DIETARY UPTAKE AND BIOTRANSFORMATION OF PHTHALATE ESTERS IN STAGHORN SCULPIN

by

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ABSTRACT

Phthalate esters (PEs) are a group of organic chemicals used mainly as plasticizers. Due to their widespread use and their ability to leach from various products, PEs are considered ubiquitous environmental contaminants. Phthalate di-esters (DPEs) and their mono-ester metabolites (MPEs) have been linked to a variety of toxic effects, including endocrine disruption.

Despite a wide range of Kows, previous work has shown that DPEs do not biomagnify in marine food webs. Biotransformation is believed to limit DPE bioaccumulation, but the specific role of metabolism in limiting DPE accumulation via the diet is not well understood.

This study examines the dietary uptake and biotransformation of phthalate esters in Staghorn sculpin (*Leptocottus armatus*). Sculpin were fed a diet containing DPEs and PCBs, and PCBs, DPEs and MPE metabolites were measured in the food, stomach, intestine, liver and muscle tissue over time.

Results show that phthalate di-esters are extensively metabolized to phthalate mono-esters in the sculpin stomach and intestines. Diffusion gradients between the gut and internal tissues indicate the potential for both DPE and MPE dietary uptake. Significant increases of DMP, BBP, DnOP and MEHP above background levels were measured in sculpin tissues over time, but steady state concentrations remained low. Significant dietary uptake was not detected for all other DPE and MPE congeners. DPEs did not biomagnify.

Gut metabolism appears to reduce the dietary uptake of phthalate di-esters and is believed to explain the lack of DPE biomagnification observed in marine food webs. However, un-metabolized DPEs and the MPEs produced by gut metabolism may still be absorbed across the gut wall. DPE and MPE absorption may be balanced by rapid elimination to produce the low steady state levels observed in the tissues. The gross flux of DPEs and MPEs across the gut wall may therefore be relatively high.

Four mid-Kow MPEs (MBP, MBzP, MEHP and MOP) were detected in fish tissues (liver and muscle). For these congeners, MPEs & DPEs were found at relatively equal concentrations in sculpin muscle. Similar patterns may exist in wild fish.

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1. INTRODUCTION

1.1 Introduction to PEs

Phthalate esters (PEs) are a group of organic chemicals used mainly as plasticizers to increase the flexibility and durability of plastics. PEs also have many non-plastic uses, in products such as insect repellents, perfumes, adhesives, photographic film, upholstery, food packaging and paints (Pierce, Mathur et al. 1980). More than 4 million tonnes of phthalate esters are produced worldwide each year (Furtman 1996; Parkerton and Konkel 2000a), making them some of the most highly produced and commercially significant synthetic chemicals in the world.

As plasticizers, PEs are not physically bound to the polymer matrix, and can thus migrate out of plastics and leach into the environment (Graham 1973). Phthalates are also emitted to the air and water from various industries (Staples, Parkerton et al. 2000; Parkerton and Konkel 2000a) and are known to leach from landfills (Ejlertsson, Meyerson et al. 1996; Jonsson, Ejlertsson et al. 2003). PEs have been measured in water, sediment, air, dust, and biota samples from various locations around the world (Rudel, Brody et al. 2001; Suzuki, Yaguchi et al. 2001; Lin, Ikonomou et al. 2003), and are now considered ubiquitous environmental contaminants.

The generalized phthalate ester structure is shown in **Figure 1**. Different PE congeners vary in the length and branching patterns of the two alkyl chains. PEs exhibit a wide range of chemical properties, with log Kow (the log octanol-water partition coefficient) ranging from 1.6 for dimethyl phthalate (DMP) up to 10 for di-iso-decyl phthalate (C10) (Cousins and Mackay 2000). The phthalate ester congeners used in this study and a selection of their chemical properties are listed in **Table 1**.

The high log Kows (i.e. >5) of many PE congeners suggest that PEs have the potential to biomagnify in aquatic organisms (Connolly and Pedersen 1988; Gobas, Wilcockson et al. 1999; Russell, Gobas et al. 1999). Biomagnification results from chemical accumulation via the diet, and is measured as a progressive increase in lipid normalized concentration at each step of the food chain (i.e. $C_{fish}>C_{diet}$) (Gobas and Morrison 2000). However, a recent field study found no evidence of PE biomagnification in a marine food web (Mackintosh, Maldonado et al. 2003). Numerous water exposure studies also

document that PE bioconcentration (accumulation via the gills) is less than expected from Kow (Staples, Peterson et al. 1997). Metabolism is widely believed to reduce PE bioaccumulation (Staples, Peterson et al. 1997; Parkerton and Konkel 2000b), but the specific role of metabolism in limiting the dietary uptake and accumulation of PEs in fish is not well understood.

1.2 Phthalate ester metabolism and toxicity

The biotransformation of phthalate esters has been well studied in mammalian species, but limited research exists for aquatic organisms. Phthalate di-esters (DPEs) are hydrolyzed to phthalate mono-esters (MPEs) by esterases and lipases in the internal tissues (**Figure 2**), where they may then be further hydrolyzed to phthalic acid or oxidized, glucuronidated and excreted in the urine or bile (Albro, Corbett et al. 1982; Woodward 1988; Barron, Albro et al. 1995). DEHP, the most widely studied DPE, is known to hydrolyze to MEHP in the liver, kidney, blood and intestinal wall in many species, including humans (Woodward 1988), and DEHP hydrolysis has also been detected at the fish gill (Barron, Schultz et al. 1989). To date, MPEs have been measured directly in urine, blood, bile and saliva, but only indirectly in tissues (i.e. as a fraction of total radioactivity following the administration of radiolabelled compounds) due to a lack of analytical methods. Since the half lives for DPEs and MPEs within biota appear to be relatively short (hours to days), high levels of DPEs and MPEs are not expected to persist in exposed organisms (Woodward 1988).

DPE metabolism has also been measured in the gastro-intestinal tract of mammals. Gastric enzymes, including pancreatic lipase, have been shown to hydrolyze DPEs to MPEs *in vitro* (Albro and Thomas 1973; Lake, Phillips et al. 1977; Rowland, Cottrell et al. 1977). Further degradation of MPEs in the gut (e.g. to phthalic acid) is believed to be minimal. DPEs and especially MPEs appear to be well absorbed from the mammalian gut, with up to 90% of a dietary dose of DPEs recovered in rat urine within a few days (measured as total radioactivity – i.e. including all metabolites) (Williams and Blanchfield 1974). However, the extent of DPE gut metabolism *in vivo* and the implications of this process for the dietary uptake of both DPEs and MPEs have not been investigated. DPE gut metabolism has also not been studied previously in fish.

Both DPEs and MPEs have been linked to a number of toxic effects, including endocrine disruption (Stahlschmidt, Allner et al. 1997; van Wezel, van Vlaardingen et al. 2000). MPEs are generally considered more toxic than the parent compounds (Lake, Gray et al. 1987; Grasso, Heindel et al. 1993; Foster, Cattley et al. 2000; Ema 2002). Understanding the fate of DPEs and MPEs in biological organisms is therefore important to properly assess the risks associated with DPE levels in the environment.

1.3 Phthalate esters and policy

The bioaccumulation potential and toxicity of phthalate esters is of considerable legal and regulatory importance. Both international legislation (UNECE 1979), as well as domestic legislation in Canada (CEPA 1999), the US (TSCA 1976; US EPA 1998), and Europe (UNECE 1979) include provisions for eliminating substances from commerce that are “bioaccumulative”, “persistent” and “toxic”. The metabolites from commercially produced substances (e.g. MPEs) are not currently screened or regulated. Several phthalate esters, including DMP, DEP, DnBP, BBP, DEHP and DnOP are currently listed on the ‘priority pollutants’ lists in either Canada or in the US, and management options are being considered (US EPA; CEPA 1999). DPEs are currently banned in Europe for use in toys and products intended for children under the age of three (CEU 2000).

Recent studies have reported several MPEs (MMP, MBP and MEHP) in environmental water samples (Suzuki, Yaguchi et al. 2001; Alzaga, Pena et al. 2003), but the environmental concentrations of MPEs remain largely unknown. MPE levels have not yet been determined in wild biota. Due to potential toxicity concerns, regulators, industry and consumer groups are shifting their attention to the fate and distribution of MPEs. The dietary uptake of DPEs & MPEs is of particular interest because human exposure to phthalate esters is largely through the diet (Tomita, Nakamura et al. 1977; IPCS 1999; David 2000).

1.4 Conceptual model of dietary uptake in fish

A conceptual model of dietary uptake & elimination in fish is shown in **Figure 3**. Chemical uptake occurs by absorption from the diet ($k_d.C_d$), and chemical elimination occurs via the gills ($k_2.C_b$), by fecal excretion back to the gut ($k_e.C_b$), or by metabolism ($k_m.C_b$). k_d, k_2, k_e and k_m are the first order rate constants for each process, and C_d and C_b are the concentrations in the fish and diet, respectively.

The net flux into the fish is thus:

$$\mathbf{dC_b/dt = k_d.C_d - C_b(k_2+k_e+k_m)} \qquad \textbf{Equation 1}$$

At steady state, $dC_b/dt = 0$, and the steady state fish concentration C_b is:

$$\mathbf{C_b = k_d.C_d/(k_2+k_e+k_m)} \qquad \textbf{Equation 2}$$

or

$$\mathbf{C_b = k_d.C_d/k_{e,tot}} \qquad \textbf{Equation 3}$$

where $k_2+k_e+k_m = k_{e,tot}$, the total elimination rate constant.

This model is useful for understanding how metabolism in different compartments may affect steady state fish concentrations (e.g. concentrations in wild biota). Low C_b may result from: 1. low dietary uptake (e.g. gut metabolism reduces C_d), and/or from 2. a combination of dietary uptake plus elimination (e.g. $k_d.C_d$ occurs, but is balanced by total elimination, including k_m). Either or both of these scenarios may explain the lower than expected fish concentrations observed in wild populations.

1.5 Research questions & hypotheses

This thesis addresses the following questions:

1. What role does metabolism play in limiting the dietary uptake & accumulation of DPEs? Can metabolism explain the lack of biomagnification observed in the field?
2. What is the fate of MPEs resulting from the ingestion of DPEs?

Two hypotheses (see **Figure 4**) are proposed to explain the lack of DPE biomagnification in the field:

1. DPEs are extensively metabolized in the GIT, which limits dietary uptake, and/or
2. DPEs are absorbed across the gut wall, but are metabolized in the internal tissues and eliminated from the fish.

Both hypotheses predict low steady state DPE fish concentrations, but they imply different levels of 'exposure' (if exposure = chemical uptake into the tissues). Under hypothesis 1, exposure is minimal since very little DPE crosses the gut wall. Under hypothesis 2, a larger fraction of the ingested DPE is absorbed into the tissues before being eliminated from the fish. This increase in exposure (although possibly transient if elimination is rapid) may increase the risk of toxic effects. Thus, a lack of biomagnification does not imply a lack of dietary uptake, and the gross flux across the gut wall (dietary absorption) may be relatively high, despite low steady state tissue concentrations.

To test these hypotheses, we conducted a dietary uptake experiment of DPEs & PCBs in Staghorn sculpin (*Leptocottus armatus*). PCBs are poorly metabolized in biota and are known to biomagnify in aquatic food chains (Oliver and Niimi 1988). PCBs thus served as a 'positive control' for dietary uptake, and could be compared to DPEs to provide indirect evidence of phthalate ester metabolism. Sculpin were fed a DPE and PCB spiked diet for 14 days and a control diet for a further 14 days. Samples of stomach contents, intestinal contents, liver, muscle and food were collected over time, and analyzed for PCBs, DPEs, and MPE metabolites. The observed concentration profiles over time were then used to interpret the fate of DPEs and MPEs throughout the fish.

1.6 Context of this study within larger PE research program

This study is part of a broader research program co-investigated by Frank Gobas (Simon Fraser University, Burnaby BC) and Michael Ikonomou (Institute for Ocean Sciences, Sidney BC). The research has been funded by the National Science and Engineering Research Council (NSERC), the Toxic Substances Research Initiative (TSRI), and the American Chemistry Council (ACC). The overall research program consisted of 3 phases, including:

- **Phase I:** the development of analytical methods to measure DPEs & MPEs in water, sediment and biological tissue
- **Phase II:** a field study to determine the bioaccumulation potential of DPEs in a marine food chain (Mackintosh, Maldonado et al. 2003)
- **Phase III:** Laboratory experiments to determine the mechanisms controlling the bioaccumulation of DPEs in fish.

This thesis presents the results from the dietary uptake (biomagnification) study in phase III. A parallel water uptake (bioconcentration) study constitutes part of another investigation.

1.7 Contributions of the phthalate ester research team

This study was conducted by the author at the Fisheries & Oceans Laboratories in West Vancouver. All sample extractions, clean up and analysis were performed at the Institute for Ocean Sciences in Sidney BC by members of the Contaminants Sciences Section (under the supervision of Michael Ikonomou). Joel Blair, Audrey Chong and Jody Carlow performed sample extractions and cleanup. Natasha Hoover developed the MPE analytical method, and analyzed the samples for DPEs and MPEs by GC/MS & LC/ESI-MSMS. Maike Fischer performed the PCB GC/HRMS analysis, and Tamara Fraser quantified the PCB data. Lipid and moisture analyses were performed by the author at Simon Fraser University.

2. METHODS

The methods section is divided into 3 parts, describing (i) the experimental methods, (ii) the chemical analyses, and (iii) data handling.

2.1 Experimental Methods

2.1.1 Summary

Wild Staghorn sculpin (*Leptocottus* armatus) were fed a DPE and PCB spiked diet for 14 days (the uptake phase), followed by a 14 elimination period. The experimental food contained six phthalate ester congeners (DMP, DEP, DnBP, BBP, DEHP, DnOP), one commercial PE mixture (C10), and three di-ortho PCB congeners (52, 155, 209). PCBs were included in the diet to be able to contrast the observed patterns of DPEs with those of non-metabolizing substances. Three sculpin were sacrificed on each sample day (days 0,2,3,5,10,14,16,17,19 and 24). Stomach contents, intestinal contents, liver and muscle samples were collected from each fish and analyzed for DPEs and MPEs by GC/MS and/or by LC/ESI-MSMS where applicable, and for PCBs by GC/HRMS. The observed concentration profiles over time were then used to compare the fate of PCBs and DPEs throughout the fish, and to monitor the formation of MPE metabolites.

2.1.2 Fishing & acclimation to captivity

Wild Staghorn sculpin (*Leptocottus armatis*) were collected by beach seine at Stearman's Beach in West Vancouver, BC. Fish were held in salt water flow-through tanks for several weeks to acclimate to lab conditions. During acclimation, sculpin were trained to eat a pelleted commercial fish food coated with marine oil & krill (to enhance palatability). Before the start of the experiment, fish were weaned onto uncoated pellets, and were individually weighed in order to assess growth over the experimental period.

2.1.3 Preparation of the experimental food

Experimental fish food pellets were prepared with Dr. David Higgs at the Fisheries and Oceans fish nutrition laboratory in West Vancouver. Food ingredients are listed in **Table 2**.

To introduce the compounds of interest to the experimental food, food pellets were submerged in petroleum ether spiked with solutions of known concentration of DPEs and PCBs. Spiking solutions contained DMP, DEP, DnBP, BBP, DEHP, DnOP, C10, and PCBs 52, 155 and 209. Food was tumbled with a rotoevaporator until all solvent had evaporated. Final food concentrations were approximately 10ppm for the DPEs and approximately 5ppm for the PCBs. Control food was prepared in a similar manner, but without the spiking solution. Prepared food was stored in solvent rinsed glass jars at room temperature. **Table 1** lists the chemical congeners added to the experimental food, and select chemical properties.

2.1.4 Experimental setup & laboratory conditions

The experimental set-up (**Figure 5**) consisted of twelve glass 15L aquaria, each containing 3 sculpin. Each tank was aerated with a submerged air stone, and received approximately 2L/min of sand-filtered flow-through sea water. One tank (the water uptake control tank) received the effluent water from an adjacent experimental tank instead of receiving clean sea water. This tank controlled for chemical uptake via the gills (i.e. for the possibility that DPEs leached into the water from the experimental food.)

Water temperature and water chemistry (pH, salinity and dissolved oxygen, nitrate & ammonia concentrations) were monitored to ensure that environmental conditions remained stable throughout the experiment. Measured values are summarized in **Table 3**.

Laboratory lighting followed a 16hr:8hr photoperiod to mimic natural summer conditions (July). To reduce fish stress and encourage feeding, full spectrum laboratory lights were dimmed by wrapping them loosely in black fabric.

2.1.5 Experiment & sampling

Fish in the experimental tanks were fed PCB & DPE spiked ‘experimental’ food for 14 days (the uptake phase) followed by control food for 14 days (the elimination phase). Day 0 fish were sacrificed at the beginning of the experiment, and controlled for background contaminant levels in wild sculpin. The water uptake control fish received control food throughout the experiment and controlled for chemical uptake from the water.

Fish were fed daily at approximately 1% of body weight throughout the experiment. Uneaten food pellets were counted and removed from the tank 1 hour after feeding. On average, fish consumed 67% of the administered diet, yielding a true feeding rate of approximately 0.0067g dry food/g fish.day.

Fish were sampled on days 0,2,3,5,10,14,16,17,19 & 24. The water uptake control fish were also sampled on Day 24, but are plotted at Day 30 on all figures for clarity. On each sample day, three sculpin were randomly chosen from among the experimental tanks and culled with a blow to the head. Each fish was wiped with a paper towel and weighed before dissection.

Stomach contents, intestinal contents and whole liver samples were removed from each sampled fish. Muscle samples were removed from the carcasses at IOS prior to sample extraction. Dissection instruments were rinsed with water and dichloromethane (DCM) and flamed with a Bunsen burner between samples to reduce sample contamination. Samples were placed in pre-weighed 20 ml scintillation vials doubly-rinsed with acetone, DCM and hexane. The remaining fish carcass was wrapped in aluminum foil and frozen at -40°C. Sample vials were weighed, sealed with parafilm and stored at -40°C until analysis. Each sample day generated 3 samples for each biological matrix (n=3 for muscle, liver, stomach contents, and intestinal contents), and one sample for fish food (n=1).

2.2 Analytical methods

This section presents a brief overview of the analytical methods. Detailed descriptions of the DPE and PCB analytical methods are available in (Lin, Ikonomou et al. 2003) (DPEs) and (Ikonomou, Fraser et al. 2001) (PCBs). The MPE extraction & analytical methods will be presented in (Ikonomou, Hoover et al. 2003).

Samples were processed in batches of 8-10 samples, including 2 procedural blanks per batch. Procedural blanks consisted of approximately 5g of pre-baked sodium sulfate, which followed the same extraction, clean-up and analysis procedures as the experimental samples. Surrogate internal standards (IS) were added to samples prior to extraction in order to recovery correct measured concentrations for chemical losses during extraction and clean-up. Surrogate recovery standards (RS) were added to

extracts immediately prior to instrumental analysis to calculate the recoveries of the internal standards and to validate the performance of the instrument.

Unless otherwise noted, all sample extractions and chemical analyses were performed at the Institute for Ocean Sciences (IOS) in Sidney, BC by members of the Contaminant Sciences Section.

2.2.1 Preparation of solvents and glassware

Background contamination has been a significant and often unrecognized problem in previous studies of DPEs. In the lab, DPEs may outgas from floor tiles, gloves, tubing, filter paper and protective coatings (Tepper 1973). DPEs have also been detected in solvents, including those of HPLC grade (Lin, Ikonomou et al. 2003). To reduce the background contamination in this experiment, all glassware and materials used in sample preparation, extraction and clean up were cleaned by an elaborate procedure developed in-house (Lin, Ikonomou et al. 2003). When necessary, solvents were doubly distilled to reduce DPE background levels. These precautions reduced background DPE contamination in the procedural blanks to levels in the low ng range (**Table 7**), and allowed for the quantification of trace levels of DPEs in biological samples. These blank levels are substantially lower than those reported from other DPE analysis laboratories around the world (ECPI/ACC/PEP 2002).

Glassware was detergent washed, rinsed with distilled water, acetone, toluene, doubly distilled hexane, and dichloromethane (DCM), and baked at 400 °C for at least 10 h. Cooled glassware was stored in solvent-rinsed aluminum foil. Prior to use, glassware was rinsed with iso-octane (2X), doubly distilled hexane, dichloromethane, methanol (2X) and again with dichloromethane. Mortars and pestles were cleaned using the same procedure, but were baked at 150 °C for 10 h. Alumina and sodium sulfate were baked at 200 and 450°C, respectively, for at least 24 h, and were cooled and stored in a dessicator. Other materials such as teflon stoppers, GC septa and sample vial lids, which decompose at elevated temperatures, were washed extensively with 1:1 DCM/Hexane (Lin, Ikonomou et al. 2003).

GC autosampler vials were baked at 325°C, sonicated in hexane and in DCM, dried, and stored in a solvent rinsed beaker. GC vials were covered with solvent rinsed aluminium

foil and capped with crimp style (red rubber / PTFE) lids. Pipettes were baked at 325°C and rinsed with hexane and DCM. Sample collection vials and lids were rinsed with acetone, hexane and DCM (omitted for lids) immediately before use. All cleaned glassware was stored in solvent rinsed aluminum foil. Heavy duty aluminum foil was cleaned by rinsing it with hexane and DCM in a large graduated cylinder. Foil was then dried, baked at 325°C, and stored in a clean aluminium foil packet.

2.2.2 PCB and DPE analysis

PCBs and DPEs were extracted concurrently (**Figure 6**). 0.1g - 4 g of food or biota sample (as available) was ground with pre-baked sodium sulfate and spiked with surrogate internal standards (see **Table 4**). Spiked samples were extracted by sonication with 1:1 DCM/Hexane. The extracts were blown down with nitrogen and cleaned up and fractionated with a deactivated neutral Alumina column (15% HPLC-grade water w/w). To isolate the compounds of interest, the alumina column was eluted with three 30mL fractions of (i) Hexane, containing the PCBs, (ii) 1:9 DCM/Hexane, which was discarded, and (iii) 1:1 DCM/Hexane, containing the DPEs.

The PCB fraction was cleaned up with an acid/base silica column eluted with 1:1 DCM/Hexane, followed by an activated alumina column eluted with 1:1 DCM/Hexane. The eluate was spiked with 30 μL of the PCB recovery standard solution (^{13}C PCB 111, **Table 4**), and analyzed by gas chromatography / high resolution mass spectrometry (GC/HRMS). The GC/HRMS system was a VG-Autospec High-Resolution MS, Micromass, UK, coupled to a Hewlett Packard model 5890 series II GC. Instrumental analysis conditions, the criteria used for analyte identification and the quantification procedures used are described in detail in (Ikonomou, Fraser et al. 2001).

