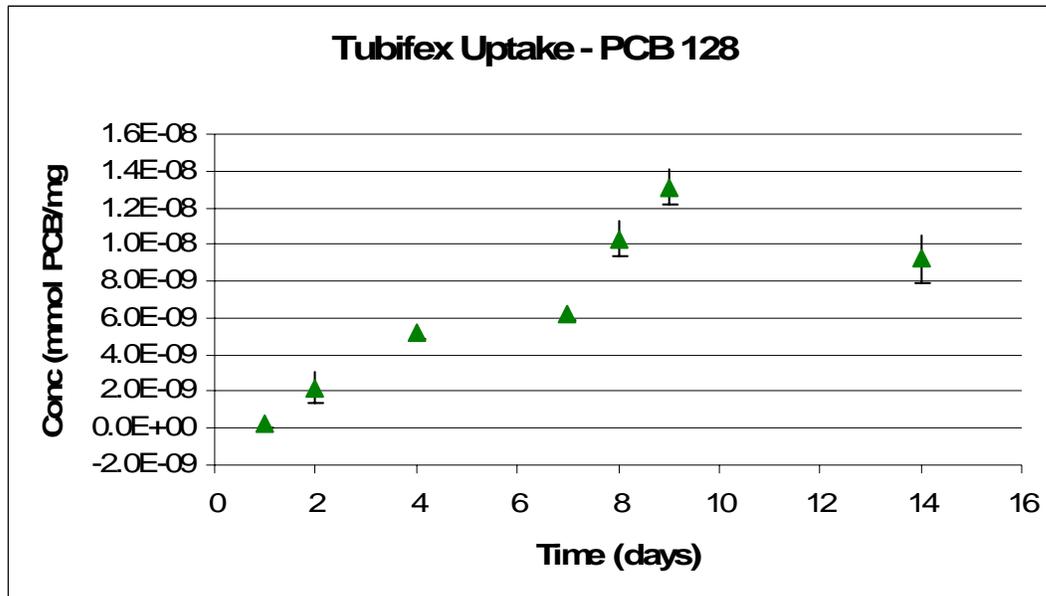


Figure 9: Uptake kinetics of PCB 128 by *Tubifex tubifex* over a 14-day exposure period.

The uptake kinetics of PCBs by *Tubifex* was similar to PCB uptake kinetics by quagga mussels. The concentration of PCBs detected in the worms generally increased slowly during the first 7 days of exposure and decreased between days 7 and 14. However, it is difficult to determine the time required to reach steady state based upon the data collected thus far. It would be beneficial to include a longer exposure duration, but we were unable to keep expose *Tubifex* beyond the 14-day exposure period without mortality.

The experimental design was such that the exposure water was renewed daily in order to account for rapid uptake of PCBs by *Tubifex*. After three days of exposure, a small amount of food was placed in the test chamber in order to allow the organisms to feed and stay healthy. Water in each beaker was spiked with the same intermediate stock solution. *Tubifex* exposures were conducted in triplicate and no mortalities were observed. Variability among replicates was consistent as suggested by error bars on the graphs.

Because it is anticipated that further investigations are required to determine steady state conditions of *Tubifex* within the exposure design, steady state values were not modeled for purposes of this final report. Rather, comparisons using PCB tissue concentrations (mmol PCB/mg lipid) were based upon the Day 9 exposure tissue

concentrations, which in general represents the maximum detected concentration measured in *Tubifex* over the range of exposure times investigated.

SPME:Tissue Uptake Ratios

Due to the difficulties in determining steady state PCB levels in SPME fibers, quagga mussels, and *Tubifex*, single point observations were used to compare PCB uptake. The point for comparison was chosen based on the average maximum concentration observed for the SPME fibers and organisms. The 128-hour exposure is used for the SPME fibers, the 7-day exposure for the quagga mussels, and the 9-day exposure for the *Tubifex*. As a means of comparing the uptake of PCBs by quagga mussels and *Tubifex*, a ratio of SPME PCB levels (mmol) to tissue concentrations (mmol PCB/mg lipid) was determined. Table 2 summarizes the SPME:organism uptake ratios for all of the PCB congeners in the test mixture.

Table 2: Comparison of SPME to organism uptake ratios for each PCB congener.

PCB Congener	SPME:QM ^a	SPME:TUB ^b
8	0.0	0.0
18	0.0	0.1
28	2.1	4.9
52	2.6	6.1
44	2.8	6.0
66	4.8	4.5
101	4.4	3.5
77	8.4	5.6
118	7.9	5.4
153	5.7	4.1
105	8.5	4.2
138	7.2	4.4
126	13.7	6.1
187	6.0	3.9
128	8.8	4.4
201	5.0	4.1
180	6.9	12.9
170	8.0	5.8
195	7.8	4.1
206	7.1	4.3
209	3.5	2.7

- a. Ratio of 128-hr SPME concentrations to 7-day quagga mussel tissue concentrations.
- b. Ratio of 128-hr SPME concentrations to 9-day *Tubifex* tissue concentrations.

The ratio of SPME:tissue concentrations varies among congeners as well as between organisms. For instance, PCB 126 has an uptake ratio of 13.7 for SPME:QM, which is more than two times the ratio of 6.1 for SPME:TUB. We observed a range of ratios for quagga mussel uptake from 0 for PCB 8 to 13.7 for PCB 127. The ratios for *Tubifex* range from 0 for PCB 8 to 12.9 for PCB 180.

The general trend observed in SPME:tissue PCB ratios is for lower ratios for PCBs with lower numbers of chlorine atoms (e.g., PCB 8, 18), increasing to highest ratios with the mid-chlorinated PCBs, and decreasing again with the most chlorinated PCB 209. This trend would be expected as lower chlorinated PCBs are more water soluble and therefore partition less to the hydrophobic phases in both the SPME fiber (polydimethylsiloxane) and organisms (neutral lipid). Mid-chlorinated PCBs as a group exhibit the greatest uptake due to increased hydrophobicity. The most chlorinated PCB congener, 209, is extremely hydrophobic and difficult to dissolve in water, so despite partitioning to lipid very easily, supply in solution was limited, resulting in a lower mass of PCB partitioning to both the fiber and organism lipid. We would expect to see a range of ratios within the organism for different PCBs due to the inherent differences in physical/chemical properties of the congeners (molecular weight, Log Kow, solubility, stereochemistry). SPME:tissue PCB ratios also differed between organisms. Since the uptake of PCBs by the SPME fiber was constant for each PCB congener, the observed difference in ratios was due to differences in PCB uptake by the organisms. PCB uptake was lower in quagga mussels compared to *Tubifex* and this may be attributed to the exposure duration of organisms in the test chambers. Quagga mussels were only exposed to PCBs for a total of eight days and the mussels were not continuously exposed to the PCBs since mussels often close their shells and don't siphon water. This results in variable exposure of the mussels to the test water. However, *Tubifex* were continuously exposed to PCBs in the test water and PCB accumulation would be expected to be higher.

CHALLENGES AND LESSONS LEARNED

As indicated in the interim report (February 2007) and alluded to briefly above, various challenges were encountered throughout the project in working with the aquatic organisms. We were unable to maintain viable cultures of quagga mussels in the laboratory for longer than approximately four months. At about the time that the test system was calibrated for exposure of mussels to PCBs, the mussel culture crashed. Due to seasonal weather conditions, we were unable to continue the experiments with the mussels and decided to continue the project with *Tubifex* until we were able to collect more mussels in the spring of 2007. This was the reason for the requested no-cost extension for the project.

Various pilot studies were conducted with each organism in order to determine the appropriate exposure concentration and exposure duration. Several experiments had to be repeated because mortality was observed in both of the organisms prior to the end of the planned exposure duration. We hypothesized that this could be due to pH and/or chlorine levels in the water as well as lack of food. Originally, the experiments were designed such that organisms would not be fed during the exposure period, minimizing the loss and/or interaction of the PCB mixture with the food. However, it was determined that the organisms could not be sustained for longer exposure durations without food. We decided to provide small amounts of food and ensure consistent water conditions by adopting a static renewal exposure system. Exposure water was replaced every day to replenish PCB levels in the exposure system. Prior to renewing the water on every third day of exposure, food was added to the system and the organisms were allowed to feed to two hours. Even with this type of exposure system, we were unable to keep either organism alive for the longest intended exposure duration.

Because of the inability to sustain the organisms for longer exposures, it was difficult to determine the time required for steady state based upon the data collected. Thus, future work to perfect the experimental design and attempts to extend the length of exposure would be a beneficial addition to the current data set in order to gain a more clear understanding of the results obtained.

As mentioned, it is also unclear based upon the data what represents true equilibrium conditions for the SPME exposures. The 256-hour exposure duration was intended to

exhibit that we considered steady state conditions. However, we observed a slight decrease in SPME concentrations at this longer exposure duration compared to organisms exposed for 128 hours. Thus, additional experiments that extend the exposure durations for SPME fibers would also be beneficial in order to gain a better understanding of the uptake and in order to determine the time required for SPME fibers to reach steady state.

There are various levels of uncertainty associated with working with a mixture of congeners. Each of the 21 congeners in the mixture has a different water solubility and Log Kow value. Although mixture exposure is likely to occur in real world scenarios, it is unclear whether there are interactions between PCB congeners in the mixture and how this would affect uptake kinetics. It would be beneficial to conduct additional experiments with single or few congeners rather than a mixture of 21 and compare results obtained with the mixture to see if the assumption of independence is supported. However, exposure to mixtures is most likely to occur in a field situation, so exposure to a mixture of congeners would be more representative of what is actually observed in the environment.

In order to apply SPMEs in field exposure scenarios, it would be necessary to conduct the same experiments with organisms exposed at different concentrations of PCBs than we used in this study. Although different exposure concentrations were originally proposed for this study, the problems encountered with maintaining the quagga mussel did not allow testing with mussels year round. In order to ensure that a useful data set would be generated, we decided to include *Tubifex* as an alternate test organism.