The DPE fraction was concentrated under nitrogen and spiked with 50 ng of the surrogate phthalate ester recovery standards (RS) (DEP-d_4 and BBP-d_4) (see **Table 4**). Individual DPE congeners (DMP, DEP, DnBP, BBP, DEHP and DnOP) were analyzed by Low-Resolution GC/MS (Trace GC/Voyager MS from Thermo Finnigan). Following GC/MS analysis, extracts were evaporated to dryness, reconstituted in methanol, and analyzed for C6-C10 phthalate isomeric mixtures by LC/ESI-MSMS. The HPLC system used was a Beckman Model 126 VG Quattro, Micromass, UK. This separate LC/ESI-MSMS analysis was required to properly quantify PE isomeric mixtures and avoid

interferences found in previous GC/MS analyses. Instrumental analysis conditions and quantification procedures are described in detail in (Lin, Ikonomou et al. 2003). DPE concentrations were quantified using isotope dilution.

2.2.3 MPE analysis

The phthalate mono-esters (MPEs) analyzed in this study, and their molecular weights are listed in **Table 5**. Other chemical properties of MPEs (e.g. Kows) have not yet been published.

All food and biota samples were re-extracted for MPEs analysis (**Figure 7**). 0.1-5g of food or biota sample (as available) was ground with sodium sulphate, and spiked with 600 ng of surrogate MPE internal standards ($^{13}C_2$-MBP and $^{13}C_2$-MEHP, **Table 4**). Samples were extracted by sonication with 1:1 DCM/Acetone, evaporated to dryness, and re-suspended in acetonitrile with 5-6mL of sodium phosphate acidic buffer (pH = 2).

MPE extracts were cleaned up with an SPE Oasis cartridge (6cc, 500mg) eluted with 5mL acetonitrile and 5mL ethyl acetate. The eluate was evaporated under nitrogen, re-suspended in 1:1 DCM/Hexane, and eluted through a gel permeation chromatography column (Biobeads SX-3) with 1:1 DCM/Hexane. The eluate was evaporated under nitrogen, re-suspended in methanol, and spiked with the $^{13}C_2$-MiNP (mono-iso-nonyl phthalate) recovery standard (see **Table 4**). Extracts were analyzed by the same LC/ESI-MSMS system used for the DPE analysis. The MPEs of interest were quantified using the isotope dilution approach. Instrumental analysis conditions are described in detail in (Ikonomou, Hoover et al. 2003).

2.2.4 Lipid and moisture determinations

Lipid and moisture determinations were performed by the author at Simon Fraser University. At least 3 samples of each sample matrix (food, stomach contents, intestinal contents, muscle and liver) were analyzed for lipid and moisture content. Results were used to lipid normalize the concentration data, and to calculate the dry to wet food concentration ratio (R) (see section 3.2.1 below).

For lipid determinations, approximately 2g of each matrix (food, stomach, intestine, liver and muscle) was measured into a pre-weighed aluminum weighboat, transferred to a

mortar, and ground with approximately 20g of anhydrous sodium sulfate. Ground samples were transferred to a glass extraction column, packed from bottom to top with a small amount of glass wool, 5 g of sodium sulfate, the ground sample, followed by another 5 g of sodium sulfate. The column was eluted with 100mL 1:1 DCM:Hexane into a 250 mL round bottom flask, and left overnight. The eluate was partially evaporated using a rotoevaporator, transferred to a pre-weighed beaker using DCM:Hexane, and evaporated to dryness under nitrogen. Beakers were placed in a 35°C vented oven for 1 -2 days, cooled completely in a dessicator, and reweighed.

The lipid content (L) of each matrix was then calculated as:

L = (weight of lipid / weight of original sample)

For moisture determinations, 1-2 g of each matrix was measured accurately into a pre-weighed aluminum weighboat. The sample was dried in a 35°C vented oven for a minimum of 48 hours, cooled completely in a dessicator, and reweighed.

The moisture content (W) was then calculated as:

W = [(wet sample weight – dry sample weight)/ wet sample weight]

Table 6 lists the mean lipid and moisture contents (+/- 1 standard deviation) for each sample matrix.

2.3 Data handling and screening

2.3.1 Recovery and blank corrections / lipid normalizations

Samples were processed in batches of 4-10 samples and 2 procedural blanks. (MPE batches contained only 1 procedural blank). Procedural blanks consisted of pre-baked sodium sulfate which followed the same extraction, clean up and analysis procedures as the food and biota samples. Procedural blanks are therefore a measure of background contamination from sample extraction and analysis (solvents, glassware etc). Mean blank amounts (ng) for DPEs and PCBs (i.e. means across all sample matrices) are listed in **Table 7**. Mean blank amounts for MPEs are listed in **Table 8**.

Each sample was spiked with surrogate internal standards (IS) prior to extraction and with surrogate recovery standards (RS) prior to instrumental analysis. After quantification, measured sample concentrations were corrected for the % recovery of the internal standard to account for analyte loss during sample extraction and clean up. The mean recoveries of the internal standards are listed in **Table 9**.

Recovery corrected concentrations were then blank corrected with the mean concentration of the 2 procedural blanks for each batch. This blank correction step normalized the data for background contamination in glassware, solvents, sample handling etc.

Following blank correction, all data were lipid normalized by dividing the wet weight concentration in a matrix by the lipid content (L) measured in that matrix, i.e.

$C_{Lipid} = C_{wet\ wt}/L$

2.3.2 MRL screening

In contrast to previous phthalate ester work in our lab, blank corrected data in this experiment were not further screened against the Minimum Reportable Limit (MRL), defined as 3 standard deviations of the blanks (ng). (When data have not been blank corrected, the MRL = mean blank + 3SD). The reasons for omitting the MRL screening step are discussed briefly below.

The purpose of MRL screening is to remove ‘false positives’ from the data set, i.e. to screen out low values which may reflect background contamination rather than true sample concentrations. This approach guards against reporting sample concentrations unless they are ‘well above’ the background levels.

However, the definition of what is ‘well above’ the background (i.e. the MRL criterion) is arbitrarily chosen. 99% of a normal distribution is within 3SD of the mean, leaving 0.5% at each tail. With MRL=3SD, blank-corrected sample concentrations must be higher than 99.5% of the blank distribution to be considered ‘different from’ the mean blank (i.e. a=0.005 to reject the null hypothesis that the sample concentration = the mean blank concentration). This is an extremely conservative screening criterion, and may cause many sample concentrations to be incorrectly removed from the data set.

As in statistical hypothesis testing, the choice of an MRL implies a trade off between our willingness to accept false positives (i.e. incorrectly concluding that a sample > blank, a Type I error) and false negatives (i.e. incorrectly concluding that a sample < blank, a Type II error). A conservative (i.e. high) MRL implies a low willingness to tolerate false positives, but a higher willingness to tolerate false negatives. This means that many ‘real’ low data values may be incorrectly screened out of the data set.

The selection of an appropriate screening criterion will be different depending on the desired balance between false positives and false negatives. In this study, the goal was to compare sample concentrations over time in response to a DPE gradient in the diet. In this case, valuable information about trends over time may be lost by incorrectly screening out low data values (e.g. Day 0 concentrations) (i.e. making a Type II error). By contrast, Type I errors may be of little consequence since incorrectly retaining low data values will add variability to the time trends, but are unlikely to produce spurious trends. Thus, a lower screening criterion (a lower MRL) was considered appropriate to retain more low values in the data set.

For this study, data blank-correction was assumed to adequately remove background contamination from the reported data. To minimize the chance of Type II errors, no additional MRL screening was done on the blank-corrected data.

For interest, the overall MRLs (ng/sample) and matrix specific MRLs (ng/g sample) are presented in **Tables 7 & 8**, and are plotted at Day 32 in **Figures 8, 9,12 and 13**.

2.3.3 Statistical analyses

Statistics were calculated using JMP IN 4.0 software (Sall, Lehman et al. 2001). For DPEs, one-sided t-tests were used to detect significant uptake into fish tissues (Day 0 vs the mean uptake phase tissue concentrations). Regression analyses were used to test for significant uptake of PCBs, (testing ß= 0 across the linear part of the uptake phase), and for detecting significant elimination from fish tissues (testing ß = 0 across the elimination phase). The mean losses of PCBs and DPEs in the gastro-intestinal tract (in the stomach and in the intestine) were tested across all congeners using ANOVAs. Statistical significance was then verified using the Tukey Kramer Honestly Significant Difference test (HSD) to adjust for multiple comparisons. All concentration data were log transformed prior to statistical analyses to stabilize variances.

3. RESULTS & DISCUSSION

The results & discussion section is divided into 3 parts describing: (i) a description of PCB and DPE trends over time, (ii) evidence for DPE gut metabolism, and (iii) a comparison of DPE and MPEs in all matrices.

The mean wet weight PCB, DPE and MPE concentrations (ng/g) over time are reported in the **Appendix**. Note that DPEs and MPEs should be compared as molar concentrations (e.g. nmol/g) because of differences in molecular weights.

3.1 PCB & DPE trends over time

Figure 8 shows the measured concentrations of PCBs and DPEs over time in all matrices (food, stomach, intestine, liver and muscle). Concentrations are shown in units of ng/g lipid to highlight thermodynamic gradients between the gastro-intestinal tract (stomach and intestines) and the internal tissues (liver and muscle). Mean wet weight concentrations are summarized in the **Appendix (Tables 17-25, 35-43)**.

3.1.1 PCBs in the gastro-intestinal tract (GIT)

PCB concentrations increased approximately 10,000 fold between the control food (Day 0) and the experimental food (uptake phase, Days 2-14) (**Figure 8**). In both the stomach and intestine, PCB concentrations increased significantly during the uptake phase in response to the experimental food (t-test testing Day 0 vs the mean stomach or intestinal concentrations across the uptake phase, $p<0.05$). During elimination, the gut concentrations did not return to background levels, suggesting that PCBs were being eliminated from the fish tissues back into the gastro-intestinal tract (fecal elimination).

In the gut, mean PCB concentrations across the uptake phase dropped between the food, the stomach and the intestine, ie. $C_d > C_s > C_i$ (**Figure 8**). PCB concentrations dropped approximately 3 fold between the food and the stomach, and a further 1-2 fold between the stomach and intestine. Since PCBs are not expected to metabolize in the fish gut, these concentration drops can be attributed to PCB dietary absorption as food moves along the GIT. ($C_s < C_d$ indicates absorption from the stomach, and $C_i < C_s$ indicates absorption from the intestine). These data therefore suggest that PCBs are absorbed from both the stomach and the intestine of Staghorn sculpin.

3.1.2 PCB gut to tissue gradients

The thermodynamic potential for dietary uptake can also be observed in **Figure 8**. Chemicals diffuse across the gut wall in response to thermodynamic gradients between the gut contents and the internal tissues. These gradients are created by a combination of food digestion and food absorption, which increases the lipid normalized concentration (an indirect measure of chemical activity) in the gut as food moves along the GIT. This 'gastro-intestinal magnification' provides the potential for diffusive flux across the gut wall (dietary uptake). This mechanism is believed to explain how persistent organic pollutants such as PCBs are able to increase in lipid normalized concentration (i.e. biomagnify) at each step of the food web (Connolly and Pedersen 1988). Higher lipid normalized concentrations in the gut than in the internal fish tissues therefore indicate the potential for dietary uptake.

For all three PCBs, gut concentrations exceeded tissue concentrations across the uptake phase (both C_s and $C_i > C_L$ and C_m), indicating the presence of diffusion gradients between the gut and the internal tissues of the fish (**Figure 8**). PCB absorption across the gut wall is therefore expected to occur.

3.1.3 PCB uptake and elimination in sculpin tissues

Figure 8 shows that PCB concentrations in the liver and muscle increased approximately 1,000-10,000 fold across the uptake phase (i.e. from Day 0 to Day 14). On a normal scale (**Figure 8** is on a log scale), tissue concentrations are expected to increase linearly at the beginning of the uptake phase (when $C_b(k_2+k_e+k_m)$ is low, see **equation 1**) and then curve down to reach an asymptote, the steady state tissue concentration (when dietary uptake = elimination, and the net flux into the fish is zero). The regression slope (ß) across the linear portion of the uptake phase is a measure of the chemical flux into each tissue (e.g. $C_b/t = k_d.C_d$, in ng/g fish.day). These slopes can be tested statistically to determine if the observed uptake is statistically different from zero. In a dietary experiment, non-zero slopes indicate significant uptake via the diet.

For PCBs, the uptake slopes (on a normal scale) were linear across the entire uptake phase. The uptake slopes into muscle and liver (Day 0-14) were all statistically different from zero (ß=0, $p<0.05$), indicating that significant dietary uptake for PCBs occurred

during the experiment. PCB uptake fluxes (ng/g lipid.day) into the liver and the muscle are summarized in **Tables 10 & 11**.

During the elimination phase, tissue concentrations are expected to decline linearly on a logarithmic scale, representing the sum of gill elimination (k_2), fecal egestion (k_e), and metabolism (k_m, for metabolized chemicals), (**Equation 1**). The total elimination rate ($k_{e,tot} = k_2+k_e+k_m$) can be measured as the negative slope of the regression across the elimination phase on a logarithmic scale (e.g. dC_L/dt, from Day 14-24). Significant elimination occurs if the slope of this line is statistically different from zero. The PCB total elimination rate constants from sculpin liver & muscle are shown in **Tables 10 & 11**. Elimination rate constants ranged from 0.002 to 0.05 (1/day) in the liver and 0.07-0.09 (1/day) in the muscle. For all three PCBs, the elimination slopes were not statistically different from zero (ß=0, p>0.05), suggesting that PCBs are eliminated relatively slowly from sculpin tissues. However, the muscle elimination rates observed in this study are somewhat higher than the whole body elimination rates reported previously in guppies ($k_{e,tot}$ = 0.005 – 0.016 1/day for PCBs 209 and 52 respectively) (Gobas, Clark et al. 1989).

Since PCB tissue concentrations did not exceed food concentrations at the end of the uptake phase (C_L and $C_m < C_d$ at Day 14), 'true' biomagnification of PCBs was not observed during this experiment. However, tissue concentrations were still increasing at Day 14, indicating that the experiment was too short for fish to reach steady state with PCB levels the experimental food. The expected steady state PCB tissue concentrations were calculated by dividing the observed uptake fluxes by the elimination rate constants (see **Equation 3**). PCB biomagnification factors (BMFs) were then estimated by dividing the tissue concentration at steady state by the PCB concentration in the dry diet (C_L/C_d and C_m/C_d), on a lipid weight basis. BMFs > 1 indicate biomagnification. The values used for these calculations are summarized in **Tables 10 & 11**.

Tables 10 & 11 show that the estimated BMFs for PCBs are all < 1, except for PCB 155 in the liver. These results are surprising, since many PCB congeners are well known to biomagnify in fish (Oliver and Niimi 1988). The relatively high elimination rate constants and low BMFs calculated for PCBs in this study may suggest that Staghorn sculpin have the ability to metabolize PCBs to some extent. Metabolism of PCBs 101, 110 and 149,

and metabolism of up to 10% of the total PCB body burden has been measured previously in Deepwater sculpin (Stapleton, Letcher et al. 2001).

Although PCBs did not biomagnify in this experiment, statistically significant dietary uptake was observed for all three PCBs into the internal tissues of the sculpin. Thus, the PCBs served as positive control for chemical uptake via the diet.

3.1.4 DPEs in the gastro-intestinal tract (GIT)

DPE concentrations over time in all matrices (ng/g lipid) are also shown in **Figure 8**. DPE food concentrations increased significantly (approximately 10-100 fold) between the control food (at Day 0) and the experimental food (administered during the uptake phase) (t-test, $p<0.05$). Food concentrations increased the least (approximately 10 fold) for DnBP and DEHP, due to high background levels in the control food (**Appendix X**). In the stomach, all DPEs except DEHP increased significantly above background levels during the uptake phase (using a t-test to test Day 0 vs the mean C_s across the uptake phase, $p<0.05$), indicating that DPE stomach concentrations increased in response to DPE levels in the experimental food. DPE stomach concentrations decreased to background levels during the elimination phase.

By contrast, DPE concentrations in the intestine remained virtually constant over time, despite significant changes in food concentration at Day 0 and Day 15. For all DPE congeners, C_i during the uptake phase was not statistically different from the Day 0 intestinal concentrations (t-test, $p>0.05$). Increasing DPE concentrations in the experimental food therefore had no measurable effect on DPE concentrations in the intestine. This observation suggests that virtually all of the ingested DPEs are removed from the gut before reaching the feces (intestine).

As discussed above for PCBs, the mean lipid normalized DPE concentrations during the uptake phase dropped between the food, the stomach and the intestine ($C_d > C_s > C_i$) (**Figure 8**). For all DPEs, concentrations decreased approximately 4-8 fold between the food and the stomach, and a further 4-150 fold in the intestine. For all DPE congeners, the greatest concentration drop occurred between the stomach and the intestine. For most DPEs, these concentration decreases along the GIT were substantially greater than those observed for PCBs. This suggests that a process in addition to dietary

absorption is reducing DPE concentrations in the sculpin gut. For DPEs, chemical loss along the GIT is believed to reflect a combination of dietary absorption and gut metabolism. The differences between PCB and DPE 'losses' along the GIT are examined more closely below.

3.1.5 DPE gut to tissue gradients

As seen for PCBs above, DPE concentrations during the uptake phase were higher in the gastro-intestinal tract than in the internal tissues (C_s and C_i > C_L and C_m) (**Figure 8**). These patterns indicate that thermodynamic gradients exist between the fish gut and the internal tissues for all DPE congeners. For DPEs, these gradients were substantially smaller in the intestine than in the stomach because of the comparatively low DPE intestinal concentrations. These gut to tissue gradients suggest that DPE dietary uptake is expected to occur, despite the substantial DPE losses observed along the sculpin gastro-intestinal tract.

3.1.6 Evidence for DPE biomagnification

Unlike PCBs, DPE tissue concentrations increased very slightly, if at all, over the uptake phase (**Figure 8**). For the purpose of this analysis, DPE steady state concentrations in the liver and muscle were estimated as the mean concentrations across the uptake phase (Day 2-14). Between Day 0 and steady state, DPE concentrations increased up to 7 fold in the muscle (DnOP) and up to 2 fold in the liver (DMP and DnOP). Tissue concentrations therefore increased substantially less for DPEs (<10 fold) than for PCBs (up to 10,000 fold) during this experiment. DPE uptake & elimination from fish tissues is discussed in more detail below.

For all DPE congeners, tissue concentrations across the uptake phase remained approximately 2-3 orders of magnitude below the food concentrations. Biomagnifcation factors (BMFs) for liver and muscle were calculated by dividing the mean lipid concentration across the uptake phase (an estimate of steady state concentrations) by the concentration in the diet (e.g. $BMF_m = C_m/C_d$). DPE and PCB BMFs in the sculpin liver and muscle are summarized in **Table 12**. DPE BMFs ranged from 0.0002 (DnOP in liver) to 0.01 (DnBP in muscle), and are substantially smaller than the BMFs estimated

for PCBs. Since the BMFs of DPEs are much smaller than 1, DPEs do not appear to biomagnify in Staghorn sculpin.

This conclusion supports evidence from a recent field study, which found no DPE biomagnification in an urban marine food web (Mackintosh, Maldonado et al. 2003). The results from these two studies (lab & field) provide strong evidence that DPEs do not accumulate in marine organisms as a result of exposure in the diet. This finding is an important contribution to classifying DPEs under the existing bioaccumulation criterion ('B') in Canadian law (CEPA 1999). However, the lack of DPE biomagnification does not imply a lack of dietary uptake. This distinction is discussed below.

3.1.7 DPE uptake into sculpin tissues

DPE concentrations in liver and muscle tissue (ng/g lipid) +/- 1 SD are shown in **Figure 9**. The vertical lines at Day 14 and day 28 mark the end of the uptake phase and the elimination phase, respectively. Water uptake control fish are plotted at Day 30, and the MRL for each tissue is shown at Day 32.

For all DPE congeners (except for DEHP in the muscle), tissue concentrations did not increase significantly between Day 0 and the water uptake control, plotted at Day 30 (t-test, $p>0.05$). Water uptake therefore appears to have been minimal for most DPE congeners.

The observed DPE tissue patterns over time were relatively variable (**Figures 9)**. DMP, BBP and DnOP showed clear increases during some parts of the uptake phase, but either decreased again (e.g. DMP in the muscle), or only increased after a few days of little change (e.g. BBP in the liver). Muscle concentrations of BBP and C10 seemed to increase linearly, and DEP concentrations remained relatively constant over time. These patterns may reflect substantial variability around steady state concentrations, or suggest that the dietary uptake of DPEs is controlled by processes such as enzyme induction (e.g. in the tissues) and/or enzyme saturation (e.g. in the gut).

To determine if the observed tissue increases were statistically significant, Day 0 tissue concentrations (n=3) were tested against the mean concentrations across the uptake phase (n=15). This approach is not ideal since the sample sizes are unbalanced, and concentrations were not constant from Day 2-14. However, this method was considered

appropriate since the linear portion of the uptake phase could not be determined for most DPE congeners. (The linear portion of uptake curves can be tested for significant difference from 0 to indicate significant uptake. This was done for PCBs above). The same approach to detect significant uptake was applied across all DPE congeners.

Using this approach, three DPEs were found to increase significantly above background levels during the uptake phase. DMP, BBP and DnOP increased significantly in the muscle, and DMP and DnOP increased significantly in the liver ($p<0.05$). For all other congeners, differences in tissue concentrations over the uptake phase could not be detected. However, the power to detect statistical differences was low, mainly because of high variability in tissue concentrations across the uptake phase (**Figure 9**). Note that this variability will have been enhanced by omitting the MRL data screening step, which increases the chance of Type II errors (incorrectly retaining low data values in the data set, when they actually represent background contamination). Type II errors may have artificially increased the variability within sample days, and reduced the statistical power to detect differences among sample days. With low power, only strong relationships will be detected as significantly different (e.g. DMP, DnOP uptake).

Significant (although small) tissue increases demonstrate that dietary uptake occurs for some DPE congeners (i.e. at least for DMP & DnOP). Gut metabolism therefore does not entirely prevent DPE dietary uptake for all DPEs. As suggested by **Equation 3** above, dietary uptake plus rapid elimination (including metabolism in the tissues) can produce low steady state tissue concentrations. The ‘limited’ net dietary uptake of DPEs observed in **Figure 9** may therefore actually reflect higher gross dietary uptake than is originally evident, provided that elimination from fish tissues is rapid. DPE elimination from sculpin tissues is discussed below.

3.1.8 DPE elimination

Figure 9 shows two broad patterns for DPE elimination. First, DMP and DEP liver concentrations remained relatively constant across the elimination phase, suggesting very slow elimination from the sculpin liver. However, liver concentrations for both of these DPEs were not raised sufficiently above background levels at Day 14 to be able to measure an elimination slope. Liver elimination rates can therefore not be determined for these congeners.