In summary, test systems were developed for the exposure of quagga mussels and *Tubifex* to waterborne PCBs for determining PCB uptake. Determination of partitioning of PCBs to SPME (30 μ m PDMS) was compared by calculation fiber to organism ratios. The differential partitioning of PCBs to the SPME fiber could be explained by the relationship between PCB chlorination and water solubility. Differences in the absolute amount of PCBs accumulated by the quagga mussels and *Tubifex* may be explained by the actual organism exposure scenarios. *Tubifex* were exposed continuously for 14 days while mussel exposure was intermittent since the mussel shells were not always open and the siphoning of water was not continuous over the 8-day exposure period. Thus, *Tubifex* accumulated more PCB during their exposure period.

In order to understand the potential for biomimetic applications of SPMEs to assessing PCB uptake by quagga mussels and *Tubifex*, two additional pieces of information are required. First, it is necessary to determine steady state conditions for PCB partitioning to both fibers and organisms. Steady state PCB levels may be modeled using the existing data, but validation of steady state levels by additional time course measurements is needed. In order to predict PCB bioaccumulation in organisms, additional tests need to be conducted at different waterborne PCB concentrations. This would validate the assumption that the bioconcentration factor remains constant regardless of the external exposure concentration.

Finally, in order to make predictions regarding PCB bioaccumulation in organisms, field validation is required. SPMEs must be deployed in habitats occupied by test organisms and PCB levels in the SPME and field-collected organisms must be compared.

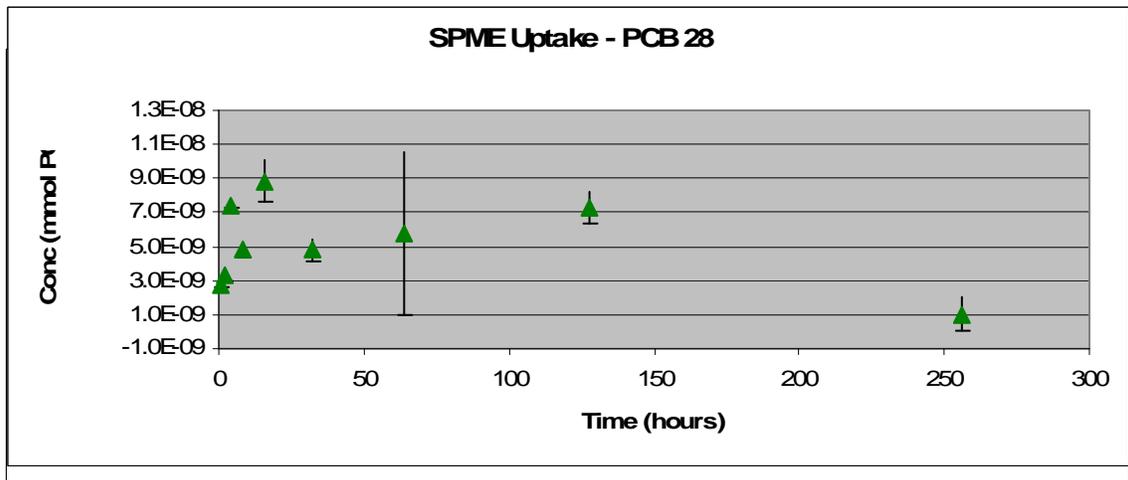
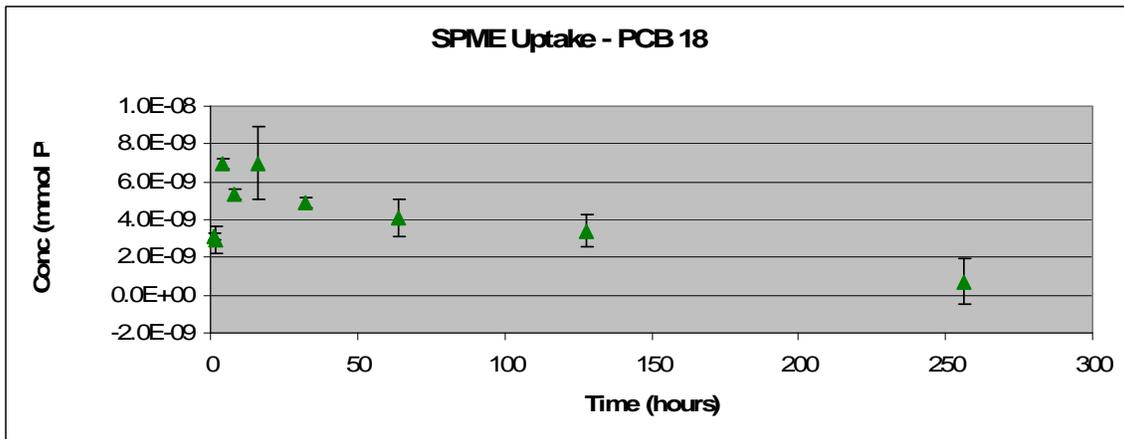
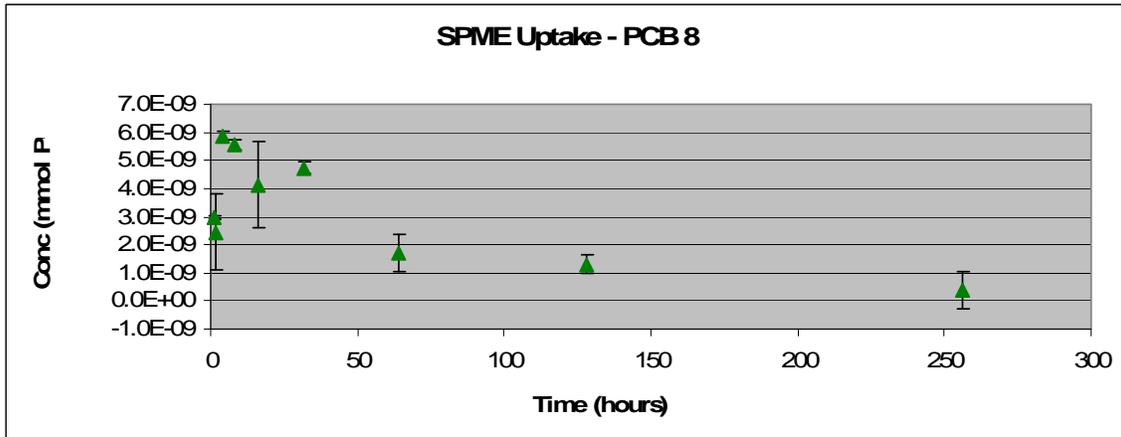
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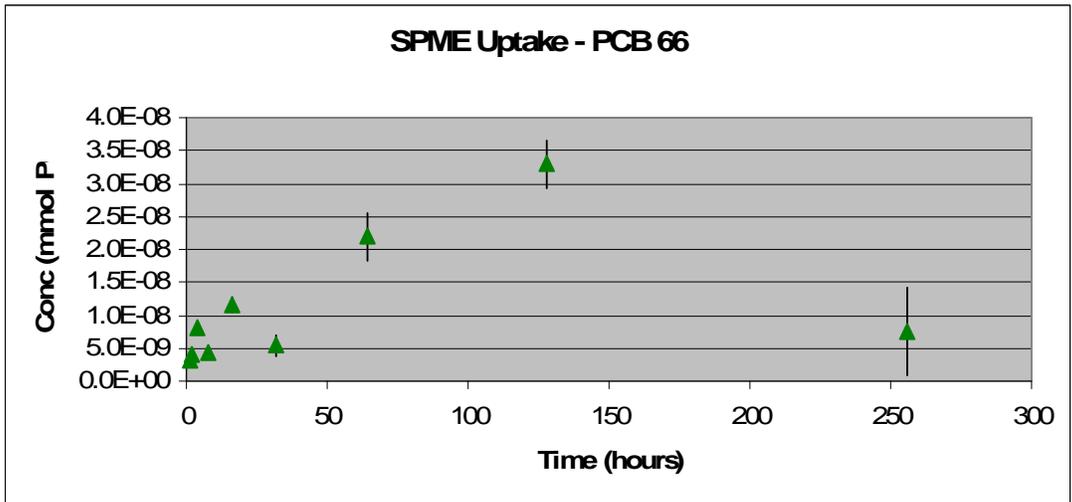
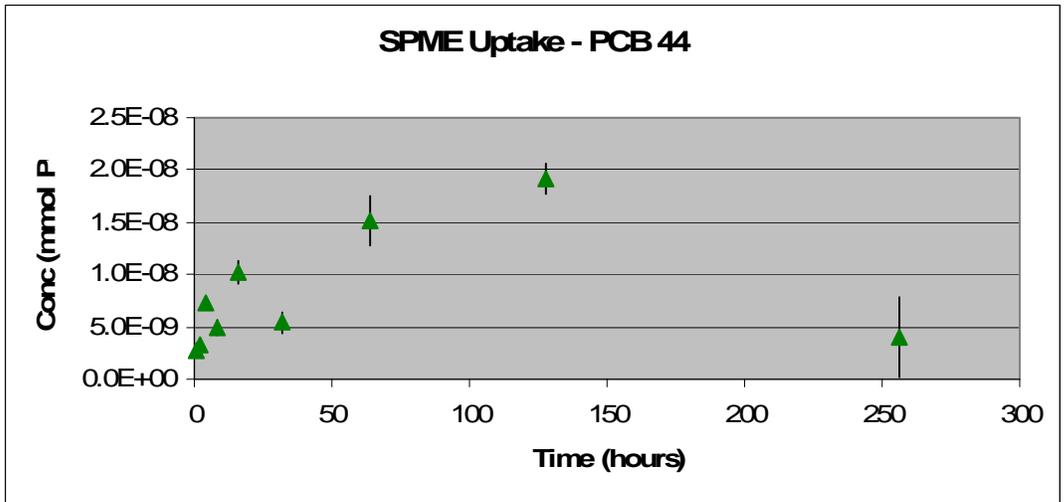
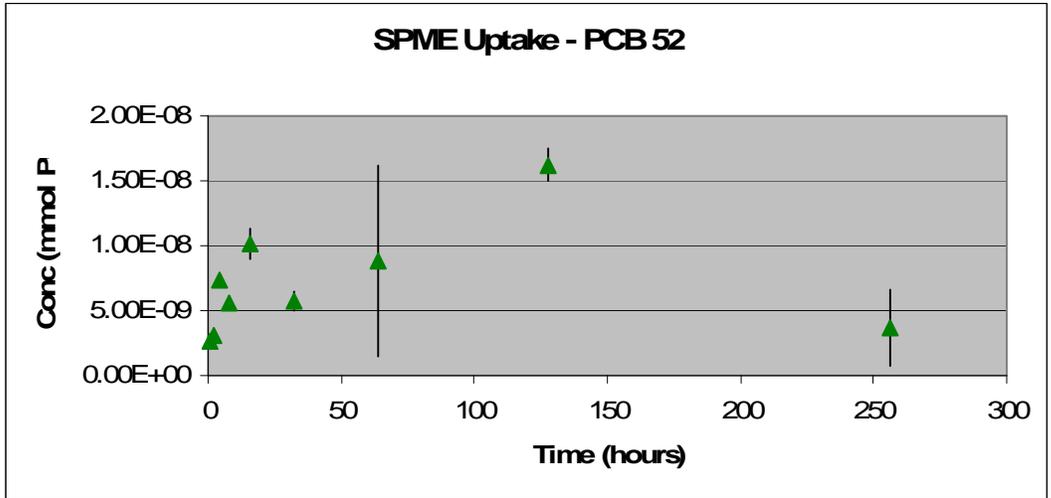
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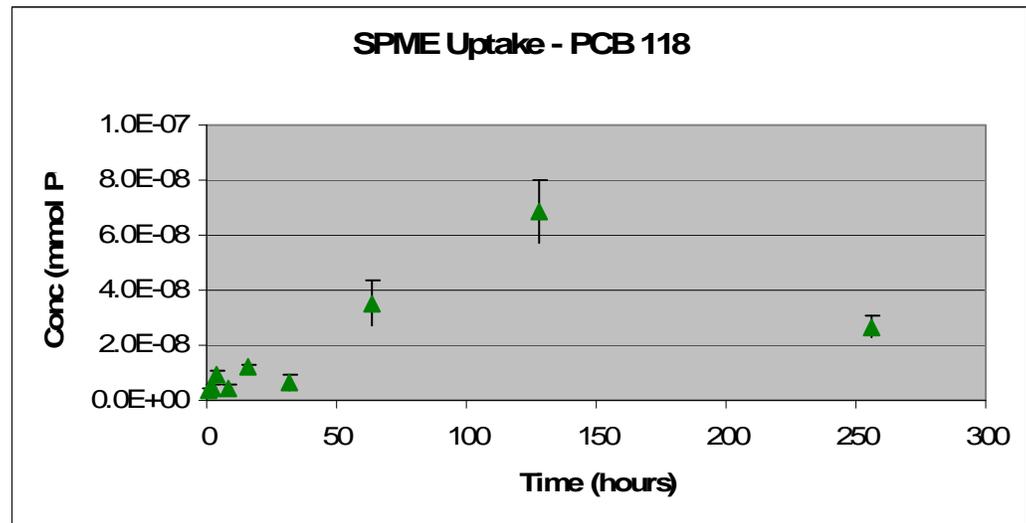
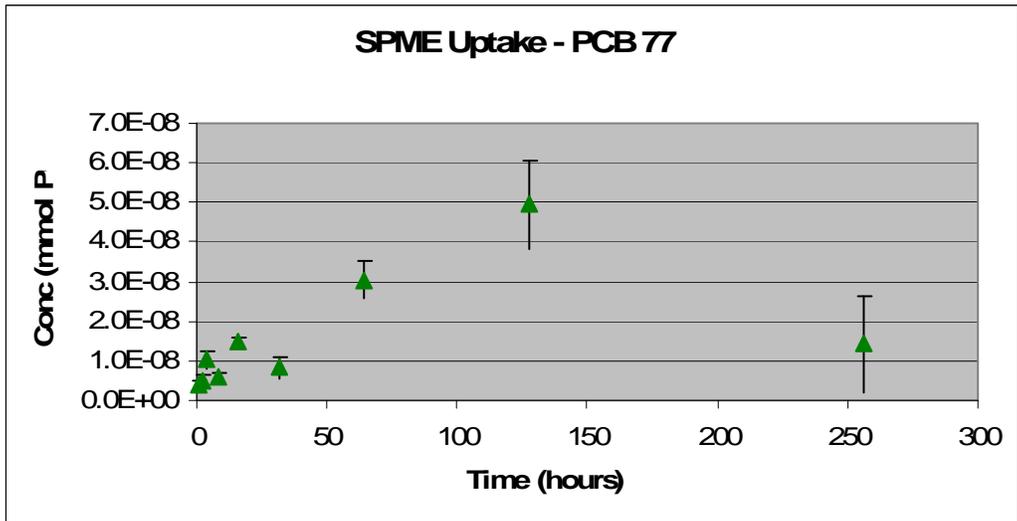
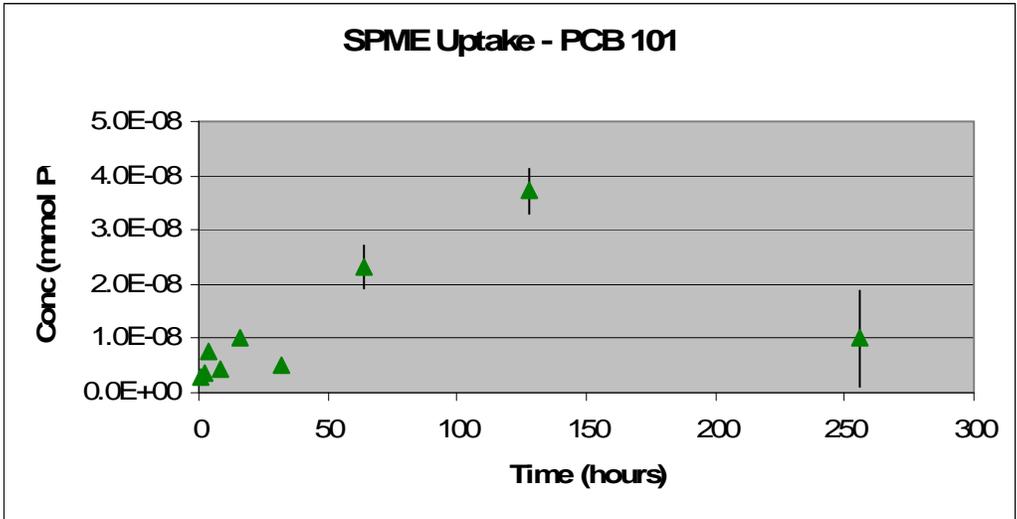
ATTACHMENT 1

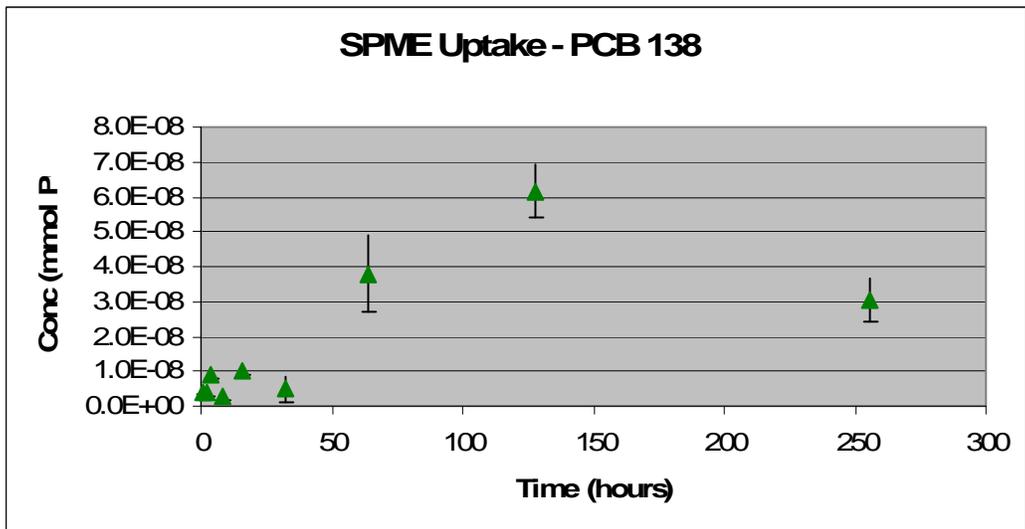
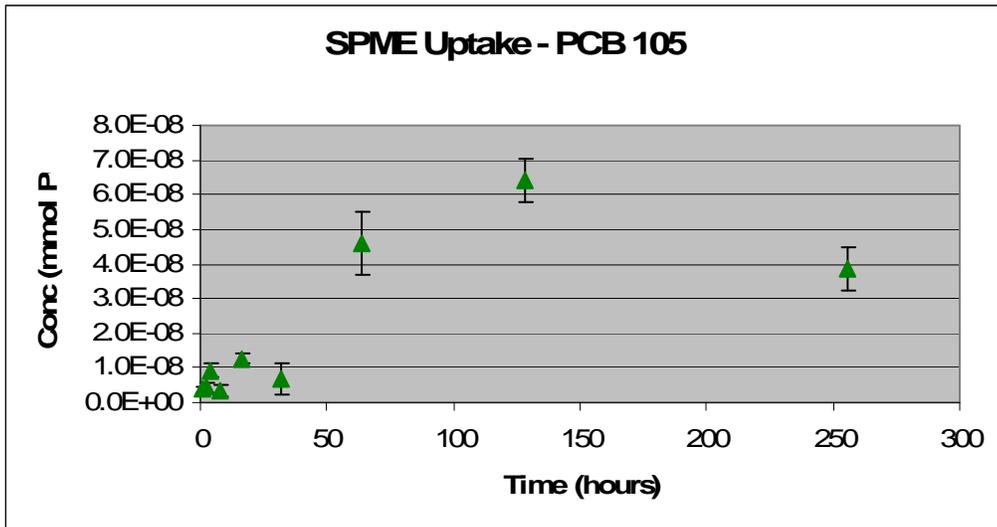
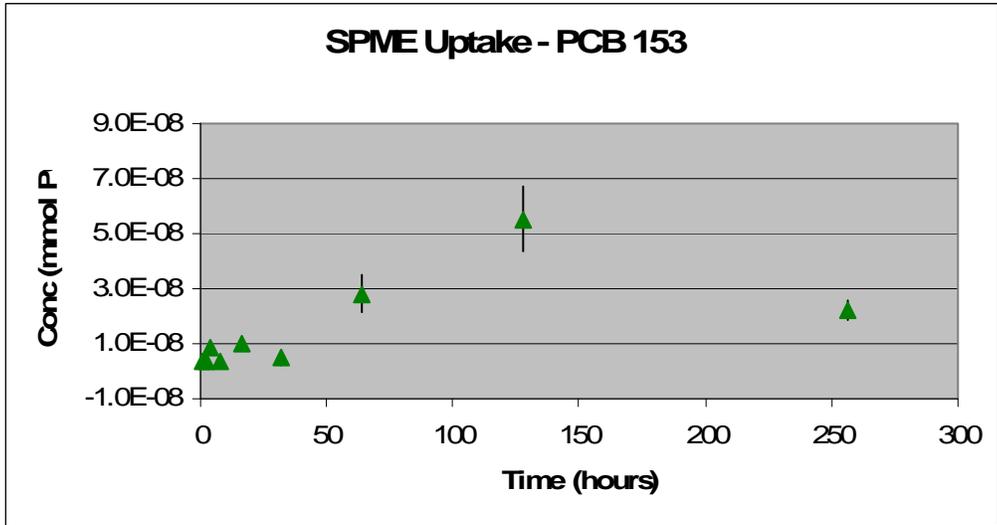
SPME, Quagga mussel, and *Tubifex tubifex* uptake of PCB congeners not used as examples in the text of the report