Secondly, concentrations of DMP, DEP, DnBP, BBP, DEHP in the muscle, and DBP, BBP and DnOP in the liver declined over the first few days (e.g. Days 14-17, or Days 14-19) but reached background levels before the end of the elimination phase. This pattern suggests that DPE elimination from fish tissues is rapid. Elimination rates were calculated for these DPE congeners by regression across the first 2-4 days of elimination, depending on the observed pattern. The number of sample days used in the elimination regression was determined separately for each congener in each tissue (**Table 13**). These elimination rates should be interpreted with caution since confidence in a regression with few data points is relatively low. If elimination is indeed rapid, a shorter elimination phase with more frequent sampling (e.g. every few hours), is required to measure DPE elimination rates accurately. Elimination rates in **Table 13** are minimum estimates, since the sampling design of this experiment may have been too 'coarse' to detect rapid declines over the first few days.

Total DPE elimination rates (ke_{tot}) ranged from 0.10–0.43 in the muscle, and 0.19-0.38 in the liver (**Table 13**), corresponding to half lives of 1.62-6.93 days in the muscle and 1.84-3.65 days in the liver ($t_{1/2} = 0.693/ke_{tot}$). These estimates are similar to DPE elimination rates reported in the literature for rat tissues (DEHP half life = 1-5 days) (Daniel and Bratt 1974).

Of all the DPE congeners, only DnBP and BBP in the liver had elimination rates that were statistically different from 0 (testing ß=0, $p<0.05$). However, all DPE elimination rates in **Table 13** (except DEHP in the muscle) are substantially higher than those observed for PCBs, suggesting that DPEs are more rapidly eliminated from the sculpin tissues. The differences in elimination rates between the two groups of compounds will partially reflect differences in Kow (DPE salt water log Kows = 1.8-10, PCB salt water log Kows = 6.1-8.5, **Table 1**). For low Kow DPEs (DMP, DEP), high gill elimination (high k2) may explain why these congeners are eliminated more rapidly than the PCBs. However, for the mid to high Kow DPEs (i.e. congeners with comparable Kows to the PCBs), higher DPE elimination rates may indicate that DPEs are metabolized to a greater extent than PCBs in sculpin tissues.

Qualitatively, the DPE elimination rates in the muscle appear to be greatest at mid Kow (**Table 10**). This may indicate that mid-Kow DPEs are most rapidly metabolized in

sculpin muscle. However, since this pattern reflects a combination of k2, ke and km, this observation cannot be directly attributed to metabolism without further analysis.

3.2 Evidence for gut metabolism

Further evidence for DPE gut metabolism is explored by (i) Comparing the fluxes of PCBs and DPEs through the GIT, (ii) Observing the formation of MPEs in the gut.

3.2.1 PCB vs DPE fluxes in the gastro-intestinal tract (GIT)

Fluxes of PCBs and DPEs through the gastro-intestinal tract were compared to look for indirect evidence of DPE gut metabolism. Chemical is assumed to enter the GIT by ingestion in the diet and leave the GIT either in the feces, by absorption across the gut wall, or by metabolism. Metabolism of PCBs in the gut is assumed not to occur.

Weight specific chemical fluxes (N, in ng/g fish.day for PCBs, and nmol/g fish.day) were calculated for the ingested diet (N_d), the stomach (N_s) and the intestine (feces) (N_f), using the following equations:

$N_d = G_{d,dry}.C_{d,dry}$

$N_s = G_{d,wet}.C_s$

$N_f = G_f.C_i$

where $C_{d,dry}$, C_s and C_i are the mean uptake phase concentrations in the dry experimental food, stomach and intestine, respectively (in ng/g matrix), and $G_{d,dry}$, $G_{d,wet}$ and G_f are the dry food feeding rate, the wet food feeding rate and the fecal egestion rate (in g matrix/g fish day), respectively. The derivation of $G_{d,dry}$, $G_{d,wet}$ and G_f are described below.

$G_{d,dry}$ is the amount of dry food consumed per gram fish per day (g dry food/g fish.day). Fish were fed at approximately 1% of body weight (V_b), but consumed only 67% of the administered food. The feeding rate, normalized per gram of fish is thus $G_{d,dry} = V_b*0.01*0.67/V_b = 0.0067$ (g dry food/g fish.day).

$G_{d,wet}$ is the amount of wet food consumed per gram fish per day (g wet food/g fish.day). Ingested dry food expands as it absorbs moisture & digestive fluids along the GIT. The volume of food reaching the stomach is therefore larger than the volume of ingested dry food. A dry to wet food conversion factor, R = 3.7, was calculated from the observed change in moisture content between dry food and stomach contents (**Table 6**). R was then used to calculate the wet food feeding rate: $G_{d,wet} = G_{d,dry}.R$.

G_f is the amount of feces produced per gram fish per day (g feces/g fish.day), i.e. the amount of ingested food that is not absorbed along the GIT. 50% food absorption has been observed in the GIT of Rainbow trout (Gobas, Wilcockson et al. 1999). Assuming similar food absorption in Staghorn sculpin, $G_f = 0.5.G_{d,wet}$.

All fluxes (N) were then expressed as a fraction of the dietary flux (the ingested dose, N_d), to allow for comparisons between PCBs and DPEs. Relative fluxes throughout the GIT (f_d, f_s and f_f) are shown in **Figure 10**, and summarized in **Tables 14 & 15.**

For PCBs, 55-63% of the ingested dose remains in the stomach, and only 8-20% is egested in the feces (i.e. recovered in the intestine) (**Figure 10**). 80-92% of ingested PCB is thus lost along the GIT ($f_d - f_f$), providing a maximum estimate for PCB dietary absorption.

For DPEs, only 21-49% remains in the stomach, and <0.5% (except 1.7% for DnBP) is egested in the feces. More than 99.5% of ingested DPE (98.3% for DnBP) is lost along the length of the GIT. These chemical losses are believed to represent the combined effects of dietary absorption and gut metabolism for DPEs.

The magnitudes of these stomach and intestinal 'losses' were compared for all PCB and DPE congeners using ANOVAs (**Figure 11**). The null hypotheses were: A. the fraction of chemical lost in stomach $(N_d-N_s)/N_d$ is the same across all congeners, and B. the fraction of chemical lost in the intestine $(N_s-N_f)/N_s$ is the same across all congeners. The tips of the mean diamonds boxes represent the 95% confidence intervals for each mean.

Statistically significant differences were verified using the Tukey Kramer Honestly Significant Difference test (HSD) to adjust for multiple comparisons.

Figure 11A shows that significantly more DMP, DEP, DnBP, DEHP and DnOP is lost in the stomach compared to PCBs. Stomach losses of BBP & C10 are lower than the other DPEs congeners, and are not significantly different from those of PCBs. Thus, the amount of DPE in the stomach (for most congeners) is substantially reduced by a process other than dietary absorption. BBP and C10 may be less well metabolized in the stomach compared to other DPEs. **Figure 11B** also shows that significantly more DPE (except DnBP) is lost from the intestine compared to PCBs. This indicates that a process in addition to dietary absorption is reducing DPE concentrations in the intestines. These DPE to PCB comparisons provide indirect evidence for DPE metabolism in the stomach and intestine of Staghorn sculpin.

3.2.2 MPE formation in the GIT

Wet weight concentrations of MPEs in all matrices are summarized in the **Appendix, Tables 26-34**.

MPE concentrations (ng/g lipid) over time are shown in **Figure 12**. MPE metabolites from all of the DPE congeners administered in the diet were recovered in the fish GIT. In general, MPE concentrations in the stomach and intestine increased during the uptake phase and decreased again during elimination, reflecting the changing DPE concentrations in the diet. (GIT patterns are less clear for MMP and MEP than for the other MPE congeners).

Of all the MPEs measured in the GIT, only two congeners (MBP and MEHP) were also detected at background levels in the experimental diet (**Appendix, Table 34**). For both of these congeners, stomach concentrations were clearly elevated (approximately 1 to 2 orders of magnitude) above background levels during the uptake phase. The increase in gut MPE concentrations for all congeners can therefore be attributed to the metabolism of DPEs administered in the diet. The formation of phthalate mono-esters in the stomach & intestine (when none or very low levels were present in the diet) provides *direct* evidence that DPEs from the diet are metabolized in the sculpin GIT.

3.2.3 MPE uptake

MPE tissue concentrations (ng/g lipid) +/- 1 SD are shown in **Figure 13**. The vertical lines at Day 14 and day 28 mark the end of the uptake phase and the elimination phase, respectively. Water uptake control fish are plotted at Day 30, and the MRL for each tissue (when MPEs were measured in the blanks) is shown at Day 32.

Although all reported MPE congeners were measured in the gut, only four MPEs were detected inside the fish. MBP, MEHP and MOP were found in the liver, and MBP, MBzP and MEHP were detected in the muscle (**Figure 13**). All of these MPEs (except MBzP) were detected in the Day 0 fish as well as across the uptake phase, suggesting that MPEs may be present at background levels in wild sculpin. MPE levels in wild fish have not yet been determined, but will be investigated in a future study.

The presence of only the 'mid-Kow' MPEs (i.e. the hydrolysis products of mid-Kow DPEs) in sculpin tissues is not understood. Mid-Kow DPEs may be best absorbed across the gut wall (dietary uptake efficiencies are generally highest at mid Kow), and then metabolized, or these MPEs may be absorbed directly from the gut. These MPEs may also have been absorbed via the gills (or their parent DPEs were absorbed and metabolized). The congener patterns in the tissues may also reflect different elimination rates across MPEs (e.g. lower elimination of mid-Kow MPEs). The reasons for why MBzP and MOP were found in only one of the two internal tissues are also not well understood, but do not appear to result from high instrumental detection limits. These possibilities require further investigation.

Figure 12 shows that for the MPE congeners with available tissue data (MBP, MBzP, MEHP and MOP), a thermodynamic gradient exists between the GIT and the internal tissues (i.e. Cs and Ci > CL and Cm). This demonstrates a potential for dietary uptake of MPEs from both the stomach and intestine of Staghorn sculpin.

However, the observed dietary uptake of MPEs is relatively minimal (**Figure 13**). Only MEHP in the liver was statistically different between Day 0 and the uptake phase (using a t-test to test Day 0 vs the mean uptake phase concentrations, $p < 0.05$). MBP & MEHP in the muscle and MBP in the liver appeared to increase slightly across the uptake phase but differences were not statistically significant. MBzP in the muscle was only

detected on three sample days, and time trends could not be established. MOP may have increased over time in the liver, but confidence in this pattern is low since the Day 0 point for this congener reflects the concentration from only 1 fish (MOP was not detected in the other 2 fish on Day 0), which is well below the MRL.

Thus, despite the diffusion gradients detected between the GIT and internal tissues, the net dietary uptake of MPEs appears to be low. This suggests that either MPEs are poorly absorbed from the sculpin GIT, or that dietary uptake of MPEs is balanced by rapid elimination (e.g. including MPE metabolism to oxidized MPEs and glucuronide conjugates) to maintain low steady state MPE concentrations in the tissues.

3.2.4 MPE elimination

MPE concentrations across the elimination phase exhibited the same two general patterns as observed for DPEs above. Either tissue concentrations remained virtually flat during elimination (e.g. MBP in the liver), or elimination was rapid over only the first few days (e.g. MBP and MEHP in the muscle). MPE elimination rates were calculated by regression across the sample days with declining concentrations (**Table 13, Figure 13**). Elimination rates could not be quantified for MBP in the liver because concentrations did not increase enough during the uptake phase to create an elimination slope. MBzP elimination was not quantified due to a lack of data during the elimination phase. MOP elimination was not calculated because of low confidence in the Day 16 data point, (which represents one fish with a concentration well below the MRL). As with the DPEs, the MPE elimination rates reported in **Table 13** are minimum estimates, since our sampling days were widely spaced compared to the rate of elimination.

MPE elimination rates ranged from 0.18 (MEHP in the liver) to 0.5 (MBP in the muscle), corresponding to half lives of 1.4-3.8 days (**Table 13**). Only MBP elimination from the muscle was significantly different from zero (testing the slope of the regression, ß = 0, $p<0.05$). However, MPE elimination appears to be comparable or faster than DPE elimination, and substantially faster than the elimination of PCBs (**Table 13**). These MPE elimination rates are similar to those previously reported in the literature, with estimated half lives of hours to days (Woodward 1988). Previous studies have also suggested that the hydrolysis of DPEs to MPEs is the rate limiting step in DPE metabolism, and that further MPE metabolism is comparatively faster (Albro and Lavenhar 1989).

The observed patterns of MPEs in the tissues (i.e. no increase across the uptake phase for some congeners, small concentration increases for others) suggest the same two hypotheses as for DPEs, above, i.e. either: 1. MPEs are poorly absorbed across the gut wall, or 2. MPE dietary absorption occurs, but elimination from the tissues (including further metabolism and excretion of MPEs) is rapid. The high elimination rates calculated in **Table 13** above provide evidence that this second hypothesis is possible.

3.2.5 MPEs, pH, and environmental fate

The dietary absorption of MPEs is expected to vary greatly between the stomach and intestine due to pH differences between these two compartments. Phthalate monoesters are weak acids which can be neutral or ionized depending on the pH of the surrounding environment. Since only neutral compounds are expected to be able to cross biological membranes (Gibson and Skett 1986; Rozman and Klassen 1996), only the neutral fraction of MPEs are expected to be able to cross the gut wall. The percent dissociation of an acid at a given pH can be predicted by the Henderson Hasselbach equation:

$$\log (A^-/HA) = pH - pKa$$

where A- is the ionized form of the acid, HA is the protonated (neutral) form of the acid, and pKa is the negative log of the dissociation constant, Ka, for a given chemical (Rozman and Klassen 1996).

MPE pKa's have not yet been published. Methods to calculate pKa exist in the literature for meta and para-substituted benzoic acids (Perrin, Dempsey et al. 1981), but not for ortho-substituted benzoic acids such as MPEs. In the absence of a better method, the SPARC online calculator was used to estimate the pKa of MPEs (SPARC 2001). pKa 4.36 (at 10C) was used to calculate the % of MPE ionized in different tissues. The estimated pH of different tissues, and the % neutral and ionized MPE are presented in **Table 16.**

Table 16 demonstrates that most of the MPE in the stomach is neutral (99.6%), whereas only 6.7% is neutral the intestine. Thus, the dietary absorption of MPEs is expected to occur primarily in the stomach.

3.3 DPEs vs MPEs in all matrices

The fractions of DPEs and MPEs measured in the sculpin stomach, intestine, liver & muscle (means across the uptake phase) are shown in **Figure 14**. DnBP and BBP are grouped together to avoid having to divide the observed MBP concentrations between these two congeners. (Both DnBP and BBP can be metabolized to MBP).

Figure 14 illustrates that MPEs from all of the DPEs administered in the diet are recovered in the sculpin stomach and intestine. In the stomach, up to 65% of total PE is recovered as MPE. The fraction of MPEs appears to vary across Kow: the % MPE increases from 31% for DMP to approximately 65% for DnBP+BBP and DEHP, and then decreases to 13% for C10. This pattern suggests that mid Kow DPEs may be metabolized to the greatest extent in the sculpin stomach (except for BBP, see **Figure 11A** above).

The MPE:DPE ratio shifts dramatically between the stomach and the intestine. Up to 99% of the total PE in the intestine is in the MPE form. This pattern reinforces previous evidence that DPEs are extensively metabolized in sculpin intestine, leaving very low levels of intact DPE in the intestine.

Figure 14 also illustrates that although all MPEs were detected in the GIT, only four MPE congeners were recovered in fish tissues. MBP, MEHP and MOP were measured in the liver, and MBP, MBzP (grouped together with MBP) and MEHP were found in the muscle. For these congeners, MPEs made up 29-66% and 52-61% of the total PE in the liver and muscle, respectively.

These tissue fractions are an estimate of the MPE:total PE body burdens in Staghorn sculpin at steady state (i.e. the mean ratio across the uptake phase). If these ratios are ‘real’, the same fractions of MPE:total PE are expected to be found in wild Staghorn sculpin. Since the methods to analyze MPEs have only recently been developed, MPE levels in wild biota have not yet been determined. **Figure 14** raises the hypothesis that MPEs may be present at approximately equal concentrations as DPEs in wild fish. If this is true, the steady state body burden of ‘total’ PEs (DPEs + MPEs) may be twice as high as predicted by the DPE levels alone. This hypothesis will be investigated further in a future study.

4. CONCLUSIONS

The following conclusions can be drawn from the observed PCB, DPE and MPE data:

1. DPEs are extensively metabolized in the stomach and intestine of Staghorn sculpin. Gut metabolism reduces the pool of DPEs available for uptake across the gut wall, but creates a pool of MPEs which may be absorbed from the GIT. The fraction of MPE found in the gut contents increases as food moves along the GIT.
2. DPEs do not biomagnify in Staghorn sculpin (BMFs for DPEs are much less than 1). This supports the conclusion from a recent field study which found no evidence of DPE biomagnification in a marine food web. Gut metabolism appears to play a large role in preventing DPE accumulation via the diet, and may explain the lack of biomagnification observed in the field.
3. Thermodynamic gradients exist between the gut (stomach and intestine) & tissues (liver and muscle) for both DPEs and MPEs. These gradients demonstrate the potential for diffusive flux into the fish. Thus, despite gut metabolism, dietary uptake of DPEs and MPEs is still expected to occur.
4. Limited dietary uptake was measured for a few DPE and MPE congeners. Tissue concentrations increased significantly between Day 0 and the uptake phase for DMP (liver & muscle), BBP (muscle only), DnOP (liver and muscle) and MEHP (liver only). All other DPE and MPEs remained relatively constant across the uptake phase. The tissue profiles for both DPEs and MPEs suggest that either:
    a. DPEs and/or MPEs are poorly absorbed across the gut wall, or
    b. DPEs and/or MPEs are absorbed across the gut wall, but are rapidly eliminated from the fish
5. Minimum estimates of tissue elimination rates were determined for DMP, DEP, DnBP, BBP, DEHP, MBP and MEHP in the muscle, and for DnBP, BBP, DnOP and MEHP in the liver. In general, elimination rates for DPEs and MPEs are higher than those found for PCBs, suggesting that PEs are more rapidly eliminated from sculpin tissues (e.g. by tissue metabolism)
6. Steady state MPE & DPE concentrations in sculpin tissues (especially in the muscle) are approximately equal. If similar patterns exist in wild fish, total PE body burdens (DPEs + MPEs) may be twice as high as currently predicted by DPE concentrations alone. This hypothesis will be investigated in a future study.

This study presents an overview of the results from a dietary uptake experiment of DPEs and PCBs in Staghorn sculpin (Leptocottus armatus). A complete analysis of the data has not been possible within the scope of this project. Ongoing work will further quantify the dietary uptake kinetics of DPEs and MPEs. A mathematical model will also be built to better understand the fate of ingested DPEs and the resulting MPEs in Staghorn sculpin.

4.1 Implications for exposure & toxicity

The combined evidence from this study and a recent field study (Mackintosh, Maldonado et al. 2003) demonstrates that phthalate di-esters do not biomagnify in aquatic organisms. Thus, DPE tissue accumulation via the diet does not occur.

However, a lack of DPE biomagnification does not imply a lack of dietary uptake. DPE and MPE fluxes across the gut wall, balanced by elimination from the fish (including tissue metabolism) may explain the limited uptake observed for some DPE and MPE congeners in this study. In this case, the ‘gross’ dietary flux across the gut wall (including both DPEs and MPEs) may be substantially higher than is initially evident from the steady state concentrations. Given that DPEs are found at low levels in wild biota, it is possible that predators may be exposed to constant, low level fluxes of some DPEs and MPEs via their prey. This may or may not be of toxicological significance.

This idea raises the question about how to define ‘relevant exposure’. Under current regulatory paradigms (which focus on bioaccumulation), the ‘standing stock’ of chemical within an organism is believed to represent the internal dose. However, it is also possible that the flux through an organism (e.g. gross dietary uptake + elimination) is a measure of the relevant dose for some modes of toxic action (e.g. endocrine disruption).This idea requires further investigation.

4.2 Study limitations

The interpretation of the data from this study is limited by the following factors:

Many of the DPE data, especially on Day 0 and during the elimination phase, were close to the levels found in the blanks. This was partly the result of having small samples (e.g. intestinal samples) with low concentrations. Future studies should consider using larger fish (e.g. to increase the amount of intestinal sample), or pooling several fish together to increase the weight of low concentration samples. Increasing the DPE dose in the diet (i.e. increasing sample concentrations) may also reduce this problem. Using deuterated DPEs rather than 'native' DPEs would also substantially reduce the problems with background contamination.

This experiment did not measure concentrations of MPE metabolites (e.g. oxidized MPEs, glucuronides, and phthalic acid). Without this information, a complete mass balance analysis is not possible.