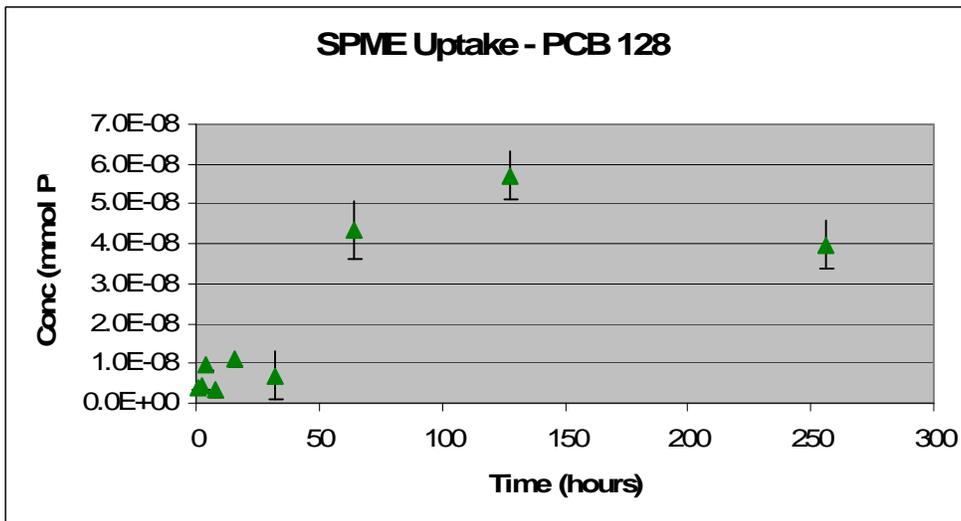
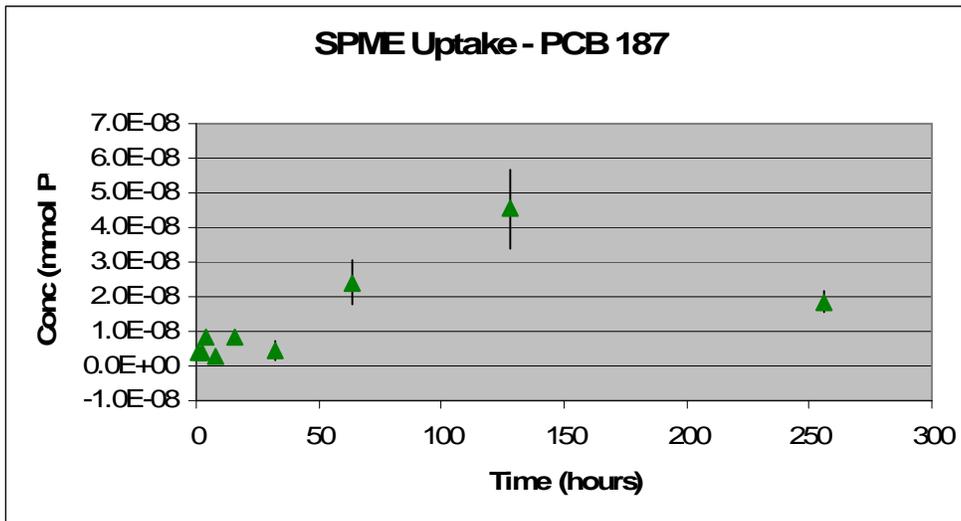
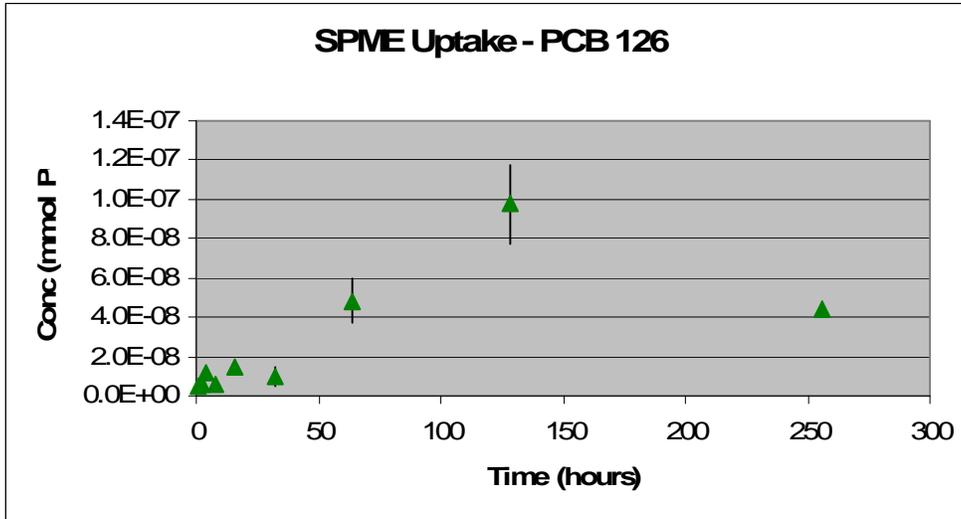
SPME Uptake Date (mmol PCB)

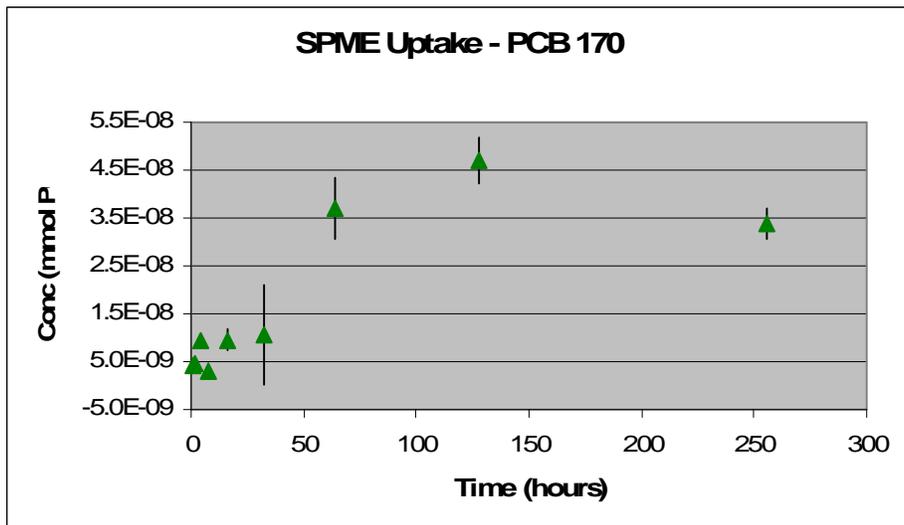
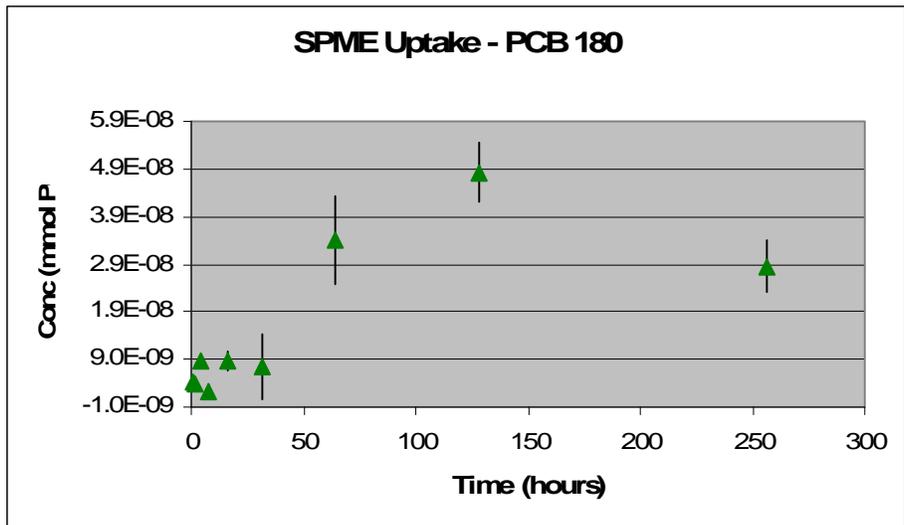
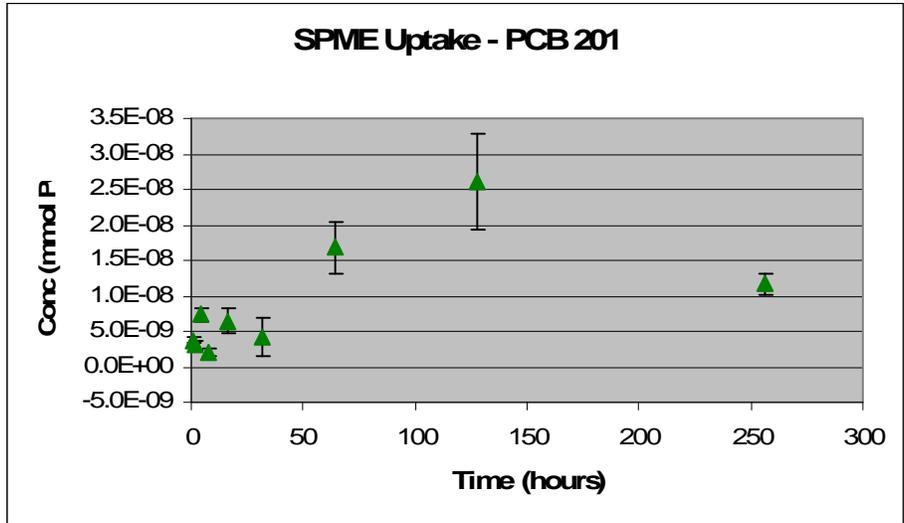


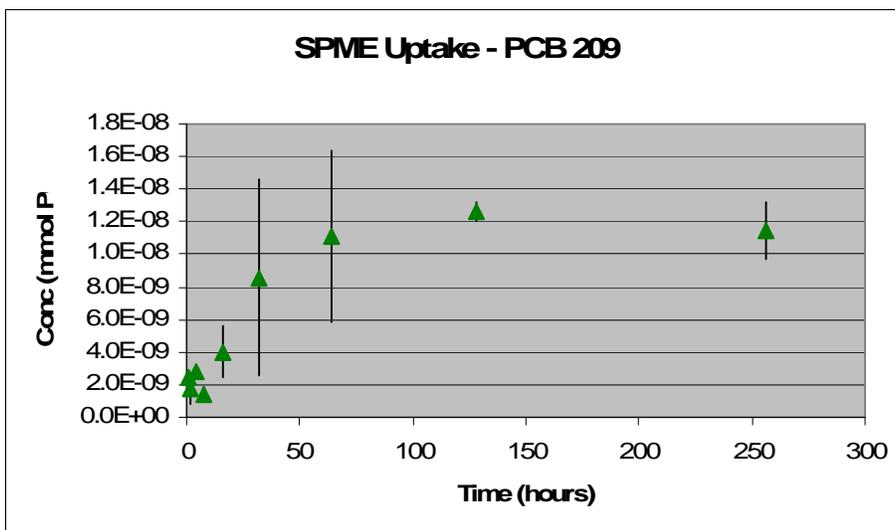
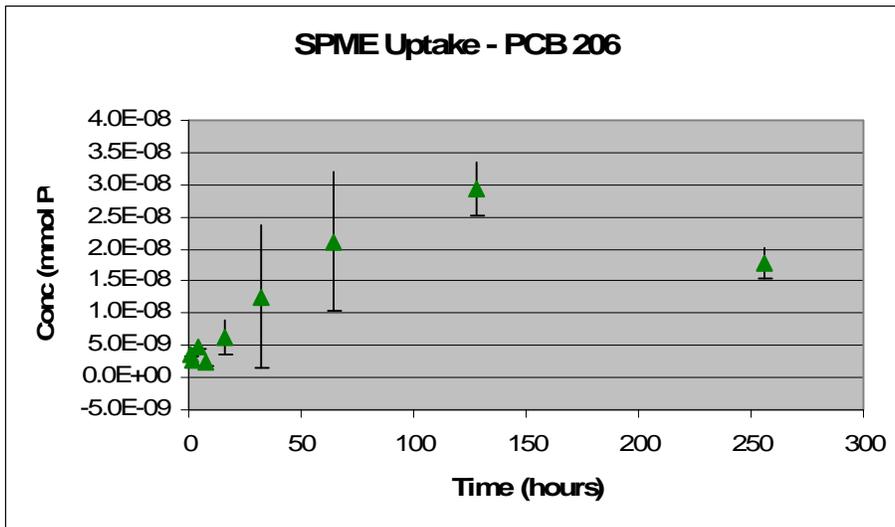
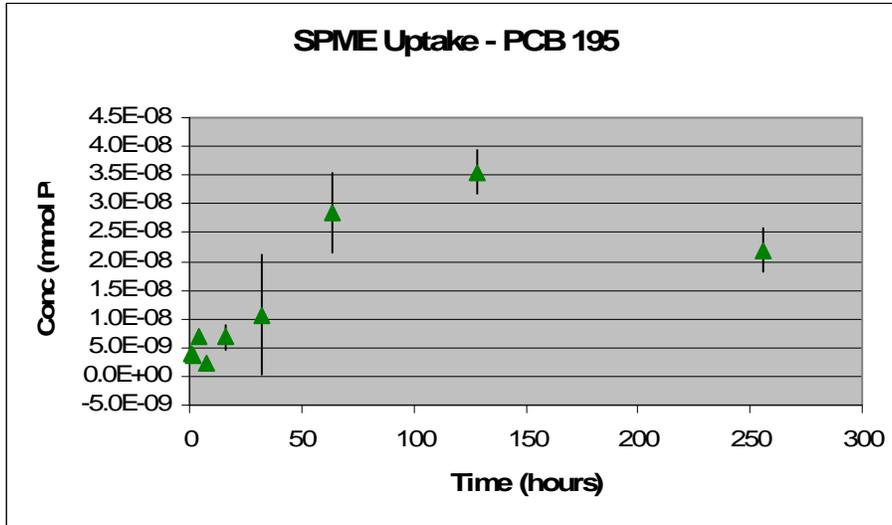




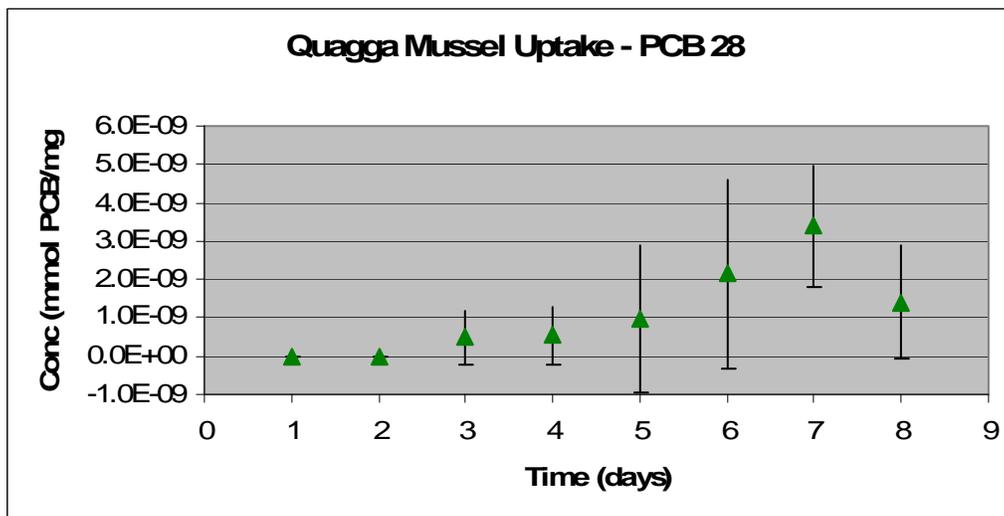
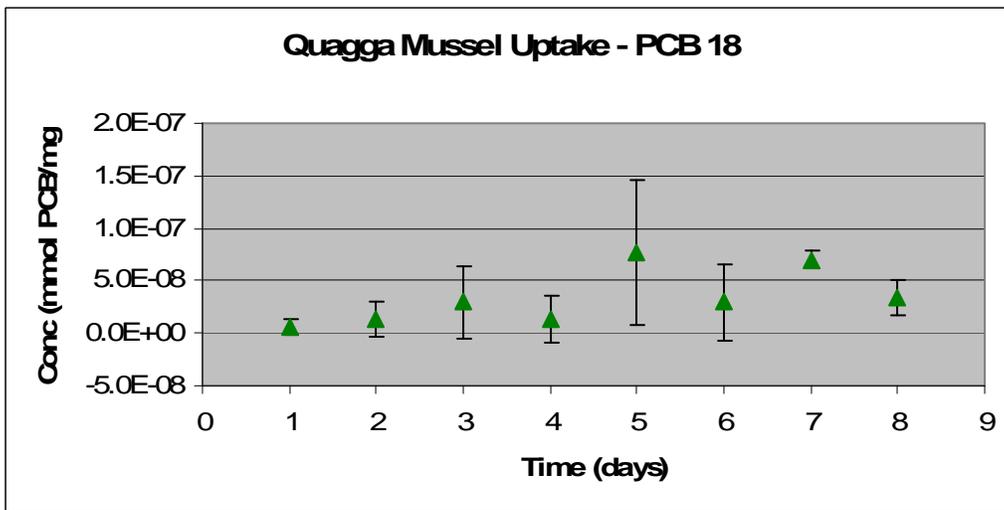
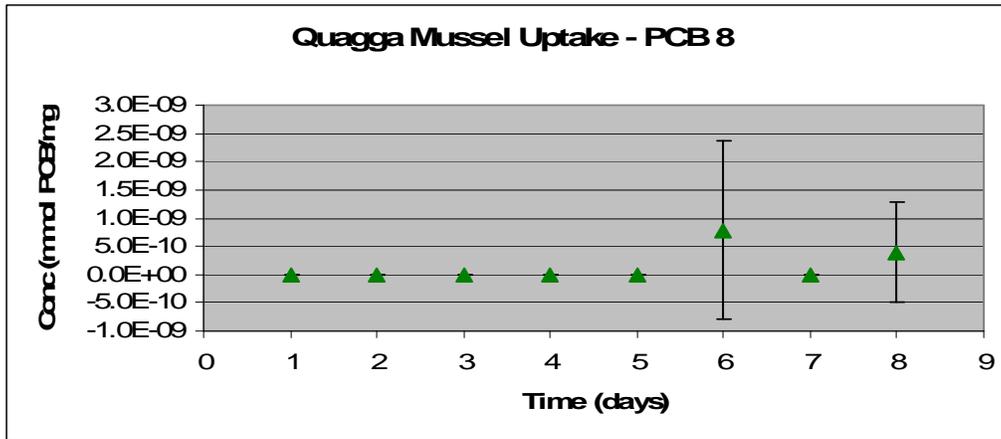