4.3 Major contributions of this study

This experiment helps to fill a number of data gaps in the literature. This study:

1. Provides dietary uptake data for DPEs, PCBs and MPEs in a marine fish species. Few DPE dietary uptake studies exist in the literature for fish, especially for marine species
2. Examines the fate of a wide range of DPE congeners. Existing studies have focused mainly DnBP and DEHP, but data for the other DPE congeners are sparse
3. Achieved low DPE levels in the blanks compared to other laboratories around the world
4. Measured the occurrence of DPE gut metabolism *in vivo* in fish. Gut metabolism has been identified previously in mammals but not in aquatic organisms
5. Applies a newly developed extraction method to measure MPEs directly in biological tissue
6. Has generated a comprehensive data set for future analysis

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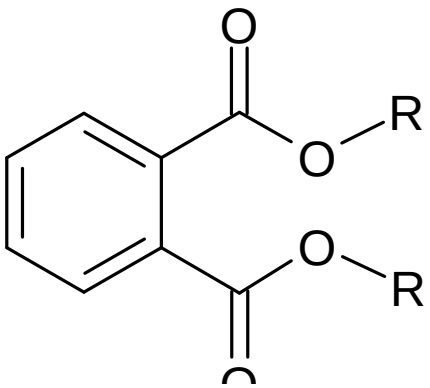
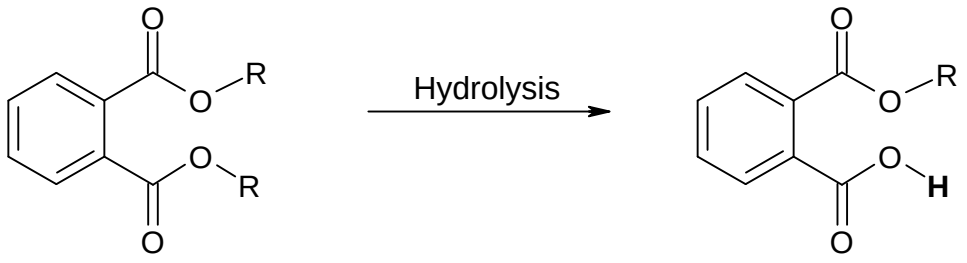


Figure 1. Generalized phthalate di-ester chemical structure. Different DPE congeners vary in the length and branching patterns of the alkyl chains (R and R')

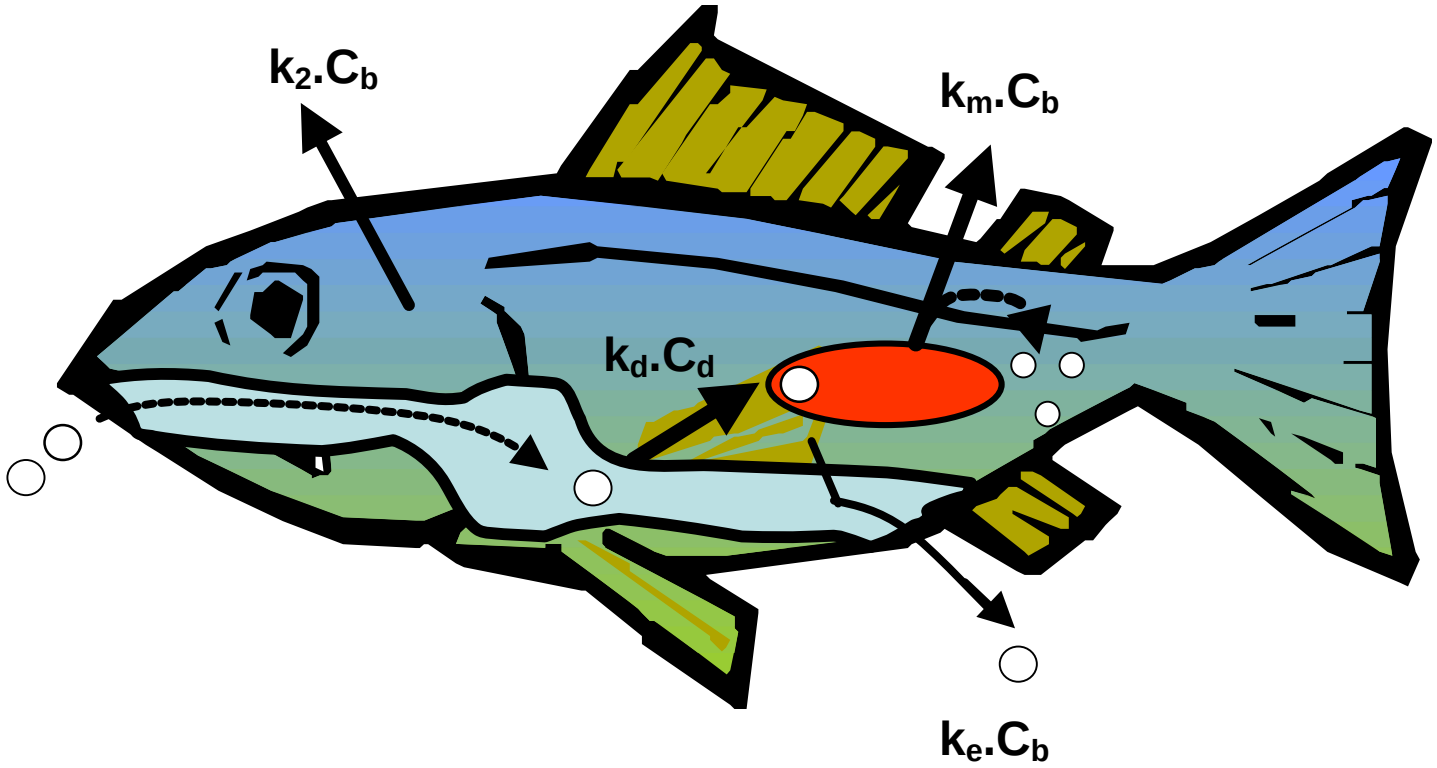
Table 1. Identification and select chemical properties (at 25°C) of phthalate ester and PCB congeners added to the experimental food. Phthalate ester properties are from (Cousins and Mackay 2000). PCB properties are from (Hawker and Connell 1988). Salt water log Kows are adjusted for decreased solubility in salt water according to (Xie, Shiu et al. 1997)

Congener Abbrev.	Congener Name	Mol. wt (g/mol)	Log Kow	Log Kow (salt water)
DMP	Dimethyl phthalate	194.2	1.61	1.80
DEP	Diethyl phthalate	222.2	2.54	2.77
DnBP	Di-n-butyl phthalate	278.4	4.27	4.58
BBP	Butyl benzyl phthalate	312.4	4.70	5.03
DEHP	Di-2-ethylhexyl phthalate	390.6	7.73	8.20
DnOP	Di-n-octyl phthalate	390.6	7.73	8.20
DiDP (C10)	Di-iso-decyl phthalate	446.7	9.46	10.01
PCB 52	2,2',5,5' tetra CB	293.0	5.84	6.08
PCB 155	2,2',4,4',6,6' hexa CB	360.9	7.18	7.46
PCB 209	2,2',3,3',4,4',5,5'6,6' deca CB	498.7	8.18	8.53



Phthalate Di-ester **Phthalate Mono-ester**

Figure 2. First step in DPE metabolism. The enzymatic cleavage of one ester bond yields a mono-ester (MPE) + an alcohol. MPEs are further oxidized, glucuronidated and excreted in the urine & bile in many species



At steady state, $C_b = k_d.C_b / (k_2+k_e+k_m)$

Figure 3. Conceptual model of dietary uptake & elimination from fish. Chemical uptake occurs by absorption from the diet ($k_d.C_d$). Elimination occurs via the gills ($k_2.C_b$), by fecal excretion back to the gut ($k_e.C_b$), or by metabolism ($k_m.C_b$). k_d, k_2, k_e and k_m are the first order rate constants for each process, and C_d and C_b are the concentrations in the fish and diet, respectively. The equation describes the fish concentration at steady state (C_b). See text for details

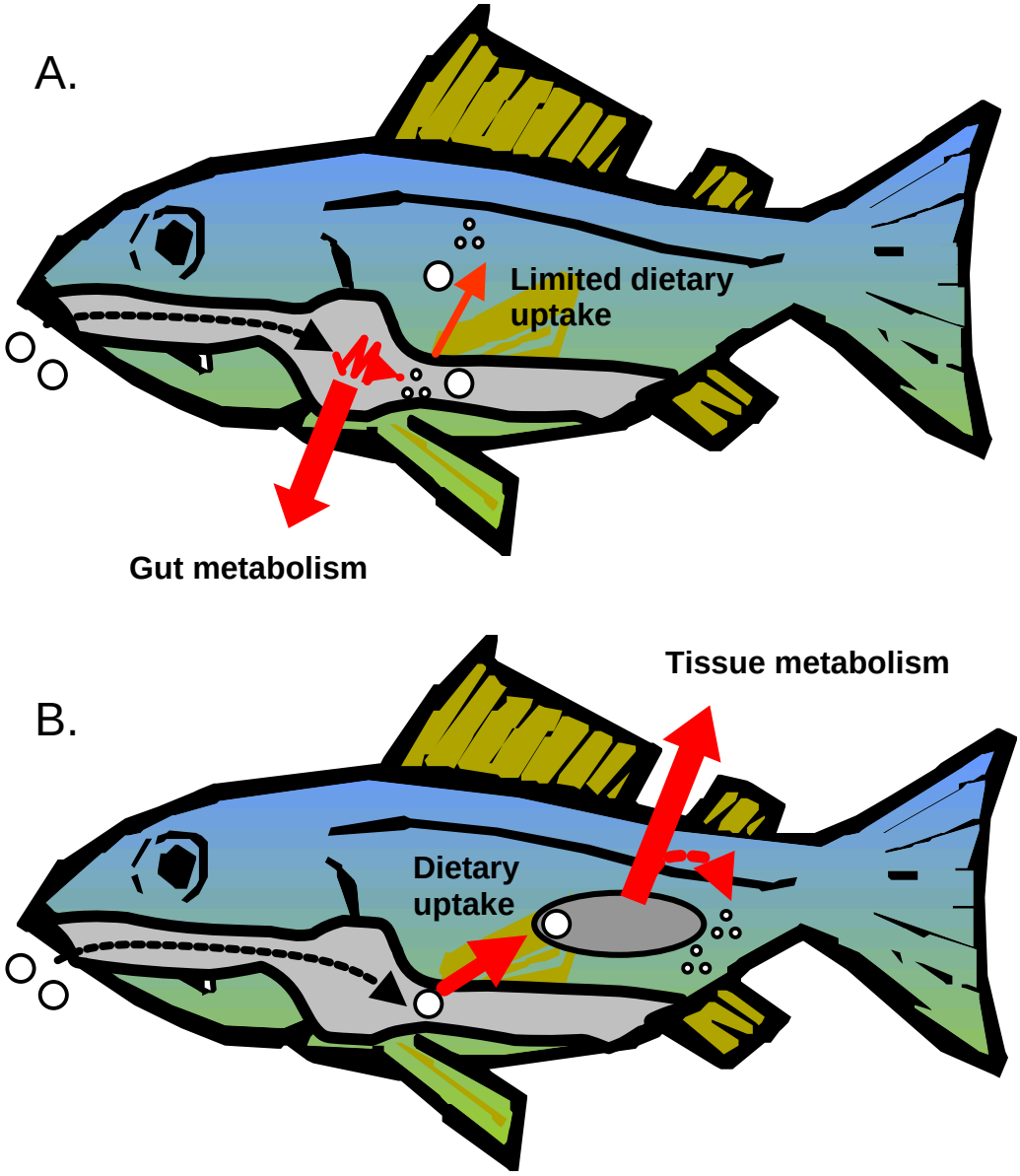


Figure 4. Hypotheses to explain the lack of biomagnification observed in the field. A. Dietary uptake is limited due to gut metabolism. B. Dietary uptake occurs, but DPEs are eliminated from the fish by metabolism in the tissues (e.g. in the liver)

Table 2. Ingredients of the fish food used during this experiment. Food pellets were 3.5mm in size

Ingredient	Wt (g)
Lt Anchovy Meal	1018.98
Blood Flour	101.92
Squid Meal	142.94
Krill Meal	200.02
Wheat Gluten Meal	141.68
Vitamin Supplement	37.64
Mineral Supplement	75.28
Soybean Lecithin	18.82
Choline Chloride (60%)	9.40
Vitamin C (Phosphate 42%)	6.72
Per mapell	15.50
Dh-Methananine	4.60
Pregelatinized Wheat	160.02
Total:	**1933.52**

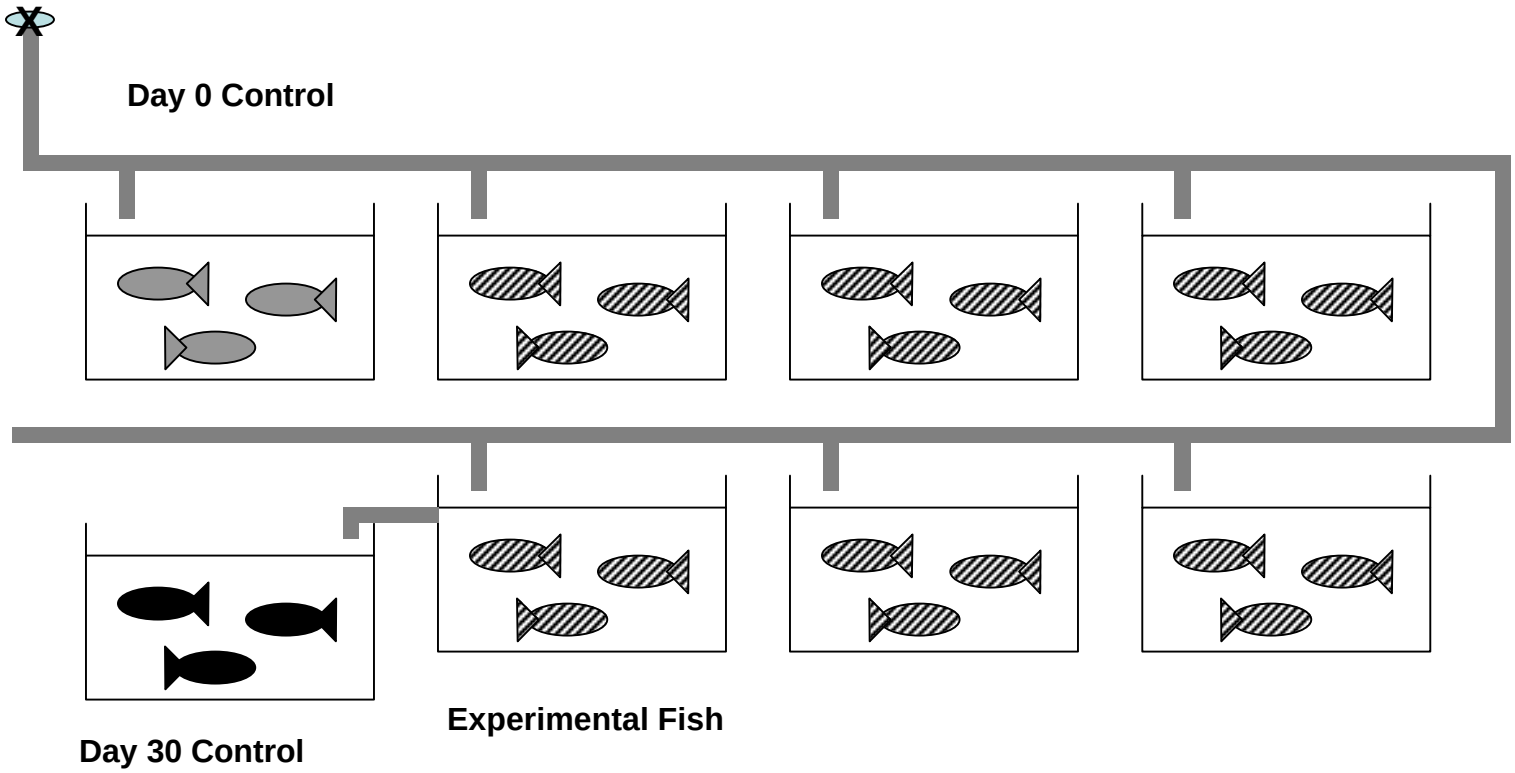


Figure 5. Experimental set-up. Experimental fish received PCB & DPE spiked experimental food for 14 days (the uptake phase), & control food for a further 14 days (the elimination phase). (Only 6 of 10 experimental tanks are shown here). Day 0 and Day 30 fish controlled for background contamination and chemical uptake from the water, respectively

Table 3. Mean water chemistry measurements taken throughout the experiment. n=4

Measurement	Units	Mean	St dev
Temperature	°C	13.0	0.6
Dissolved Oxygen	ppm	7.9	0.3
Dissolved Oxygen	% saturation	86	2.8
Nitrite	mg/L	0	-
Ammonia	mg/L	0.1	0.1
pH	-	7.9	-
Salinity	‰	31.3	2.3

Table 4. DPE, PCB and MPE internal standards (IS) added to samples prior to extraction, and recovery standards (RS) added to samples prior to instrumental analysis. Full congener names are given in Tables 1 and 5

Standard	Compounds	Amount added to sample (ng)
PE IS	DMP-d_4, DnBP-d_4, DnOP-d_4	100
PE RS	DEP-d_4, BBP-d_4	50
PCB IS	^{13}C-PCB 52, ^{13}C-PCB 101, ^{13}C-PCB 128, ^{13}C-PCB 180, ^{13}C-PCB 194, ^{13}C-PCB 208, ^{13}C-PCB 209 ^{13}C-PCB 28, ^{13}C-PCB 105, ^{13}C-PCB 118, ^{13}C-PCB 156 ^{13}C-PCB 15, , d_5-PCB 38, ^{13}C-PCB 77, ^{13}C-PCB 126, ^{13}C-PCB 169	ca. 1
PCB RS	^{13}C-PCB 111	ca. 2
MPE IS	$^{13}C_2$-MBP and $^{13}C_2$-MEHP	600
MPE RS	$^{13}C_2$-MiNP	600

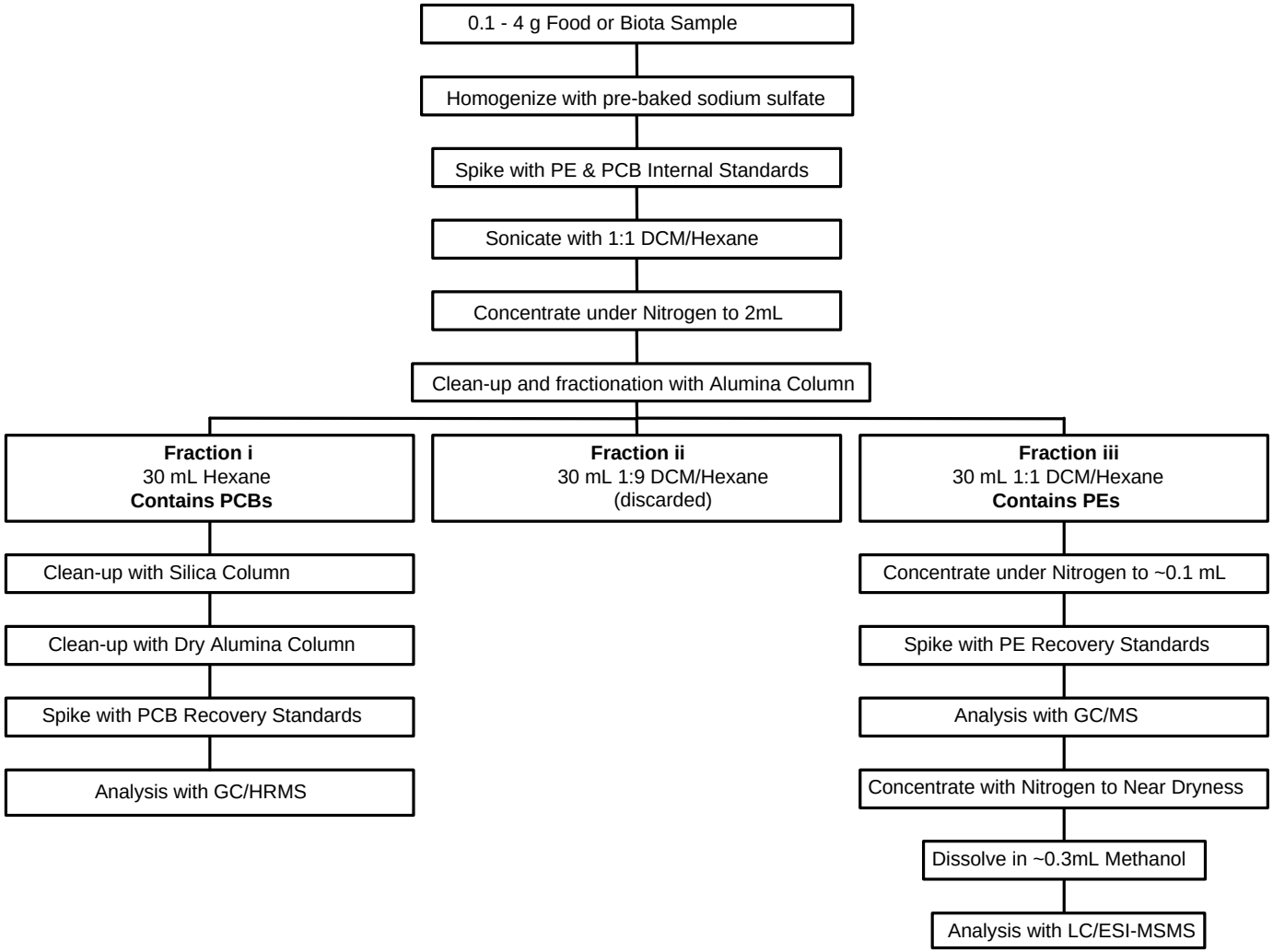


Figure 6. DPE & PCB extraction, cleanup & analysis in food and biota samples. Details are reported in (Lin, Ikonomou et al. 2003)

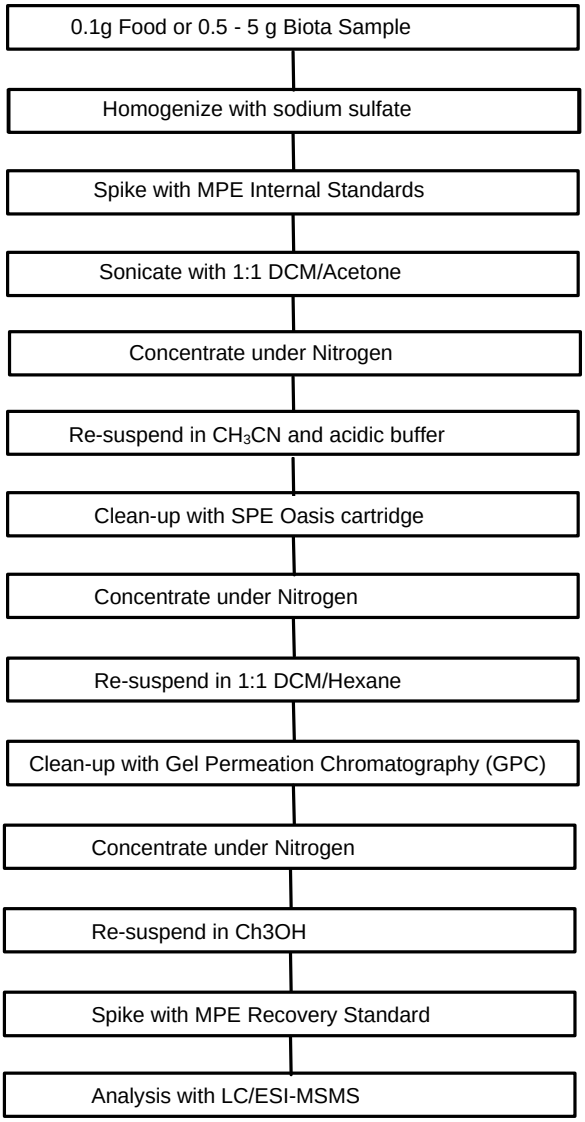


Figure 7. Summary of MPE extraction, cleanup and analysis in food and biota samples. Details will be reported in (Ikonomou, Hoover et al. 2003)

Table 5. MPE congeners analyzed in this study. Molecular weights and the parent DPE(s) are also shown. MBP can be produced by the hydrolysis of both DnBP and BBP

MPE	Congener Name	Mol. wt (g/mol)	Parent DPE
MMP	Mono-methyl phthalate	180.160	DMP
MEP	Mono-ethyl phthalate	194.187	DEP
MBP	Mono-butyl phthalate	222.240	DnBP, BBP
MBzP	Mono-benzyl phthalate	256.257	BBP
MEHP	Mono-ethylhexyl phthalate	278.347	DEHP
MOP	Mono-octyl phthalate	278.347	DnOP
MoC10	Mono-decyl phthalate	306.401	C10

Table 6. Mean lipid and moisture composition (% of wet wt +/- 1 standard deviation) of the food, stomach contents, intestinal contents, liver and muscle tissues

Matrix	% Lipid	% Moisture
Food	7.54 +/- 0.12	88.65 +/- 0.09
Stomach contents	3.49 +/- 1.06	22.53 +/- 4.49
Intestinal contents	1.91 +/- 0.59	13.22 +/- 0.76
Liver	30.89 +/- 4.96	18.58 +/- 12.88
Muscle	1.44 +/- 0.96	20.48 +/- 0.88

Table 7. Mean blank amounts (ng) in sodium sulfate blanks, 3SD (overall MRL in ng), and matrix specific MRLs (wet weight) for DPEs & PCBs. These MRLs would be used to screen blank-corrected data. For each matrix, MRL = 3SD of blanks (ng) / mean sample weight (g). See text for discussion of the MRL. PCB and DPE MRLs are plotted at Day 32 in Figures 8 and 9

			Matrix				
			Food	Stomach	Intestine	Liver	Muscle
Mean Sample weight (g)			0.08	0.31	0.47	0.50	4.39
DPE / PCB	**Mean Blank amt (ng)**	**3SD (ng)**	**MRL[b] (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**
DMP	0.46	0.53	1.19	1.72	1.14	1.07	0.12
DEP	7.76	8.62	1.09	27.99	18.50	17.40	1.96
DnBP	34.86	36.31	450.27	117.95	77.97	73.33	8.27
BBP	5.69	11.46	27.86	37.22	24.60	23.14	2.61
DEHP	8.13	16.38	118.46	53.20	35.17	33.07	3.73
DnOP	0.40	0.36	-	1.16	0.77	0.72	0.08
C10[a]	ND	-	-	-	-	-	-
PCB52	0.037	0.065	0.484	0.212	0.140	0.132	0.015
PCB155	0.019	0.061	0.104	0.200	0.132	0.124	0.014
PCB209	0.005	0.008	0.054	0.026	0.017	0.016	0.002

[b] Food MRLs were calculated using the blanks for only the food samples.