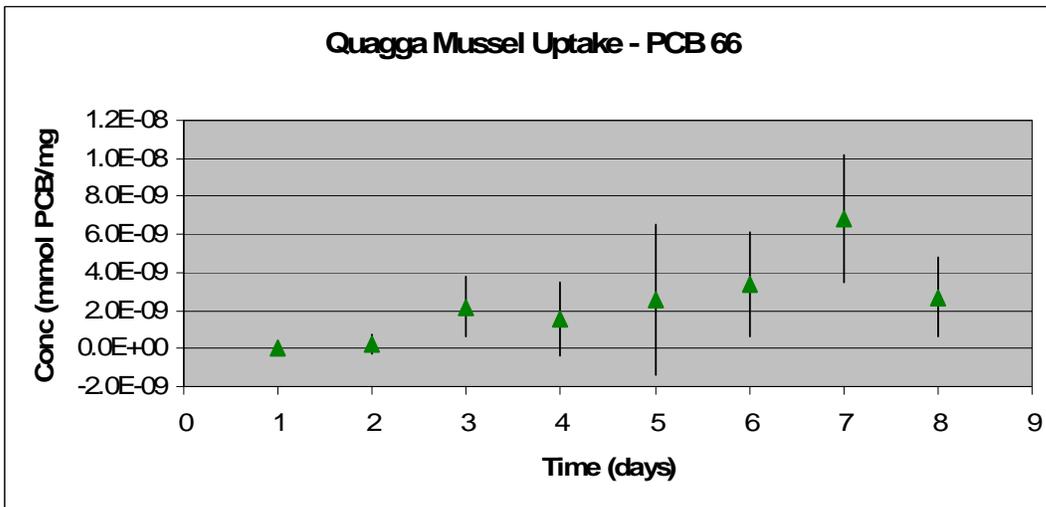
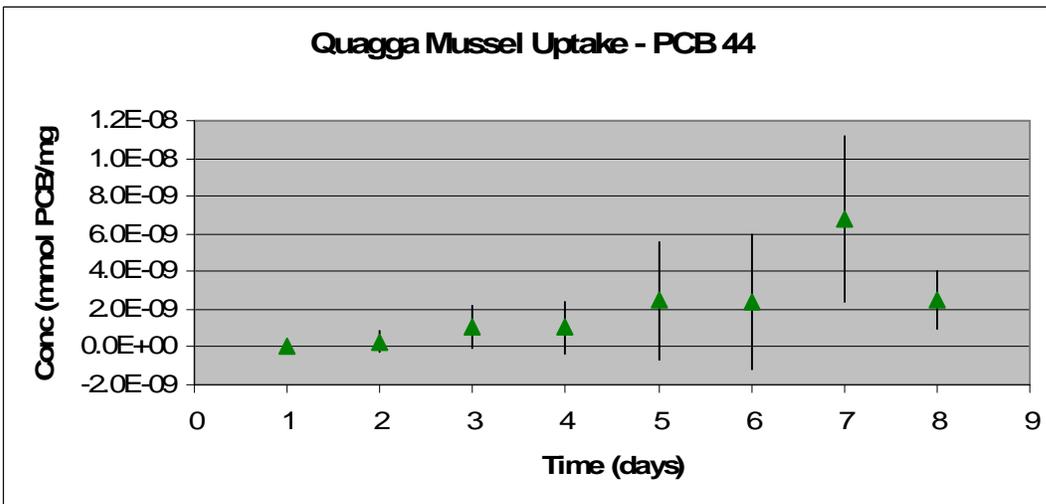
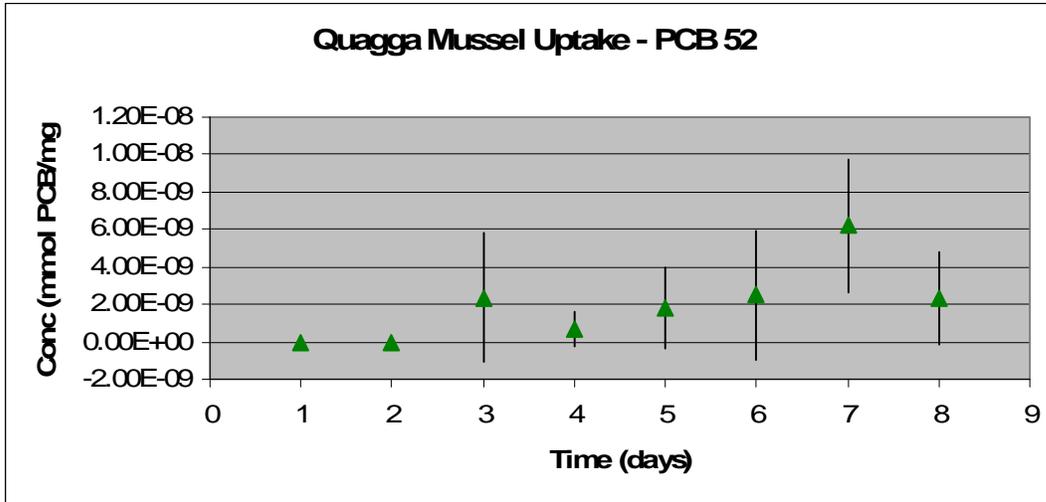


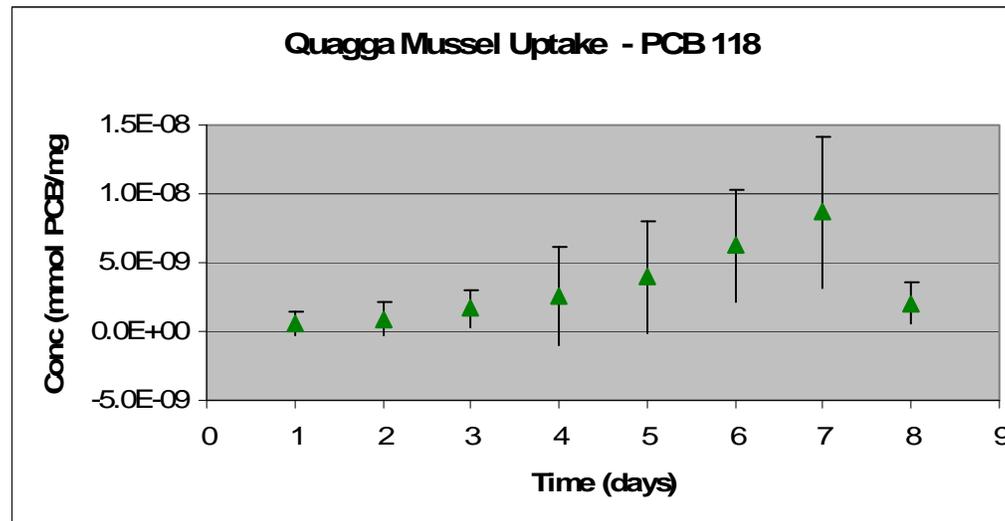
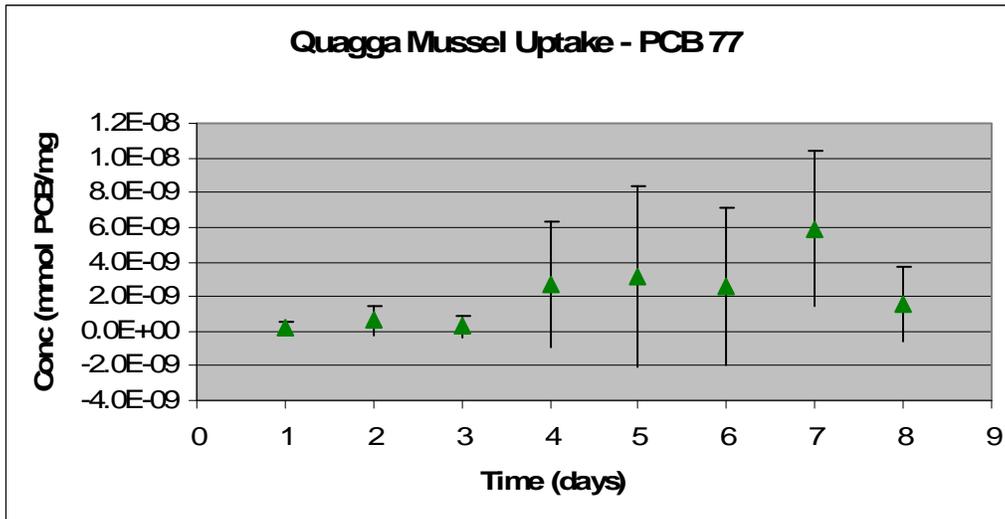
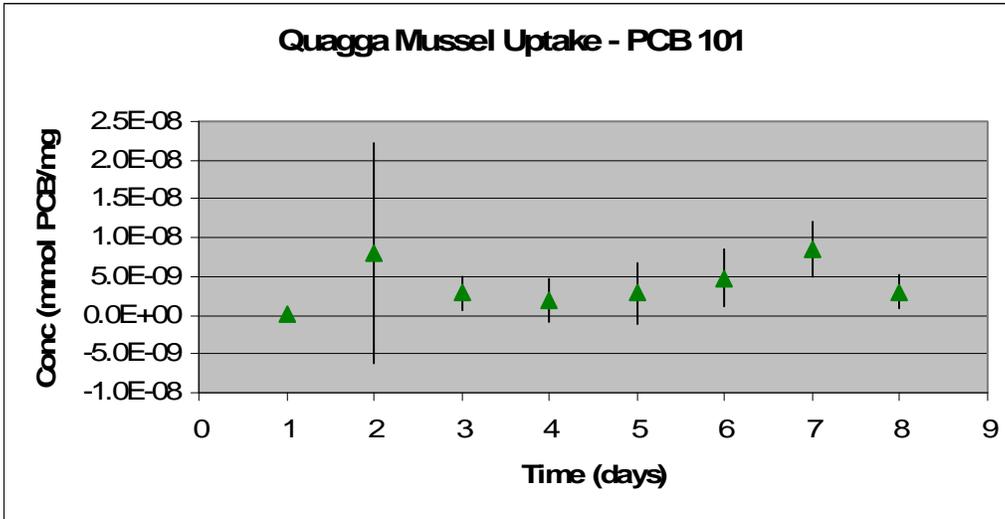


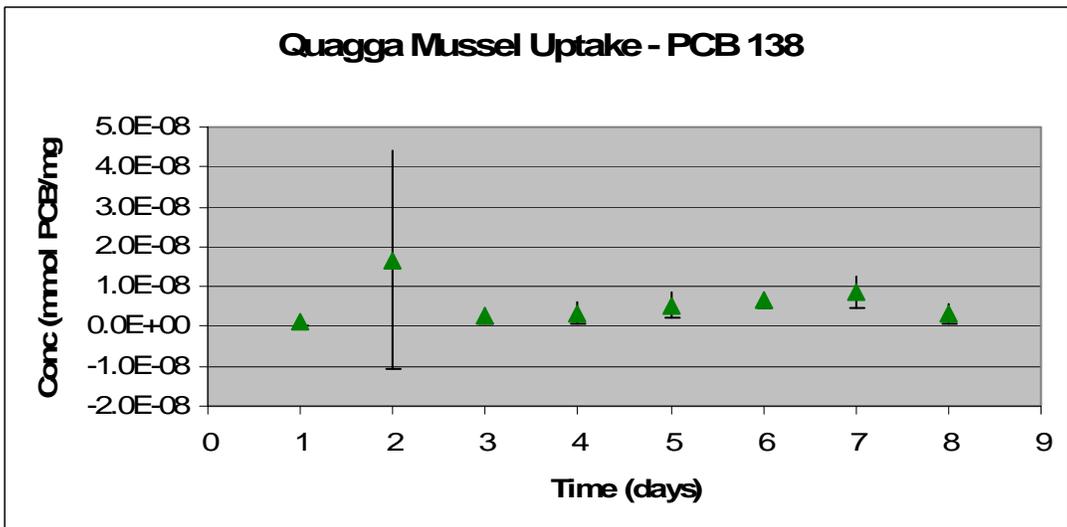
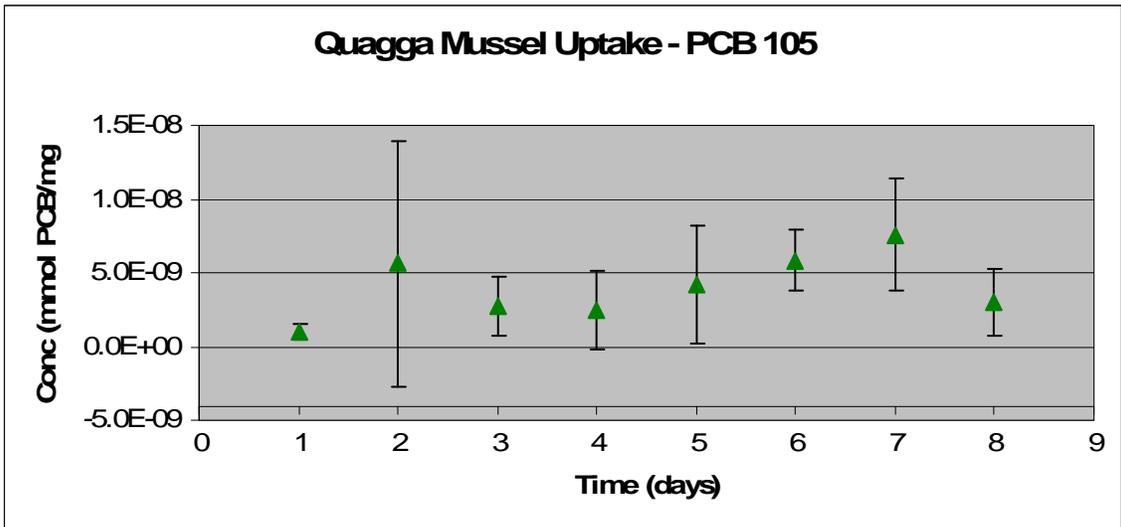
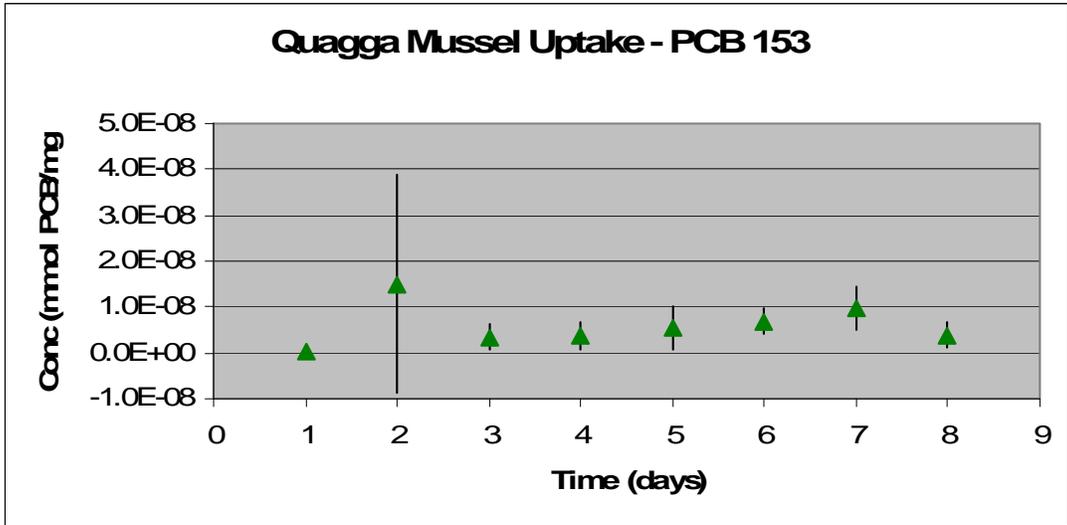


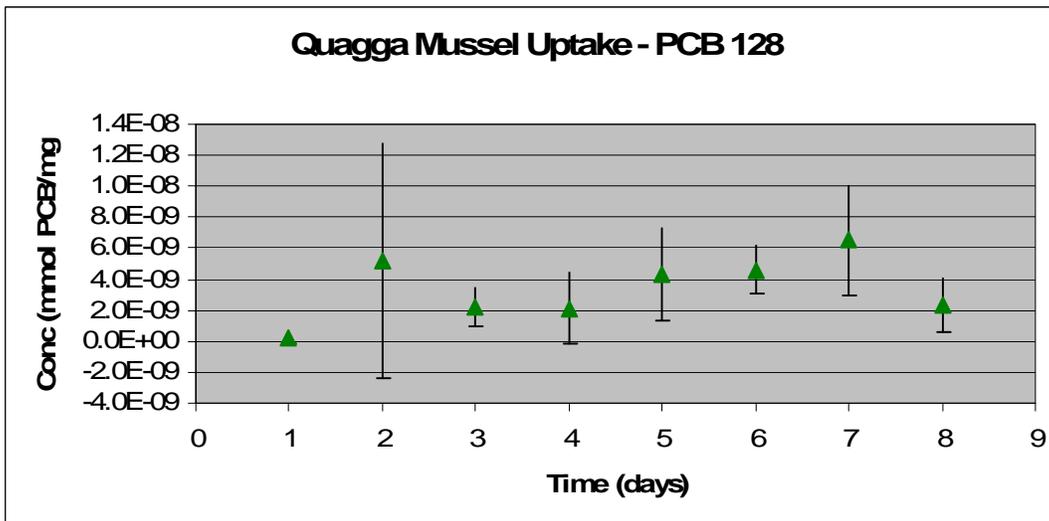
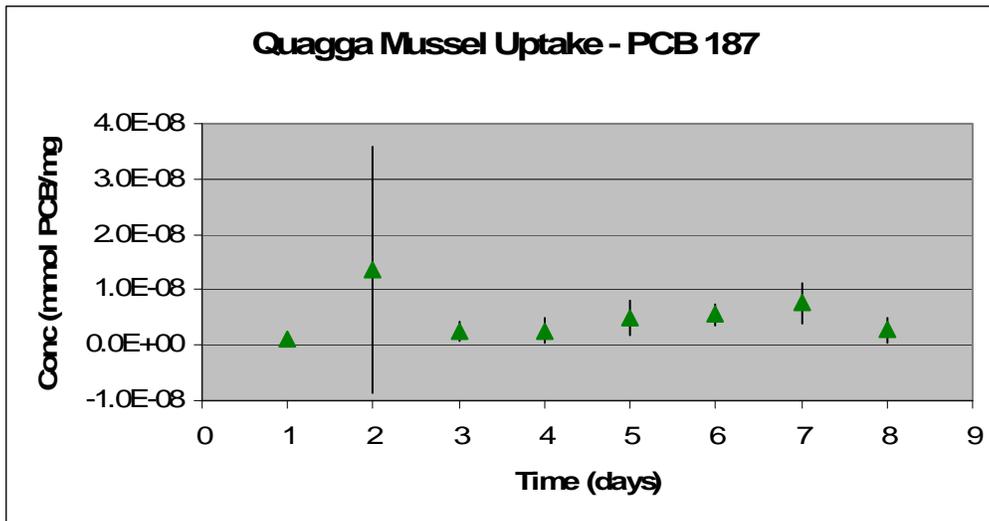
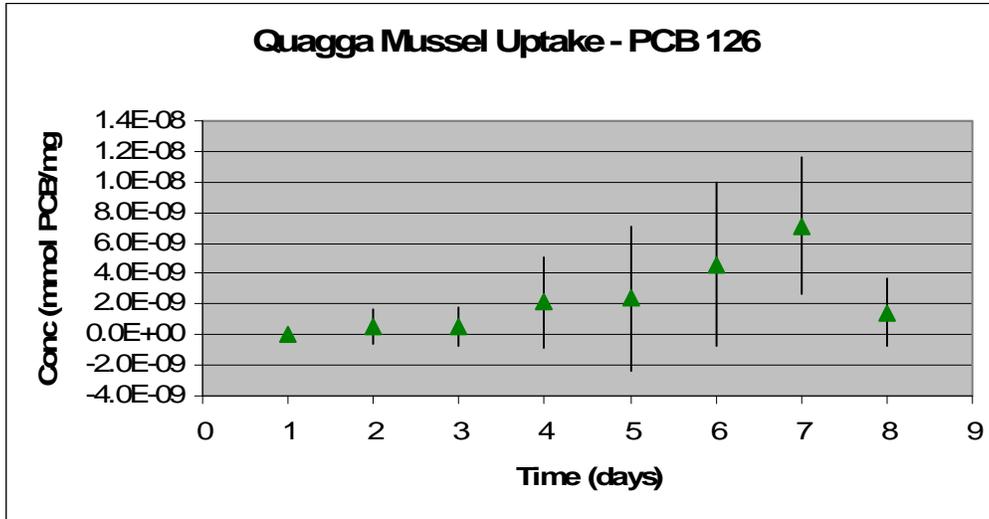
Quagga Mussel Uptake Data (mmol PCB/mg lipid)