Table 8. Mean blank amounts (ng) in sodium sulfate blanks, 3SD (overall MRL in ng), and matrix specific MRLs (wet weight) for MPEs. MRLs are shown for only three MPEs (MEHP, MOP and MoC10), since these were the only MPE congeners measured in the blanks. These MRLs would be applied to blank-corrected concentrations. See text for discussion of the MRL. MPE MRLs are plotted at Day 32 in Figures 12 and 13

			Matrix				
			Food	Stomach	Intestine	Liver	Muscle
Mean Sample weight (g)			0.07	0.55	0.46	0.54	5.58
MPE	**Mean Blank amt (ng)**	**3SD (ng)**	**MRL (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**	**MRL (ng/g)**
MMP	ND	-	-	-	-	-	-
MEP	ND	-	-	-	-	-	-
MBP	ND	-	-	-	-	-	-
MBzP	ND	-	-	-	-	-	-
MEHP	3.72	9.90	135.64	18.05	21.34	18.24	1.77
MOP	0.92	3.54	48.46	6.45	7.62	6.52	0.63
MoC10	2.45	0.36	4.94	0.66	0.78	0.66	0.06

Table 9. Mean % recoveries (+/- 1 standard deviation) of DPE, MPE and PCB surrogate internal standards across all samples & all sodium sulfate blanks.

Analytical Method	Compounds	Internal Standard	Mean % Recovery Samples	Mean % Recovery Blanks
GC/MS & LC/MSMS	DPEs	DMP-d_4	80 +/- 12	76 +/- 12
		DnBP-d_4	86 +/- 13	89 +/- 9
		DnOP-d_4	73 +/- 30	95 +/- 8
LC/MSMS	MPEs	$^{13}C_2$ MBP	75 +/- 29	44 +/- 24
		$^{13}C_2$ MEHP	63 +/- 26	58 +/- 23
GC/MS	PCBs	^{13}C PCB 52	64 +/- 17	51 +/- 17
		^{13}C PCB 128	84 +/- 16	79 +/- 18

Table 10. Observed dietary uptake fluxes, elimination rates, predicted steady state concentrations, diet concentrations & predicted biomagnification factors (BMFs) for PCBs in the Staghorn sculpin liver

Congener	Flux to liver ng/g lipid.day	$k_{e,tot,L}$ 1/day	C_L ng/g lipid	C_d ng/g lipid	BMF Liver (C_L/C_d)
PCB 52	617	0.05	13156	61804	0.21
PCB 155	717	0.002	297017	95783	3.10
PCB 209	843	0.02	37610	75209	0.50

Table 11. Observed dietary uptake fluxes, elimination rates, predicted steady state concentrations, diet concentrations & predicted biomagnification factors (BMFs) for PCBs in the Staghorn sculpin muscle

	Flux to muscle ng/g lipid.day	$k_{e,tot,m}$ 1/day	C_m ng/g lipid	C_d ng/g lipid	BMF Muscle (C_m/C_d)
PCB 52	283	0.08	3656	61804	0.06
PCB 155	446	0.09	5033	95783	0.05
PCB 209	332	0.07	4585	75209	0.06

Table 12. Estimated biomagnification factors (BMFs) for PCBs and DPEs in sculpin liver and muscle. BMF muscle = C_m/C_d, and BMF liver = C_L/C_d (using lipid normalized concentrations). BMFs are substantially smaller for DPEs than for PCBs. BMFs < 1 indicate that compounds do not biomagnify in Staghorn sculpin

Congener	BMF Muscle	BMF Liver
PCB 52	0.06	0.21
PCB 155	0.05	3.10
PCB 209	0.06	0.50
DMP	0.0010	0.0003
DEP	0.0022	0.0012
DnBP	0.0102	0.0044
BBP	0.0018	0.0013
DEHP	0.0032	0.0046
DnOP	0.0004	0.0002
C10	0.0022	

Table 13. Total elimination rate constants (ke_{tot}) for PCBs, DPEs and MPEs in sculpin muscle and liver, estimated half lives ($t_{1/2} = 0.693/ke_{tot}$), and the number of sample days used for the elimination analysis (see Figure 13). Elimination rates could not be estimated for all congeners. The reported elimination rates for DPEs and MPEs are minimum estimates (see text for details)

Congener	$k_{e,tot}$ muscle (1/day)	$k_{e,tot}$ liver (1/day)	$t_{1/2}$ muscle (days)	$t_{1/2}$ liver (days)	n_{mus}	n_{Liv}
PCB 52	0.08	0.05	8.95	14.78	5	5
PCB 155	0.09	0.002	7.82	287.08	5	5
PCB 209	0.07	0.02	9.57	30.92	5	5
DMP	0.18	-	3.79	-	3	-
DEP	0.22	-	3.22	-	3	-
DnBP	0.43	0.22	1.62	3.18	2	4
BBP	0.30	0.38	2.28	1.84	2	4
DEHP	0.10	-	6.93	-	4	-
DnOP	-	0.19	-	3.65	-	4
C10	-	-	-	-	-	-
MBP	0.50	-	1.39			3
MEHP	0.35	0.18	1.96	3.81	3	3
MOP	-	-	-	-	-	

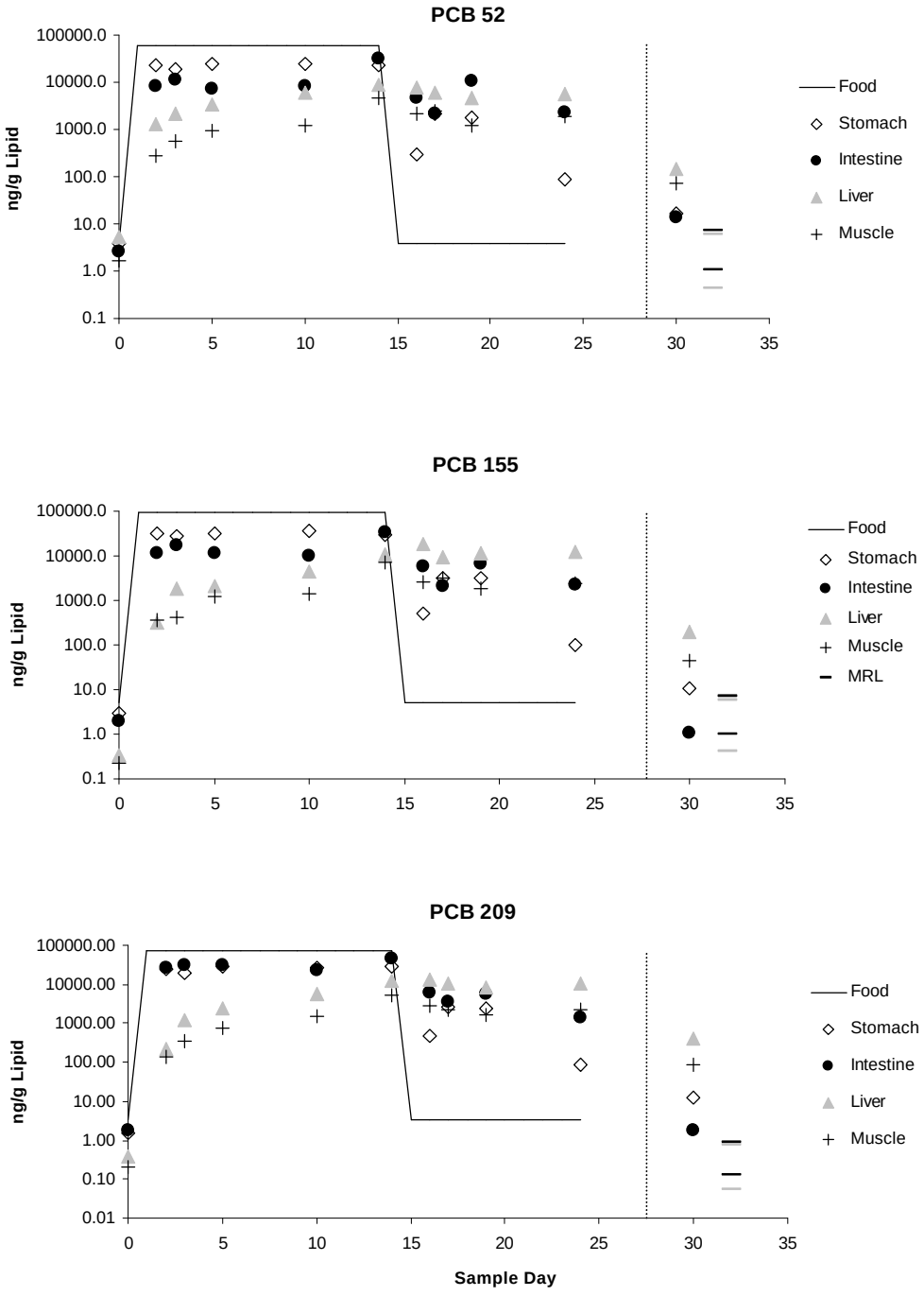
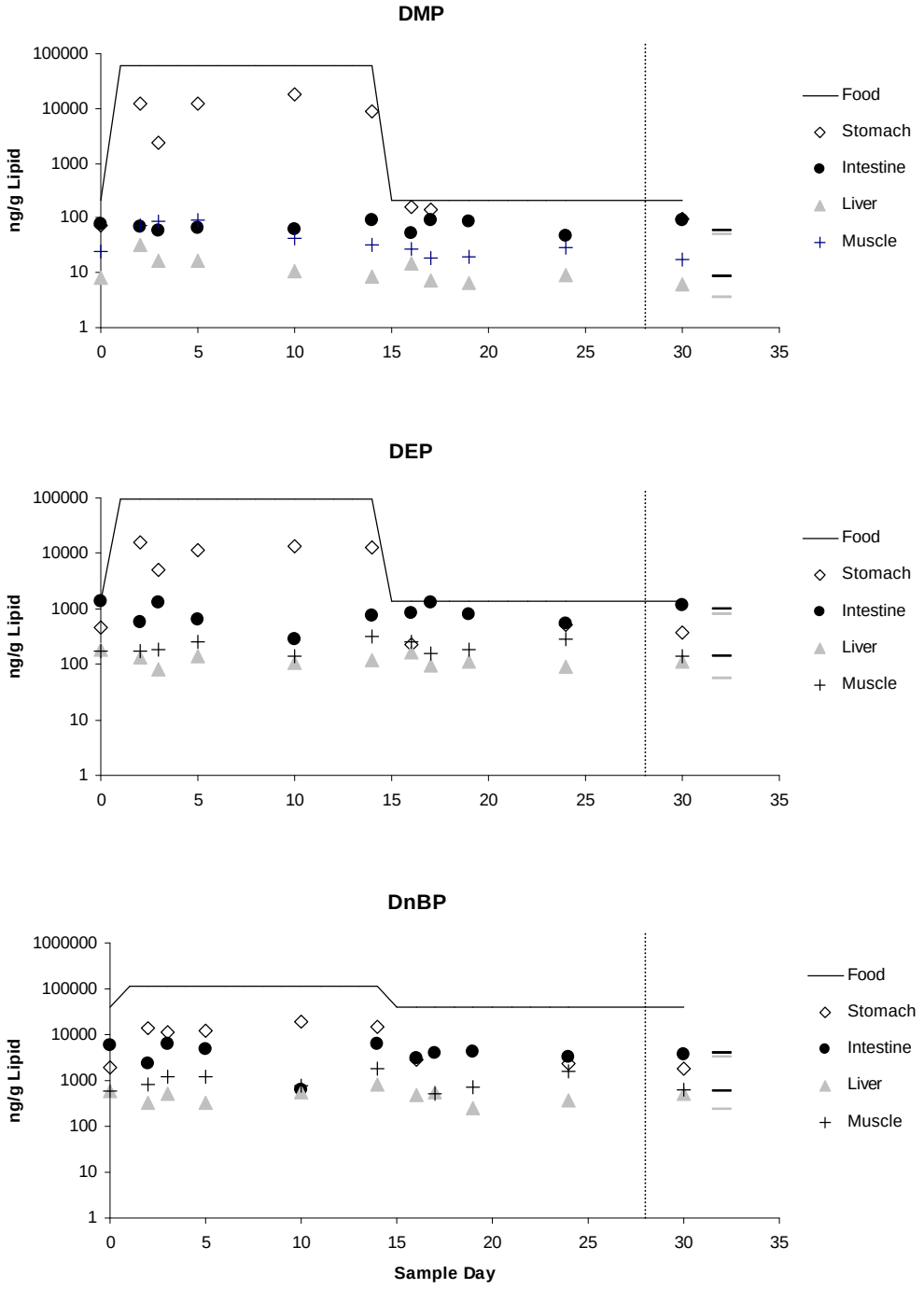


Figure 8. Mean PCB & DPE concentrations in the food, stomach, intestine, liver and muscle tissue over time (ng/g lipid, n=3 per sample day). Day 0 fish represent background levels in unexposed sculpin. Water uptake control fish are plotted at Day 30. Matrix specific MRLs (from bottom to top: liver, muscle, stomach, intestine) are plotted at day 32 (see Table 7). The vertical line marks the end of the elimination phase (day 28)



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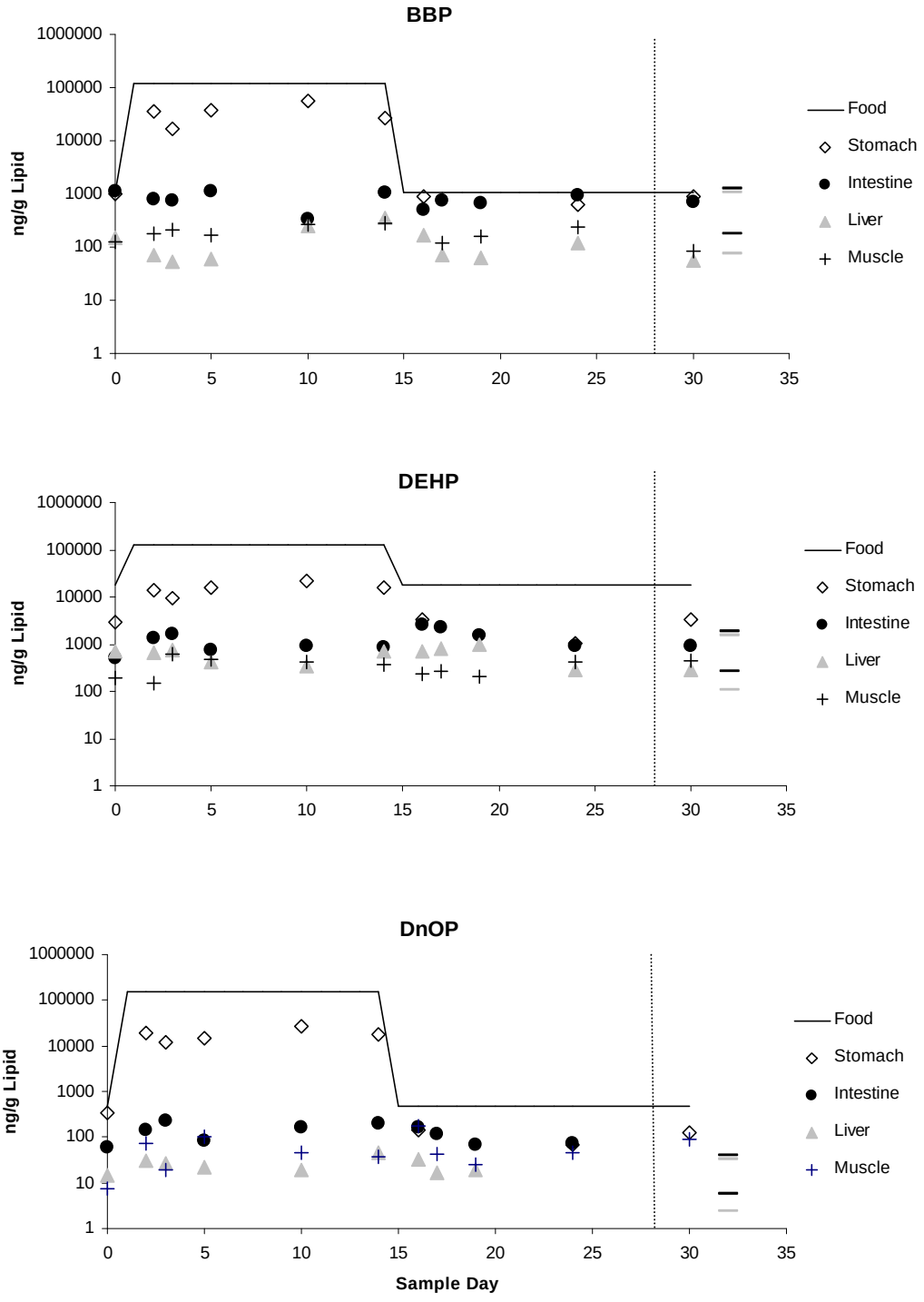

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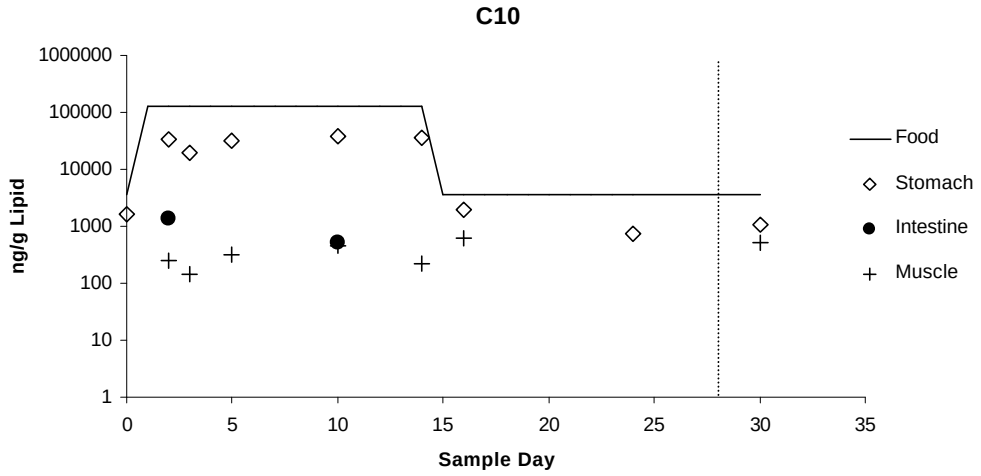