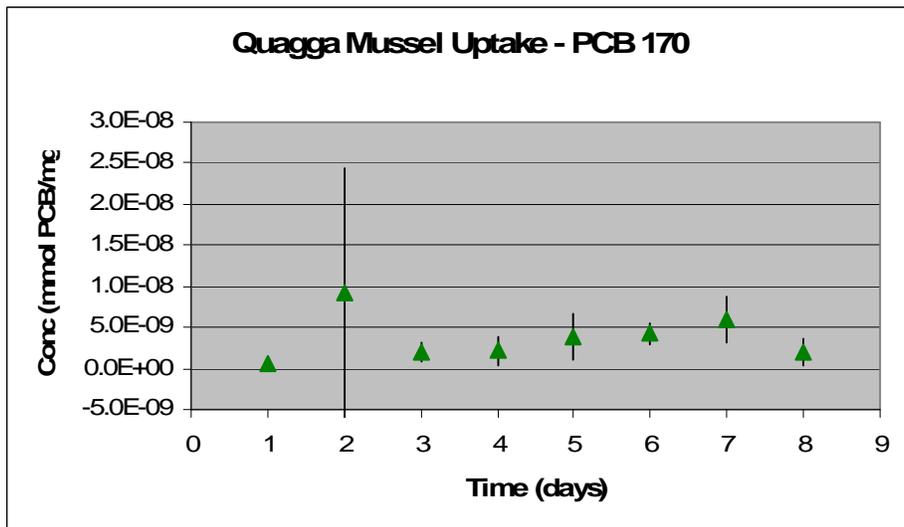
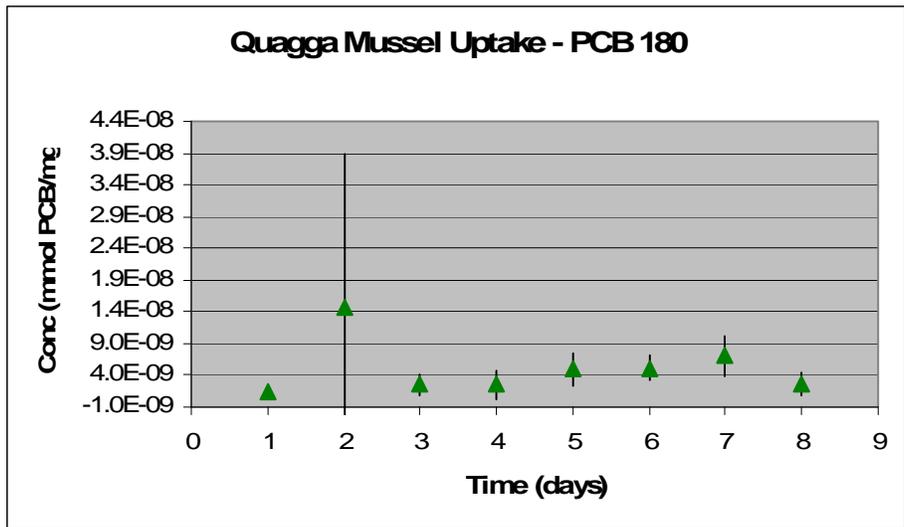
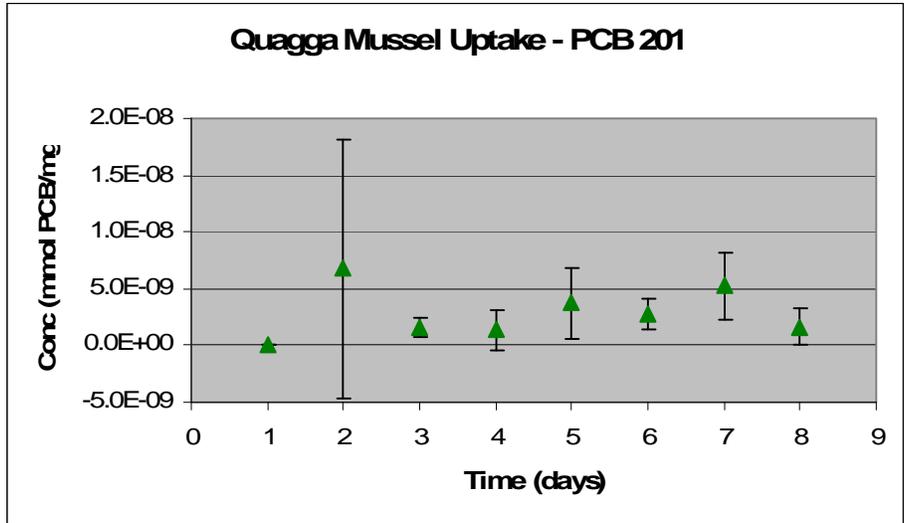


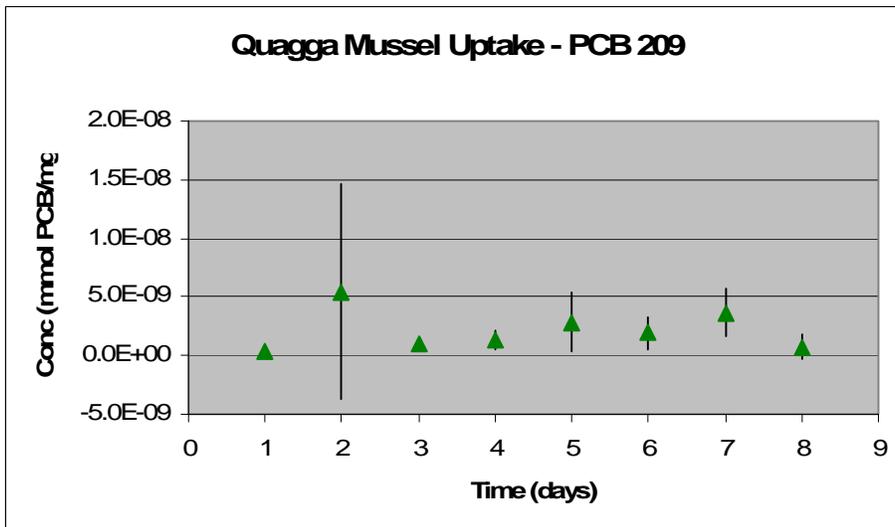
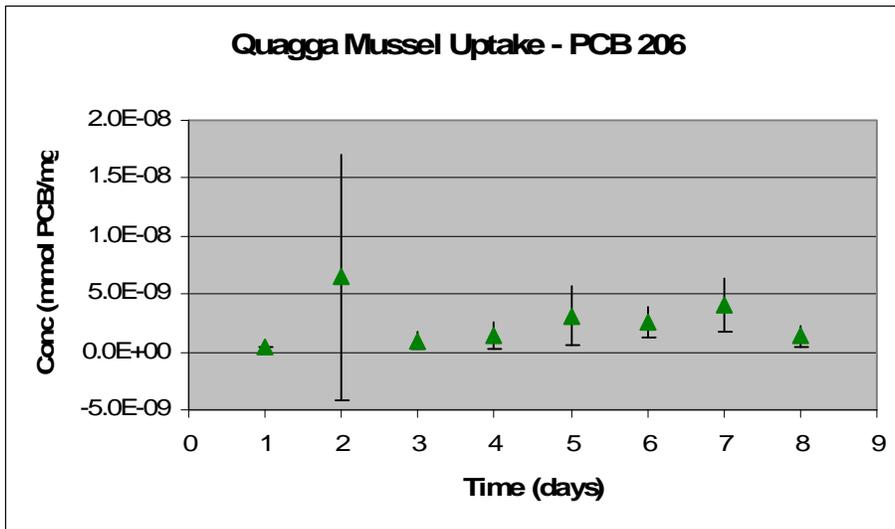
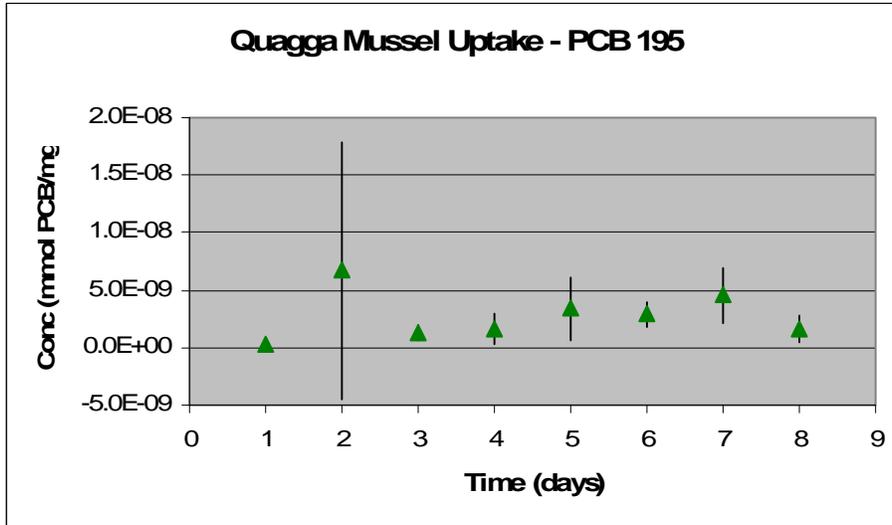












Tubifex Uptake Data (mmol PCB/mg lipid)