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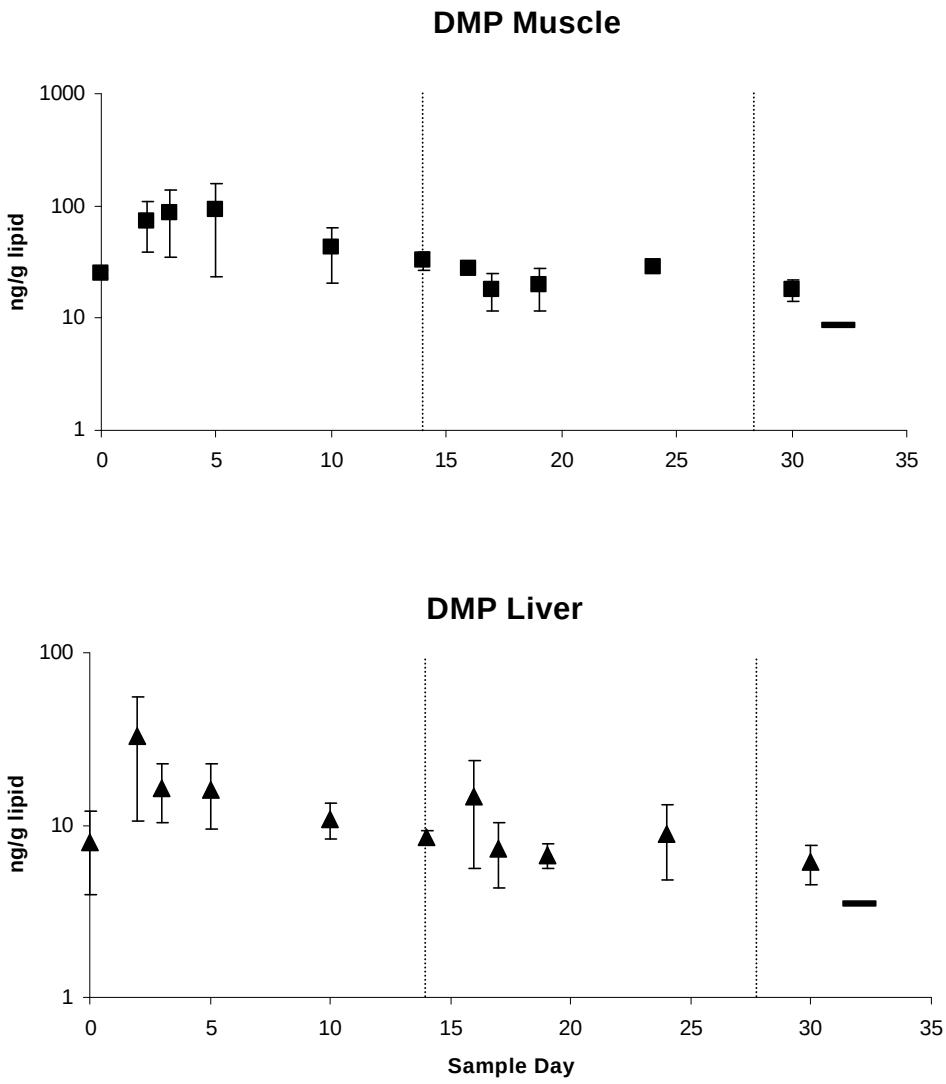
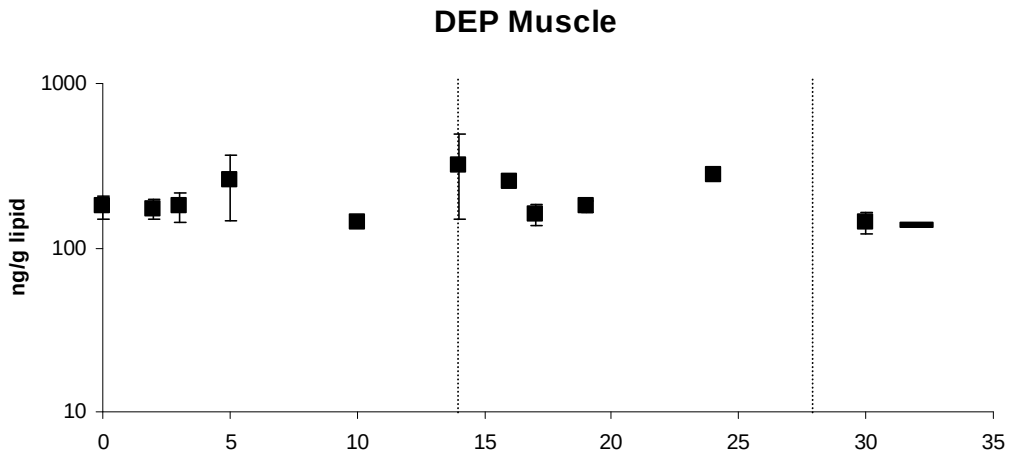
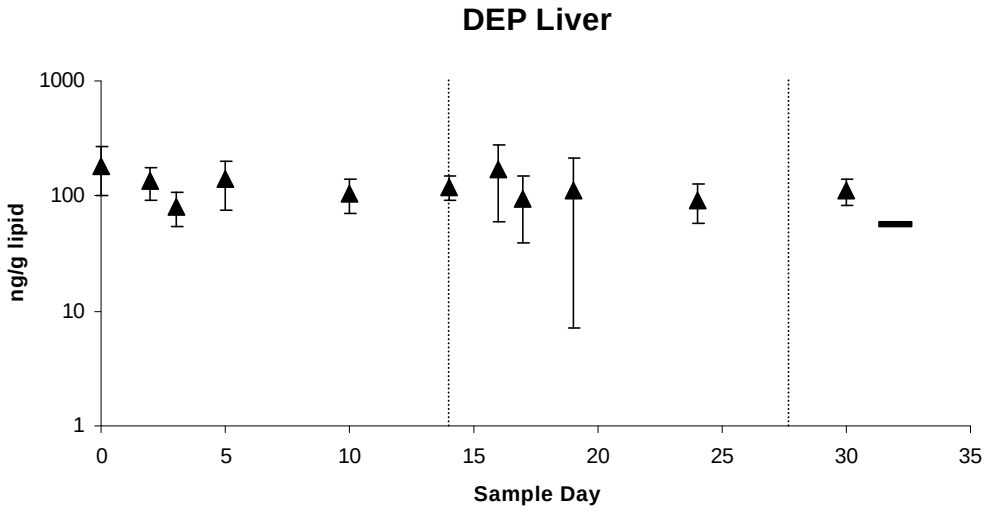


Figure 9. DPE concentrations (ng/g lipid +/- 1 standard deviation) over time in muscle (top) and liver (bottom) The dashed vertical lines represent the end of the uptake phase (Day 14) and the end of the elimination phase (day 28). Water uptake control fish are plotted at Day 30. When applicable, matrix specific MRLs are plotted at day 32 (see Table 7)





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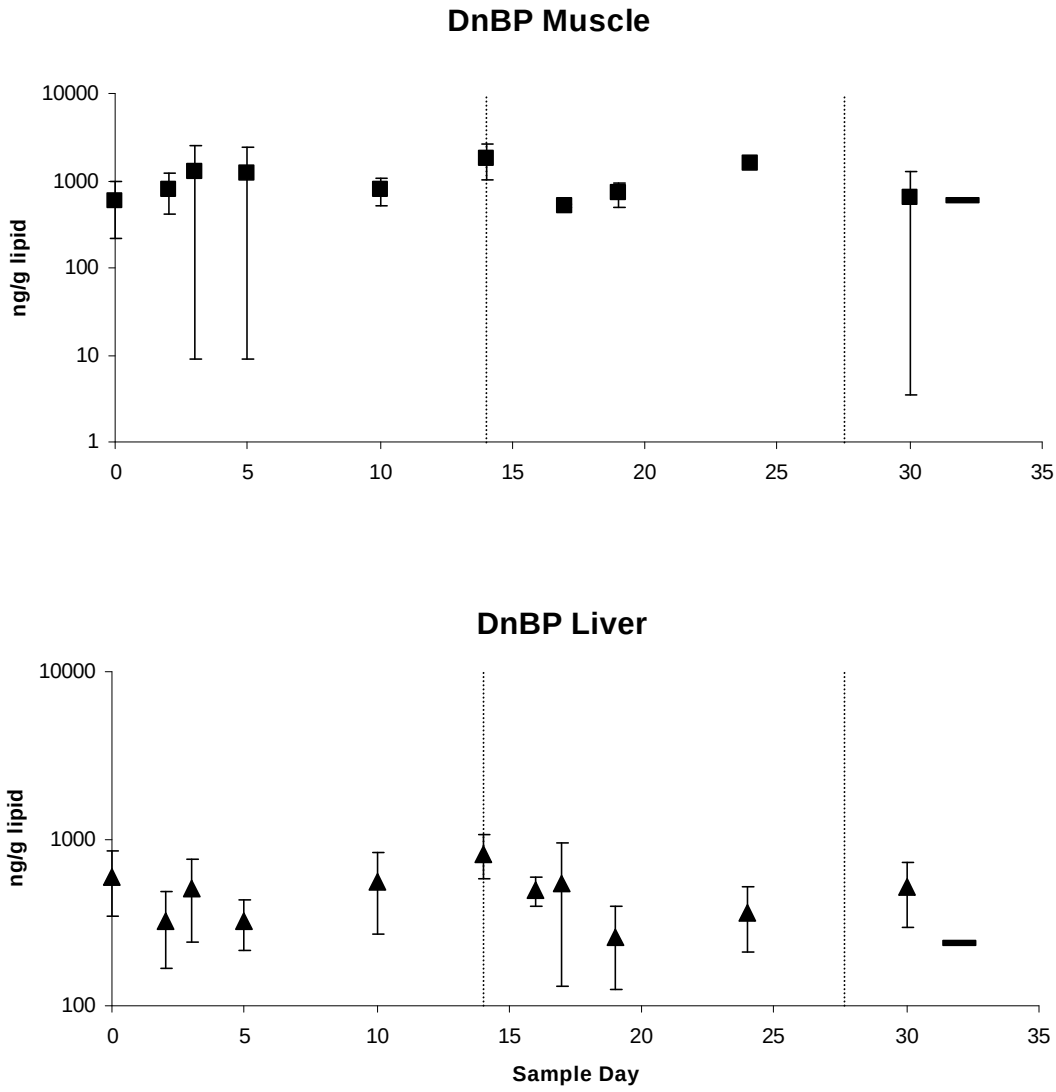

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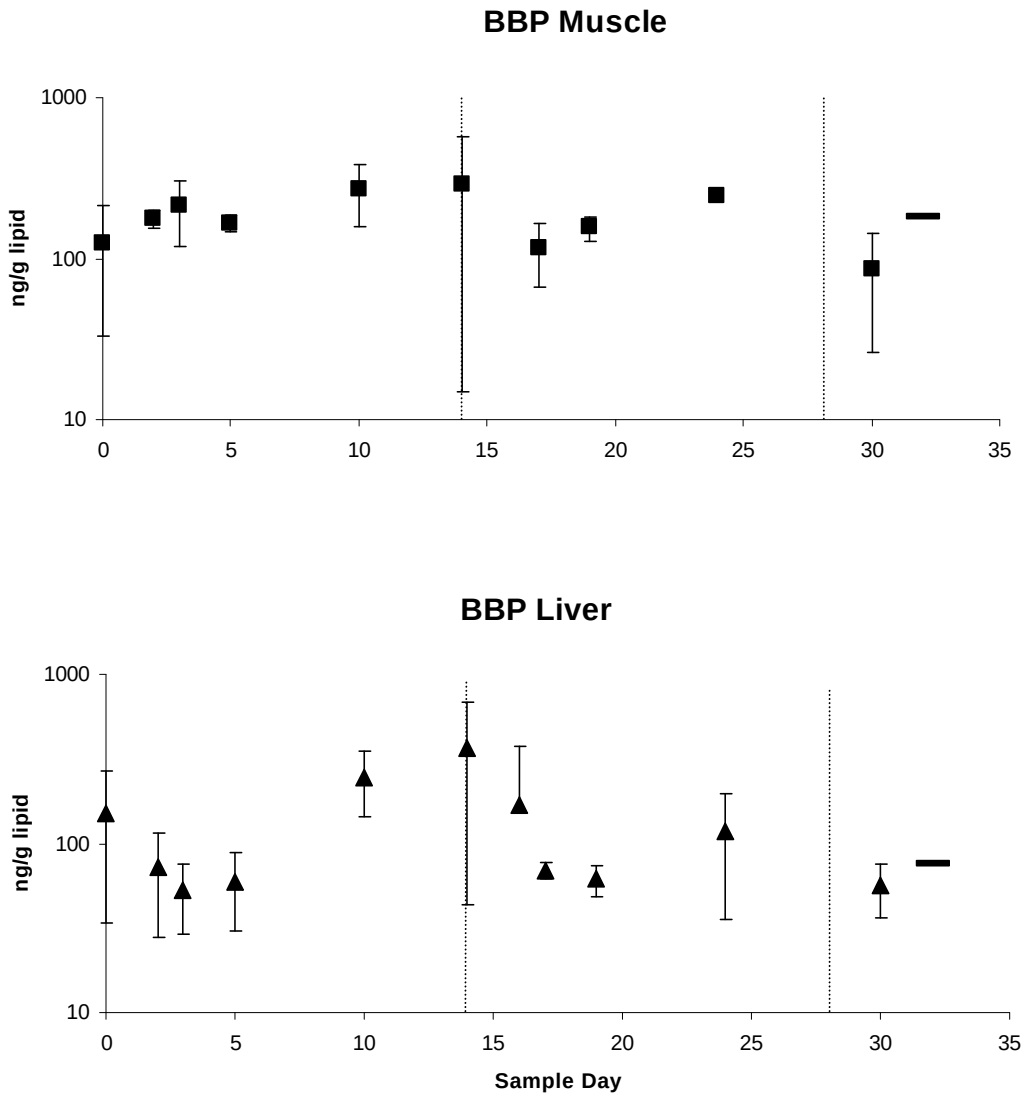

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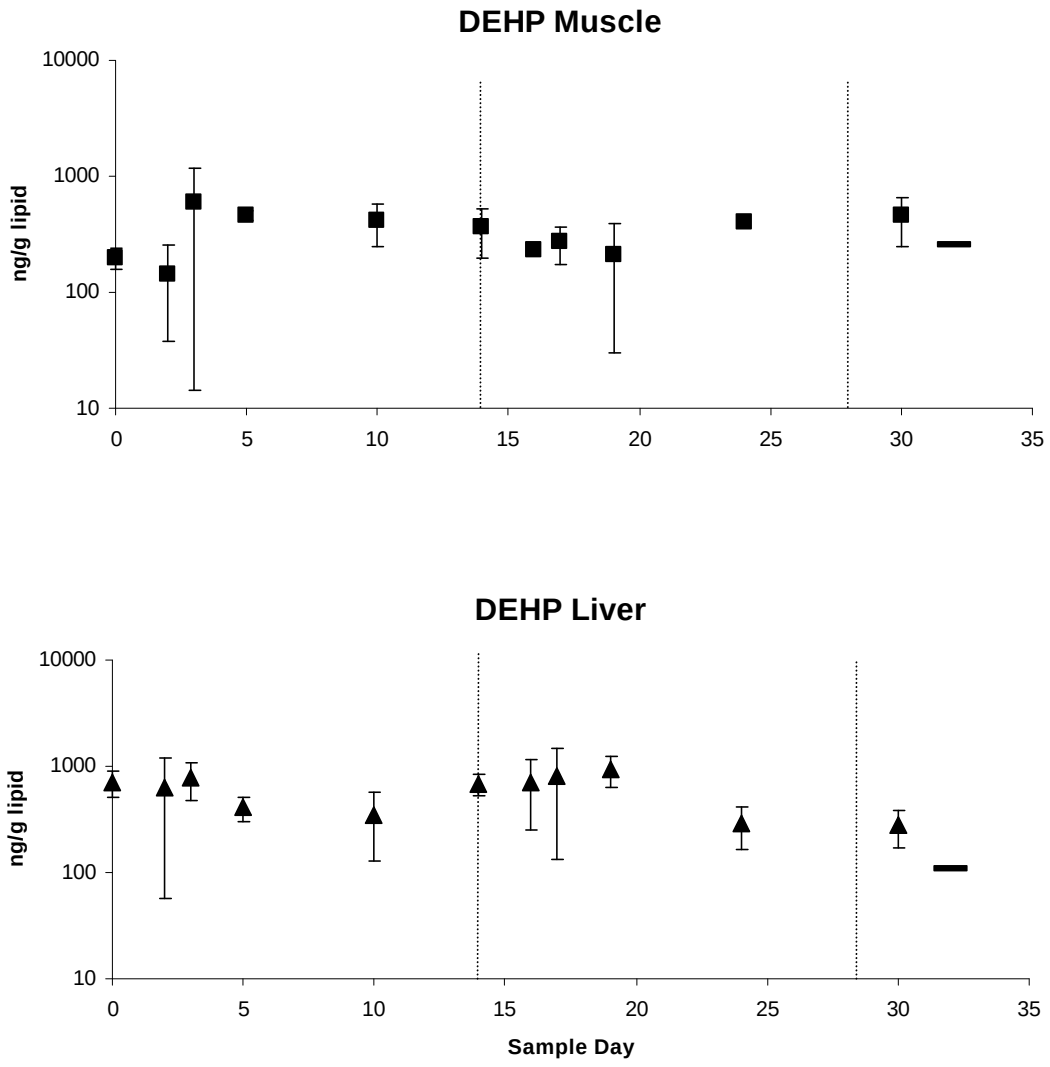

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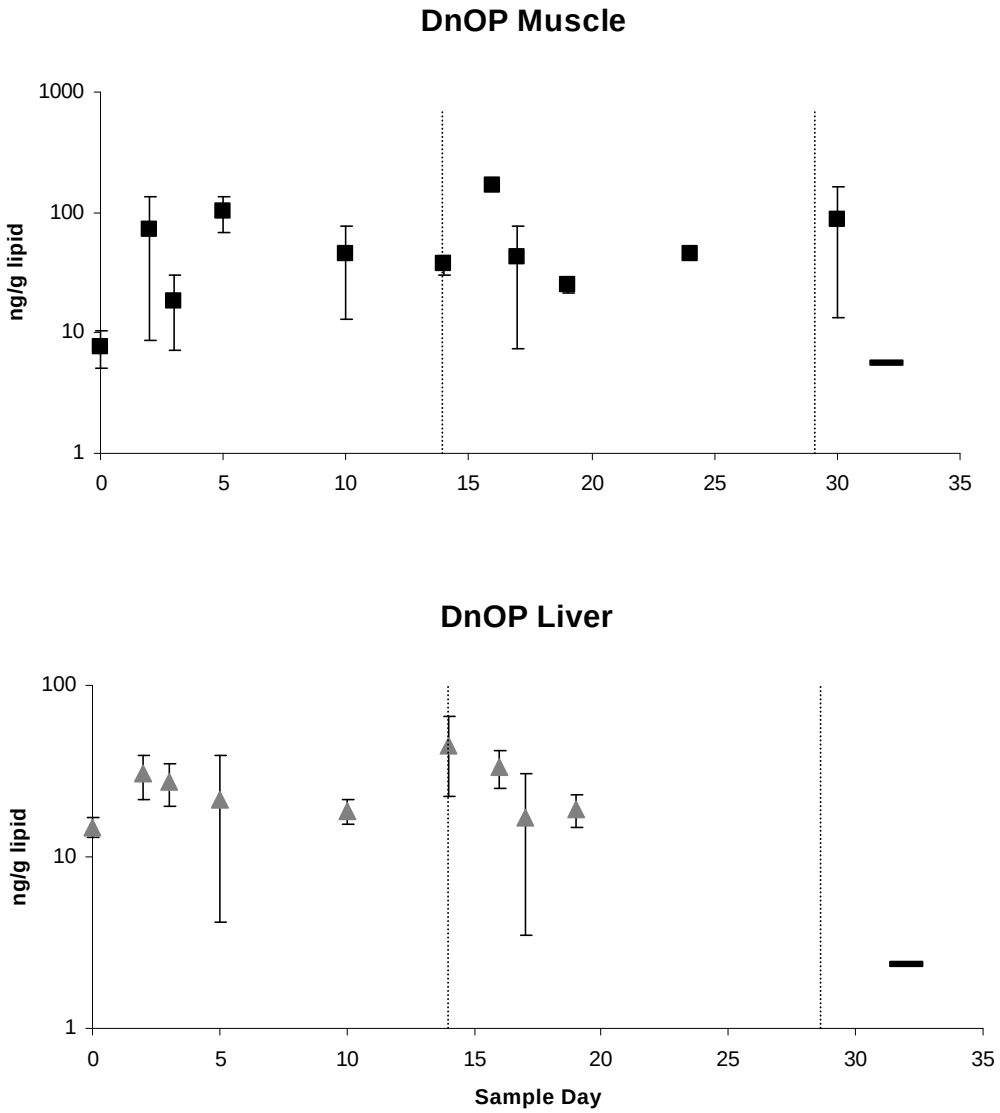

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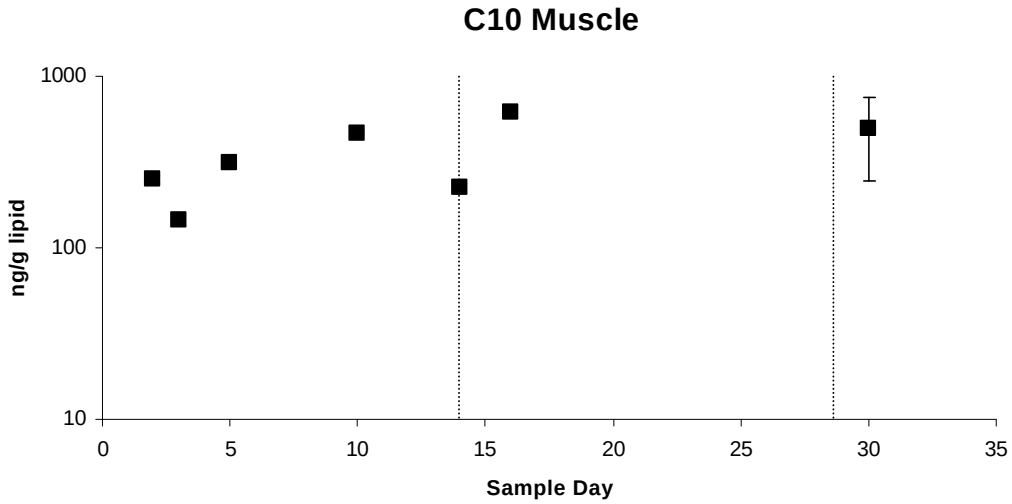


Figure 9 continued

Table 14. Fraction (f) of ingested PCB found in the diet, stomach and intestine (feces) of Staghorn sculpin. f uptake is a maximum estimate of PCB dietary absorption (f diet - f **feces)**

Congener	f Diet	f Stomach	f Intestine	f Uptake (Max)
PCB 52	100%	63%	10%	90%
PCB 155	100%	55%	8%	92%
PCB 209	100%	59%	20%	80%

Table 15. Fraction of ingested DPE measured as DPE or MPE in the diet, stomach, and intestine (feces) of Staghorn sculpin

	f Diet	f Stomach			f Intestine		
Congener	DPE	DPE	MPE	Total	DPE	MPE	Total
DMP / MMP	100%	29.7%	13.2%	42.9%	0.1%	5.8%	5.8%
DEP / MEP	100%	20.7%	16.3%	36.9%	0.3%	8.4%	8.7%
DnBP / MBP	100%	21.6%	77.6%	99.2%	1.7%	7.4%	9.0%
BBP /MBP+MBzP	100%	48.6%	50.5%	99.1%	0.3%	34.2%	34.5%
DEHP / MEHP	100%	21.2%	37.3%	58.6%	0.4%	5.2%	5.6%
DnOP / MOP	100%	20.7%	18.6%	39.2%	0.1%	1.4%	1.4%
C10 / MoC10	100%	42.7%	6.4%	49.1%	0.4%	2.2%	2.5%

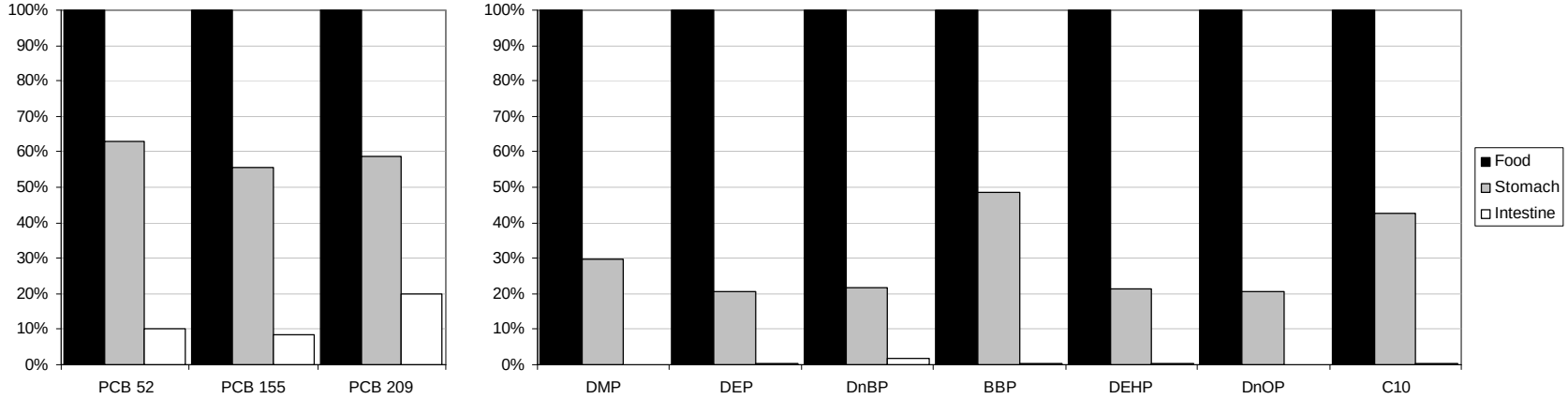


Figure 10. Fluxes of PCBs and DPEs in the diet, stomach and intestine, expressed as a % of the ingested dose

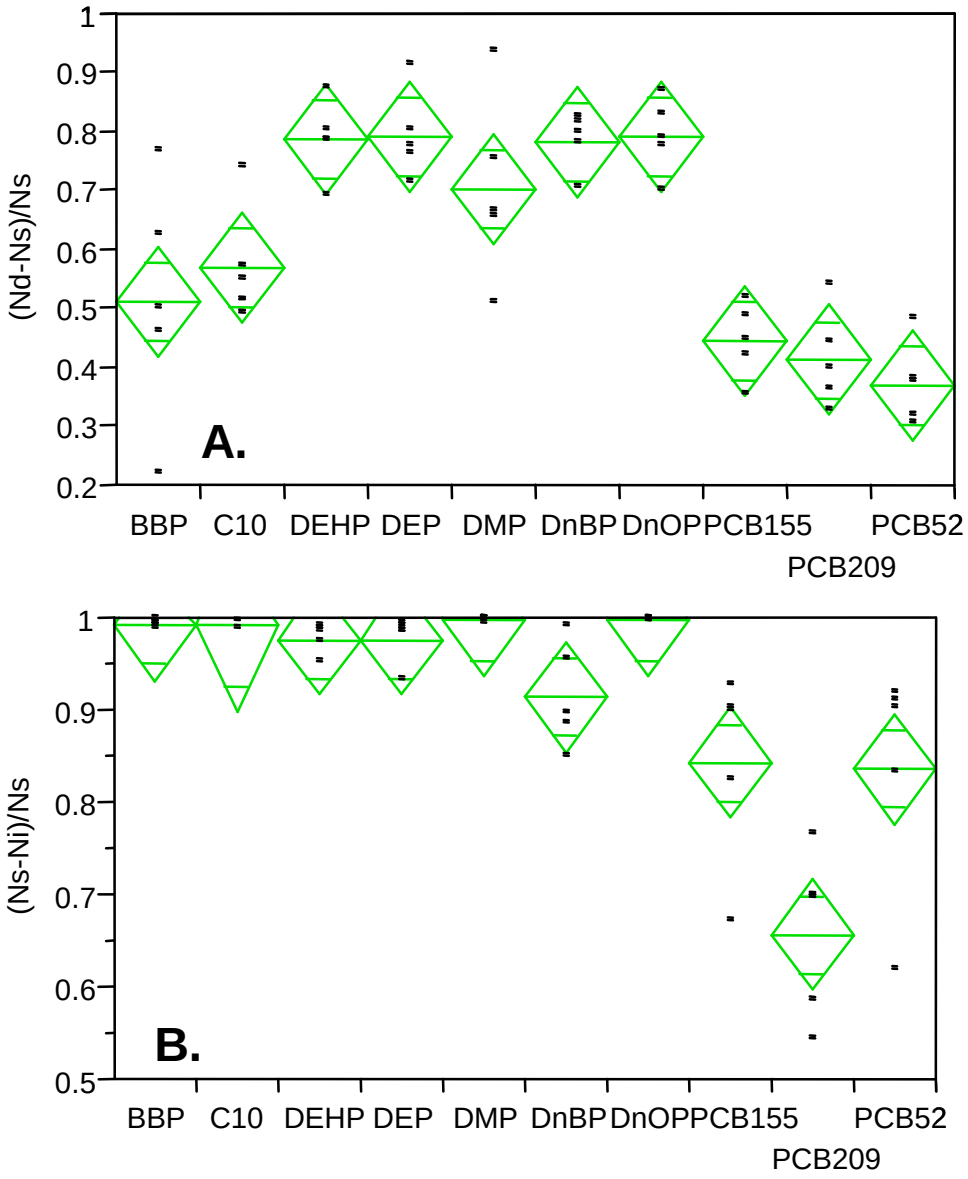


Figure 11. ANOVAs testing for differences in chemical loss along the GIT across all DPE and PCB congeners: A. Fraction of DPE lost in the stomach (Nd-Ns)/Nd. B. Fraction of DPE lost in the intestine (Ns-Ni)/Nd. This figure shows that more DPE than PCB is lost in the stomach (except BBP and C10) and in the intestine (except DnBP) of Staghorn sculpin, providing indirect evidence for DPE metabolism in both compartments

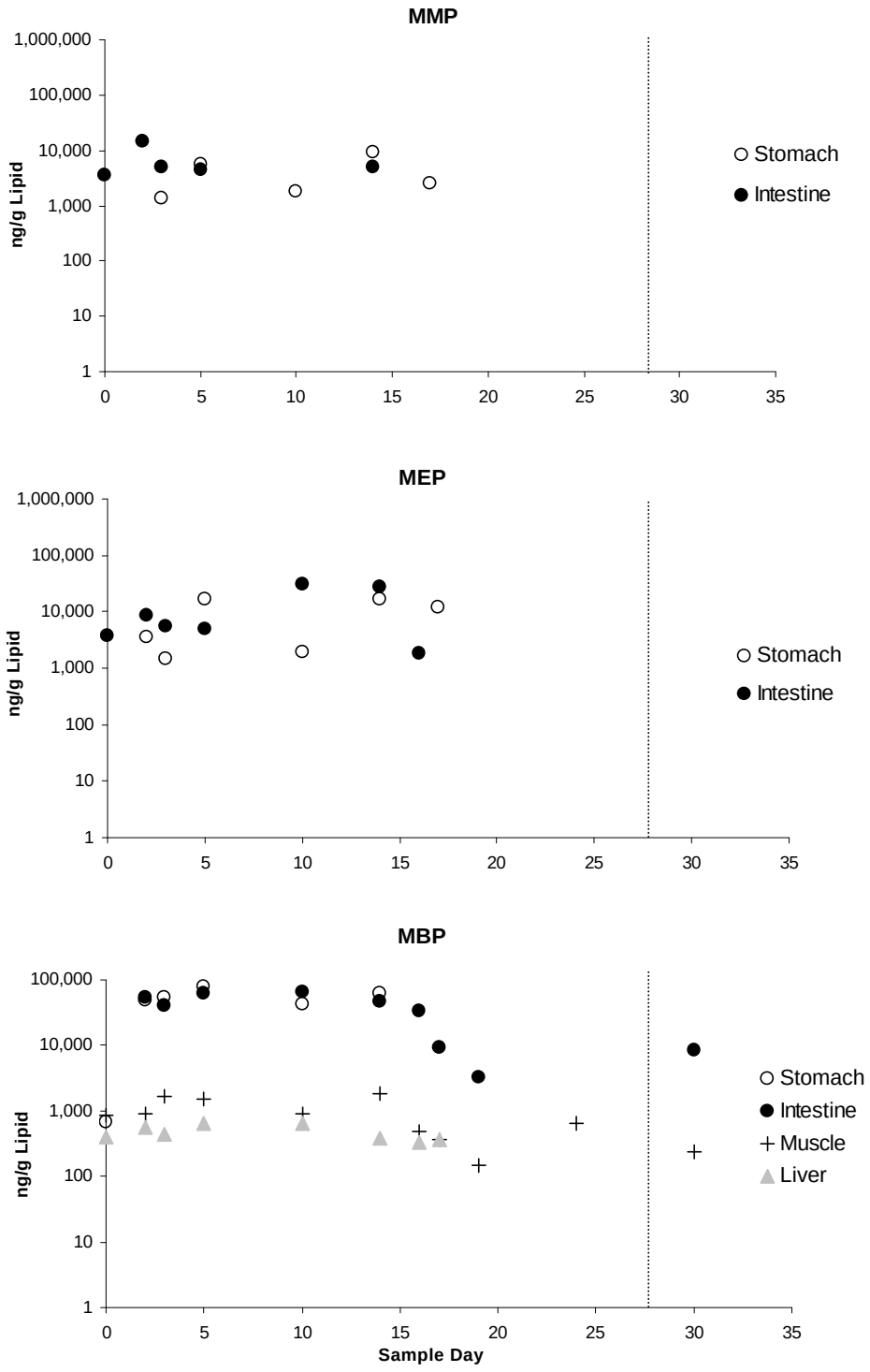
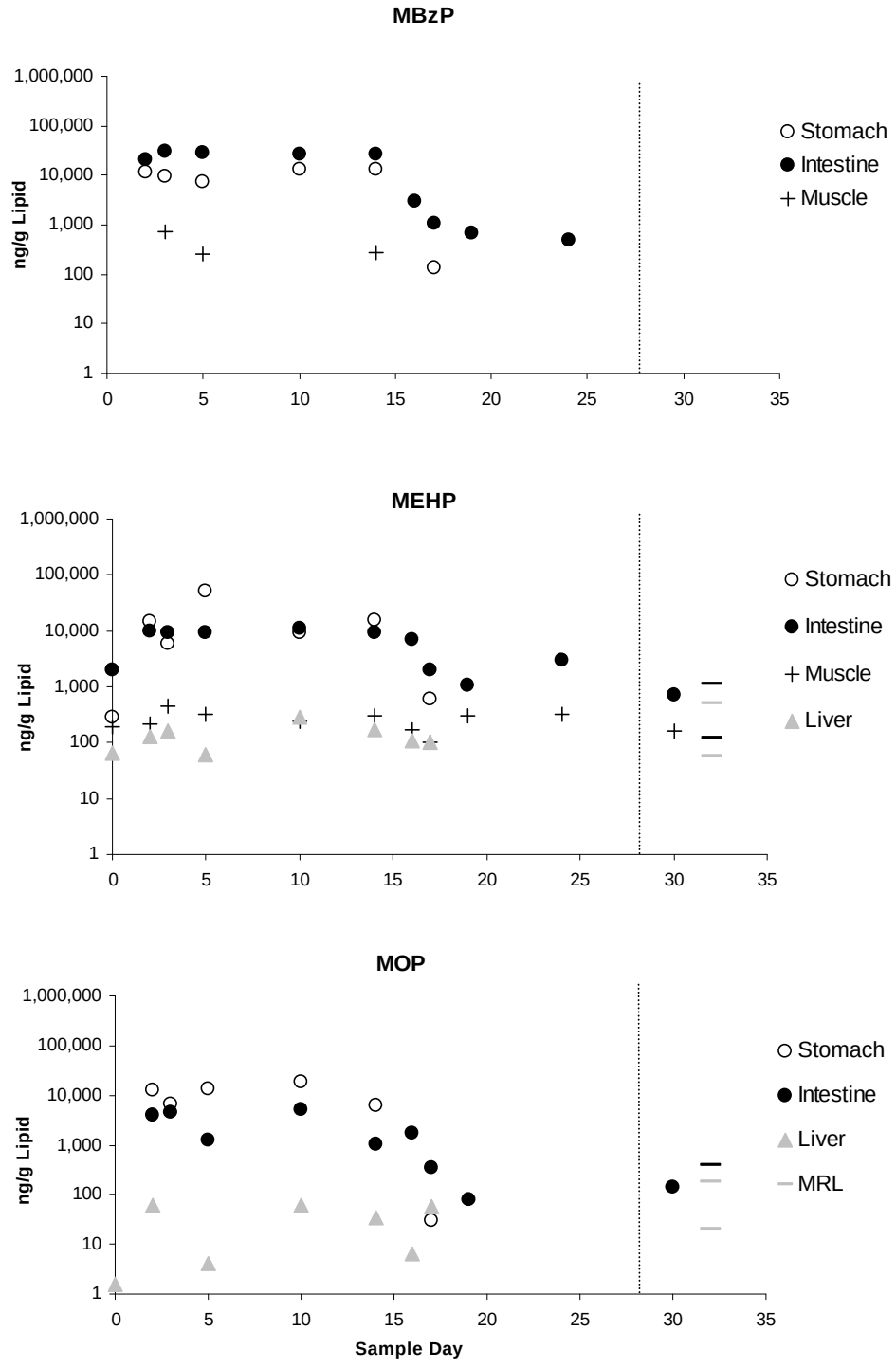


Figure 12. MPE concentrations (ng/g lipid) over time in all matrices. When applicable, matrix specific MRLs are plotted at day 32 (see Table 8)



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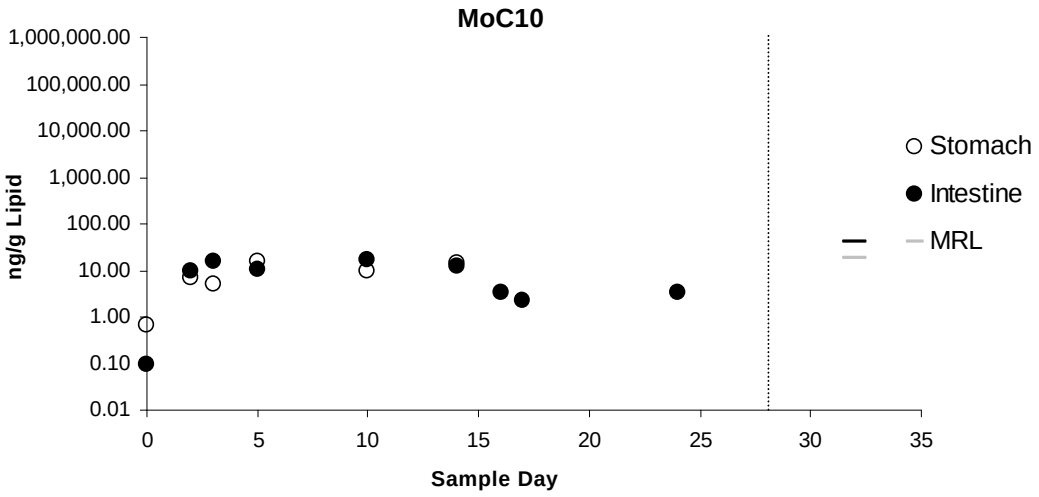

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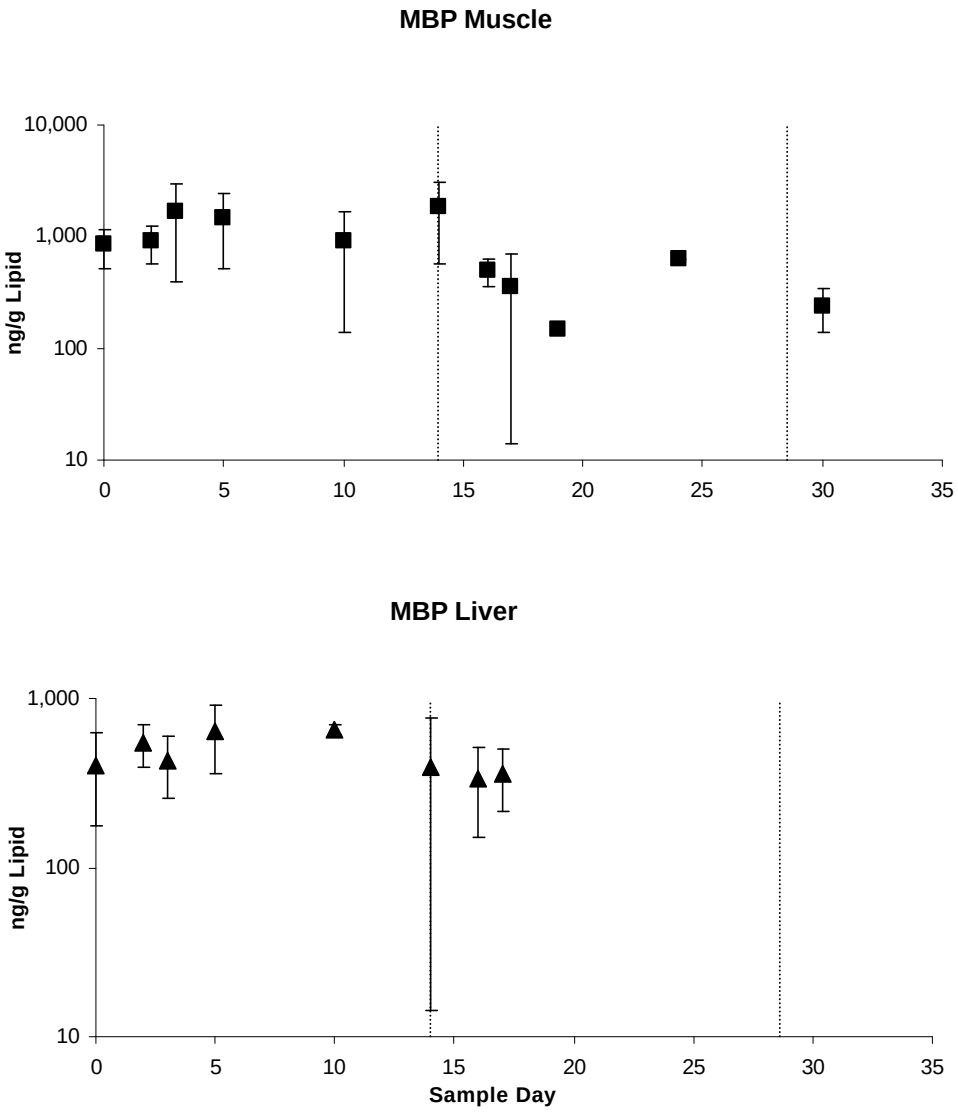
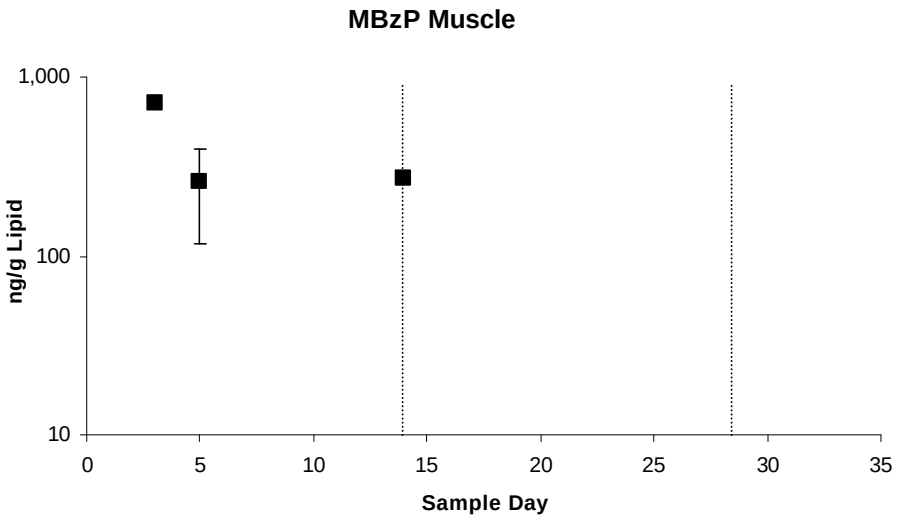


Figure 13. MPE concentrations (ng/g lipid +/- 1 standard deviation) over time in muscle (top) and liver (bottom) The vertical lines represent the end of the uptake phase (Day 14) and the end of the elimination phase (day 28). Water uptake control fish are plotted at Day 30. When applicable, matrix specific MRLs are plotted at day 32 (see Table 8)



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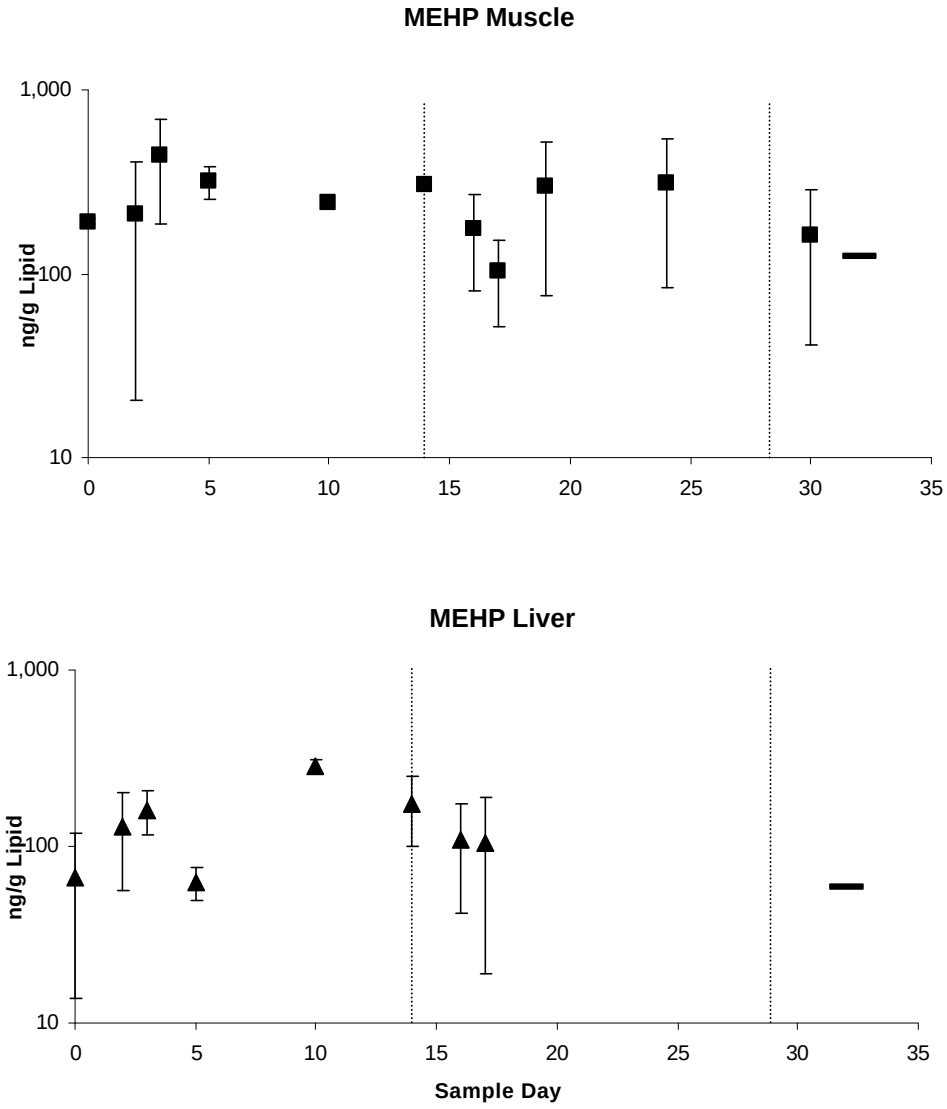

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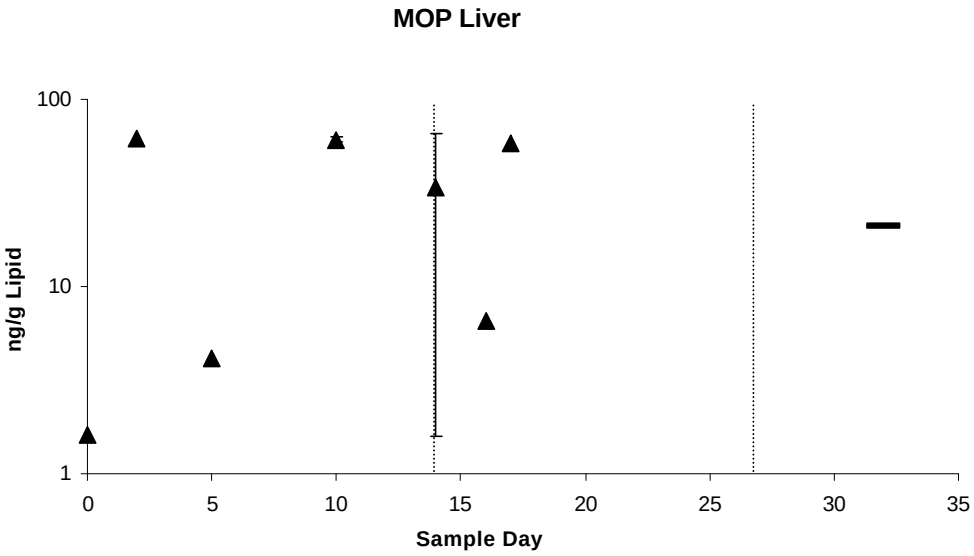

Figure 13 continued

Table 16. Fraction of neutral and ionized MPE found in the stomach and intestine, assuming pKa = 4.36 for all MPEs

Compartment	pH	% neutral	% inonized
Stomach	2.0	99.6	0.4
Intestine	5.5	6.7	93.3

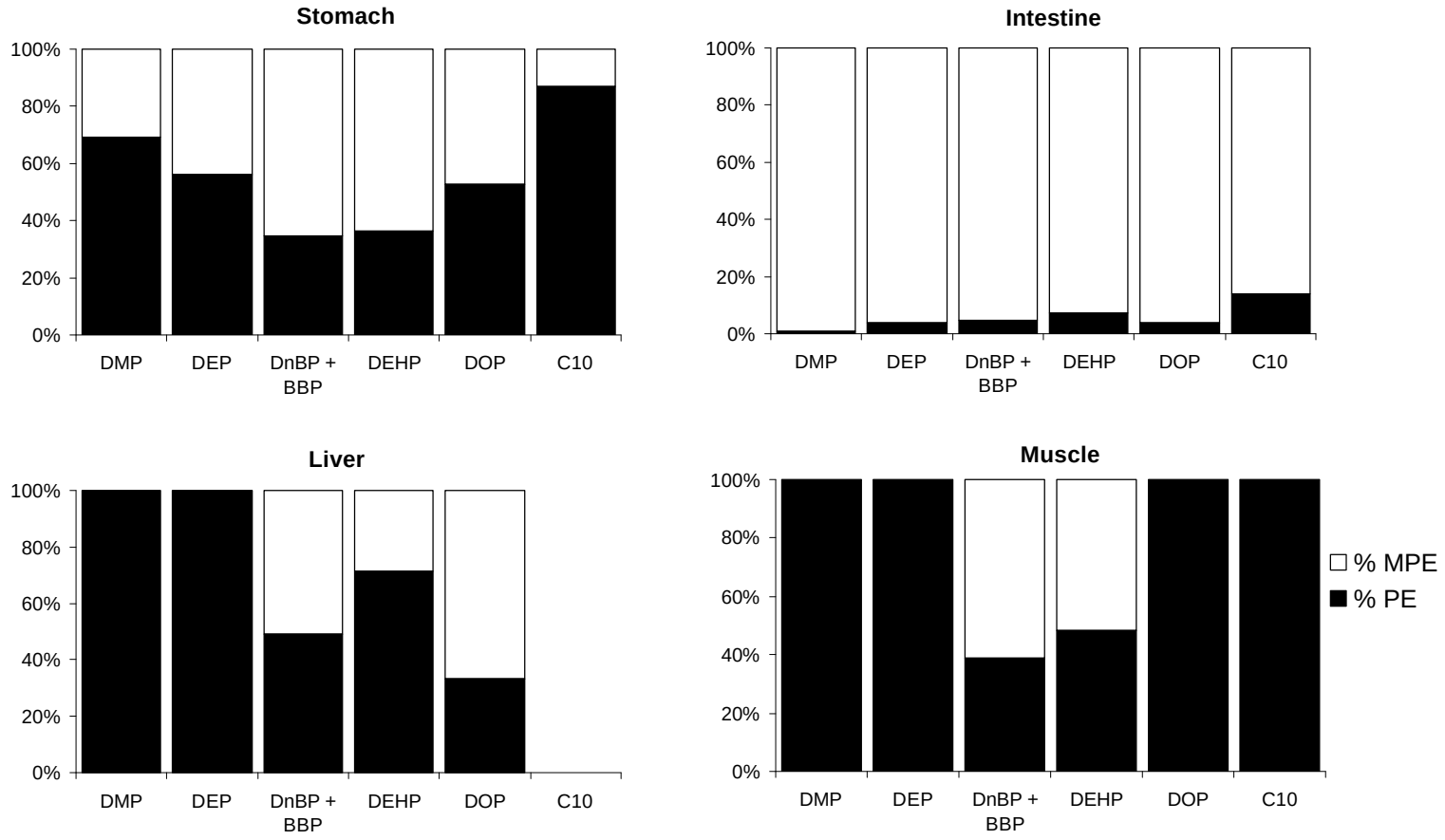

Figure 14. Fractions of total PE found as DPE and MPE in the stomach, intestine, liver and muscle of Staghorn sculpin (means across the uptake phase). DnBP and BBP are grouped together to avoid having to divide the observed MBP between these two parent compounds

6. APPENDIX

Mean wet weight concentrations and standard deviations (ng/g) for each sample day are reported below for all sample matrices. All data have been blank-corrected with the mean blanks from each batch. Data points represent the means across up to 3 samples. Missing data represent a combination of non-detects, unanalyzed samples, or outliers. Water uptake control fish (H_20) are shown at the bottom of the table. Mean uptake concentrations and standard deviations are calculated across all individual fish from Day 2-14 (n=15).

6.1 Phthalate di-esters (DPEs)

Table 17. Mean DPE muscle concentrations (ng/g) across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	0.36	2.56	8.43	1.78	2.82	0.11	
2	1.06	2.47	11.56	2.52	2.08	1.05	3.60
3	1.23	2.59	18.07	3.03	8.67	0.27	2.10
5	1.31	3.68	17.06	2.39	6.68	1.45	4.50
10	0.61	2.06	11.35	3.86	5.90	0.64	6.70
14	0.47	4.59	25.96	4.16	5.17	0.53	3.20
16	0.40	3.66			3.37	2.43	8.90
17	0.26	2.29	7.22	1.67	3.88	0.61	
19	0.28	2.57	10.19	2.24	2.98	0.36	
24	0.40	4.01	22.25	3.49	5.86	0.65	
H20	0.26	2.05	8.95	1.23	6.51	1.25	7.15
Mean uptake	**0.94**	**3.08**	**16.80**	**3.19**	**5.96**	**0.79**	**3.95**

Table 18. Standard deviations (ng/g) for mean DPE muscle concentrations across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	0.03	0.42	5.33	1.31	0.59	0.04	
2	0.50	0.32	5.52	0.31	1.54	0.92	0.14
3	0.73	0.52	19.92	1.31	10.59	0.16	
5	0.98	1.60	17.86	0.30	0.67	0.48	
10	0.31	0.14	4.08	1.59	2.33	0.46	
14	0.08	2.47	11.49	4.13	2.38	0.10	
16							
17	0.09	0.33	0.31	0.72	1.35	0.51	
19	0.11	0.21	3.22	0.39	2.55	0.06	
24							
H20	0.05	0.31	11.86	0.86	2.93	1.06	3.61
Mean uptake	**0.62**	**1.49**	**12.59**	**1.90**	**4.84**	**0.61**	**1.56**

Table 19. Mean DPE liver concentrations (ng/g) across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	2.46	56.58	183.62	46.75	217.72	4.60	
2	10.21	41.66	100.14	22.17	196.21	9.41	
3	5.08	25.11	155.33	16.27	237.98	8.38	
5	4.99	42.96	99.53	18.51	125.38	6.68	
10	3.35	32.75	168.79	76.33	106.61	5.73	
14	2.62	37.15	251.83	112.68	209.45	13.71	
16	4.49	52.17	152.39	51.92	214.46	10.40	
17	2.26	29.40	166.04	21.37	248.47	5.25	
19	2.06	34.36	79.82	19.03	289.94	5.86	
24	2.76	28.45	112.33	36.25	89.48		
H20	1.88	34.56	157.97	17.33	86.27		
Mean uptake	**5.25**	**35.92**	**155.12**	**49.19**	**175.13**	**8.75**	

Table 20. Standard deviations (ng/g) for mean DPE liver concentrations across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	1.24	25.64	76.91	36.24	59.09	0.61	
2	6.96	13.34	48.75	13.53	178.99	2.73	
3	1.86	8.42	80.47	7.19	89.16	2.28	
5	2.09	19.74	33.68	9.04	32.22	5.38	
10	0.78	10.86	86.21	31.81	66.46	0.97	
14	0.22	8.79	74.94	99.41	49.01	6.79	
16	2.75	33.41	30.44	63.03	136.96	2.58	
17	0.94	17.23	125.21	2.38	207.33	4.18	
19	0.35	32.14	41.37	3.87	98.18	1.27	
24	1.29	10.32	47.65	25.27	39.31		
H20	0.49	9.15	67.60	6.03	33.33		
Mean uptake	**3.96**	**12.82**	**81.52**	**56.69**	**97.80**	**4.14**	

Table 21. Mean DPE stomach concentrations (ng/g) across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	2.51	16.18	66.12	34.65	101.48	11.99	56.95
2	435.34	565.09	470.92	1225.17	491.46	678.82	1145.00
3	83.92	176.03	407.16	577.53	320.96	406.61	666.36
5	424.42	394.33	433.61	1331.51	545.91	521.55	1086.97
10	625.84	471.83	695.95	1920.00	774.08	911.04	1298.00
14	314.49	441.27	520.85	919.62	537.11	640.26	1236.30
16	5.45	7.80	98.65	32.05	117.59	4.84	68.90
17	5.04						
19							
24		18.48	82.58	22.53	35.16	2.32	25.60
H20	3.37	12.99	63.35	30.91	117.85	4.31	37.50
Mean uptake	**338.49**	**400.15**	**476.43**	**1083.19**	**496.95**	**588.67**	**1072.66**

Table 22. Standard deviations (ng/g) for mean DPE stomach concentrations across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	3.26	12.74	83.15	24.16	52.27	13.06	53.10
2	307.99	262.56	169.10	302.96	66.39	107.36	149.62
3	121.51	217.27	401.38	640.37	304.80	416.39	404.95
5	542.72	309.44	279.06	1201.92	449.59	454.29	995.14
10							
14	535.43	693.77	629.91	1419.95	827.58	1033.33	1528.34
16	5.53	3.65	121.36	34.73	3.47	2.29	0.85
17							
19							
24							
H20	2.97	3.80	36.81	28.04	41.70	1.38	7.92
Mean uptake	**377.57**	**368.45**	**341.91**	**899.93**	**423.33**	**513.76**	**705.41**

Table 23. Mean DPE intestine concentrations (ng/g) across sample days.

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	1.49	25.57	116.03	21.53	9.64	1.16	
2	1.33	11.22	44.13	15.08	25.37	2.67	26.10
3	1.11	24.30	123.73	14.41	30.67	4.43	
5	1.28	12.09	90.43	21.68	14.21	1.61	
10	1.22	5.30	12.06	6.33	16.93	3.18	10.10
14	1.74	14.45	120.91	20.29	15.92	3.94	
16	1.03	15.87	59.96	9.73	49.62	3.15	
17	1.76	25.02	75.00	14.46	42.59	2.28	
19	1.63	14.91	82.27	12.99	28.99	1.31	
24	0.90	10.25	62.57	17.56	17.69	1.42	
H20	1.77	21.69	69.90	13.15	17.01		
Mean uptake	**1.33**	**13.17**	**76.42**	**16.03**	**20.48**	**3.00**	**18.10**

Table 24. Standard deviations (ng/g) for mean DPE intestine concentrations across sample days

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
0	0.35	5.78	18.48	8.79	10.69	1.26	
2	0.80	6.04	36.25	5.03	14.38	0.86	
3	0.77	24.51	124.04	18.96	20.99	3.06	
5	0.34	2.89	43.14	14.30	4.15	0.09	
10	0.55	3.70	9.75	3.19	15.42	1.94	
14	0.80	1.97	23.43	0.02	1.22	1.47	
16	0.68	13.44	51.71	4.04	50.44	3.74	
17	0.28	16.64	39.45	4.50	52.39	1.42	
19	0.58	5.35	50.93	13.30	30.94	0.09	
24	0.97	14.08	86.55	12.57			
H20							
Mean uptake	**0.57**	**9.99**	**62.39**	**10.26**	**12.03**	**1.62**	**11.31**

Table 25. Mean DPE concentrations and standard deviations (ng/g) in the control food (Day 0 and Days 15-28) and the experimental food (Days 2-14)

Sample Day	DMP	DEP	DBP	BBP	DEHP	DOP	C10
Control	15.68	102.03	2920.76	80.46	1353.03	35.92	269.17
SD	0.49	13.62	673.40	16.64	419.07	15.58	74.65
Expt	4696.40	7339.51	8680.63	9094.72	9300.75	11298.16	9407.13
SD	395.40	505.40	229.12	243.48	237.70	47.30	521.82

6.2 Phthalate mono-esters (MPEs)

Table 26. Mean MPE muscle concentrations (ng/g) across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0			12.17			2.75	
2			12.95			3.05	
3			24.05	10.20		6.27	
5			21.37	3.71		4.53	
10			12.90			3.50	
14			26.50	3.90		4.40	
16			7.10			2.51	
17			5.15			1.46	
19			2.10			4.26	
24			9.05			4.47	
H20			3.45			2.34	
Mean uptake			**18.67**	**5.05**		**4.64**	

Table 27. Standard deviations (ng/g) for mean MPE muscle concentrations across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0			4.71			0.07	
2			4.89			2.76	
3			18.46			3.58	
5			14.07	2.02		0.91	
10			10.93				
14			18.24				
16			1.98			1.35	
17			5.44			0.72	
19						3.17	
24			0.07			3.27	
H20			1.48			1.75	
Mean uptake			**12.10**	**3.21**		**2.34**	

Table 28. Mean MPE liver concentrations (ng/g) across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0			124.76		0.50	20.50	
2			170.03		19.00	39.67	
3			133.23			49.80	
5			198.17		1.29	19.30	
10			201.53		18.88	87.79	
14			120.30		10.49	54.14	
16			103.15		2.02	33.32	
17			111.50		18.13	32.28	
19							
24							
H20							
Mean uptake			**167.82**		**13.17**	**50.14**	

Table 29. Standard deviations (ng/g) for mean MPE liver concentrations across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0			69.75			16.28	
2			47.62			22.33	
3			53.68			14.21	
5			87.19			3.97	
10			13.21		0.47	7.91	
14			126.43		12.84	23.44	
16			56.50			20.53	
17			44.69			26.41	
19							
24							
H20							
Mean uptake			**65.71**		**9.16**	**26.91**	

Table 30. Mean MPE stomach concentrations (ng/g) across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0			23.69			10.14	7.33
2		125.77	1710.08	394.63	428.27	510.81	77.06
3	47.15	52.21	1909.93	340.71	236.20	210.43	55.70
5	196.90	593.15	2777.47	260.25	486.44	1756.72	169.88
10	61.81	68.45	1506.51	455.96	644.78	326.72	99.12
14	318.09	572.26	2192.64	456.63	223.25	549.56	154.18
16							
17	88.84	414.42		4.63	1.08	20.59	
19							
24							
H20							
Mean uptake	**188.41**	**337.87**	**1963.68**	**387.52**	**368.87**	**645.73**	**108.98**

Table 31. Standard deviations (ng/g) for mean MPE stomach concentrations across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0							
2		89.05	681.03	178.46	421.80	185.68	47.25
3				361.55	377.03	239.09	68.89
5		347.46			238.47	376.53	36.12
10							
14	37.03	270.17	54.75	171.52	270.42	272.65	57.91
16							
17					0.90	9.42	
19							
24							
H20							
Mean uptake	**133.28**	**313.05**	**548.05**	**180.04**	**313.00**	**600.94**	**61.65**

Table 32. Mean MPE intestine concentrations (ng/g) across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0	66.22	73.33				37.03	0.56
2	270.53	160.83	1008.93	406.78	78.06	191.01	58.40
3	93.98	104.06	788.18	584.19	83.91	177.29	91.15
5	84.01	93.03	1203.69	554.85	24.41	180.46	60.41
10		579.90	1222.83	529.70	96.32	204.65	95.26
14	96.78	515.67	903.12	509.20	19.39	177.86	72.07
16		34.20	631.45	56.50	32.18	134.24	19.50
17			181.08	19.81	6.43	38.34	12.90
19			63.60	13.00	1.50	20.56	
24				9.40		56.50	20.00
H20			159.10		2.76	13.78	
Mean uptake	**128.42**	**304.28**	**1034.93**	**511.96**	**43.30**	**183.89**	**71.36**

Table 33. Standard deviations (ng/g) for mean MPE intestine concentrations across sample days

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
0						6.13	
2		196.19	689.75	399.48		32.09	
3							
5			492.65	715.33	9.97	74.36	11.41
10							
14	33.12	614.38	81.06	634.10	21.48	31.21	14.79
16			249.54	15.27	36.35	95.13	
17			88.93	0.30	5.08	20.56	7.30
19				2.69		10.50	
24						61.21	
H20							
Mean uptake	**81.32**	**343.31**	**366.58**	**473.80**	**34.59**	**40.49**	**16.54**

Table 34. Mean MPE concentrations and standard deviations (ng/g) in the control food (Day 0 and Days 15-28) and the experimental food (Days 2-14)

Sample Day	MMP	MEP	MBP	MBzP	MOP	MEHP	MoC10
Control							
SD							
Expt			67.20			23.00	
SD			9.76			4.24	

6.3 Polychlorinated biphenyls (PCBs)

Table 35. Mean PCB muscle concentrations (ng/g) across sample days.

Sample Day	PCB 52	PCB 155	PCB 209
0	2.38E-02	3.21E-03	3.05E-03
2	4.10E+00	5.07E+00	1.92E+00
3	8.28E+00	5.93E+00	5.16E+00
5	1.36E+01	1.77E+01	1.08E+01
10	1.73E+01	2.03E+01	2.13E+01
14	6.55E+01	1.04E+02	7.32E+01
16	3.04E+01	3.80E+01	4.19E+01
17	3.53E+01	4.39E+01	3.12E+01
19	1.77E+01	2.56E+01	2.40E+01
24	2.69E+01	3.45E+01	3.15E+01
H20	1.03E+00	6.51E-01	1.26E+00
Mean uptake	n/a	n/a	n/a

Table 36. Standard deviations (ng/g) for mean PCB muscle concentrations across sample days

Sample Day	PCB 52	PCB 155	PCB 209
0	5.50E-03	9.25E-04	3.23E-04
2	2.83E+00	3.65E+00	1.35E+00
3	8.04E+00	5.00E+00	4.83E+00
5	7.55E+00	1.03E+01	3.64E+00
10	1.53E+01	1.75E+01	2.46E+01
14	3.46E+01	4.24E+01	2.36E+01
16	6.97E+00	6.03E+00	1.40E+01
17	2.04E+01	3.13E+01	1.56E+01
19	1.63E+00	1.64E+00	8.73E+00
24	2.02E+01	2.14E+01	1.65E+01
H20	1.52E+00	7.94E-01	2.09E+00
Mean uptake	n/a	n/a	n/a

Table 37. Mean PCB liver concentrations (ng/g) across sample days.

Sample Day	PCB 52	PCB 155	PCB 209
0	1.64E+00	1.02E-01	1.18E-01
2	3.96E+02	9.51E+01	6.83E+01
3	6.57E+02	5.73E+02	3.61E+02
5	1.03E+03	6.55E+02	7.69E+02
10	1.80E+03	1.38E+03	1.78E+03
14	2.76E+03	3.31E+03	3.70E+03
16	2.36E+03	5.67E+03	4.00E+03
17	1.80E+03	2.79E+03	3.16E+03
19	1.48E+03	3.60E+03	2.58E+03
24	1.71E+03	3.70E+03	3.17E+03
H20	4.54E+01	6.19E+01	1.22E+02
Mean uptake	n/a	n/a	n/a

Table 38. Standard deviations (ng/g) for mean PCB liver concentrations across sample days

Sample Day	PCB 52	PCB 155	PCB 209
0	7.62E-01	2.48E-02	4.85E-02
2	2.83E+02	8.44E+01	5.83E+01
3	3.45E+02	1.78E+02	2.08E+02
5	2.26E+02	4.88E+02	4.74E+02
10	1.43E+03	1.37E+03	1.19E+03
14	1.09E+03	3.76E+03	2.48E+02
16	1.01E+03	3.15E+03	1.86E+03
17	1.44E+03	1.70E+03	3.32E+03
19	6.74E+02	1.36E+03	5.97E+02
24	1.38E+03	2.86E+03	1.38E+03
H20	7.62E-01	2.48E-02	4.85E-02
Mean uptake	n/a	n/a	n/a

Table 39. Mean PCB stomach concentrations (ng/g) across sample days.

Sample Day	PCB 52	PCB 155	PCB 209
0	1.38E-01	1.05E-01	5.22E-02
2	7.88E+02	1.08E+03	8.55E+02
3	6.50E+02	9.45E+02	7.05E+02
5	8.77E+02	1.13E+03	1.03E+03
10	8.59E+02	1.26E+03	9.25E+02
14	7.79E+02	1.00E+03	9.79E+02
16	1.07E+01	1.82E+01	1.71E+01
17	7.35E+01	1.07E+02	8.94E+01
19	6.39E+01	1.08E+02	8.42E+01
24	3.04E+00	3.46E+00	2.93E+00
H20	5.85E-01	3.84E-01	4.30E-01
Mean uptake	**7.80E+02**	**1.06E+03**	**8.96E+02**

Table 40. Standard deviations (ng/g) for mean PCB stomach concentrations across sample days

Sample Day	PCB 52	PCB 155	PCB 209
0	1.21E-02	6.01E-02	5.89E-03
2	1.07E+02	1.39E+02	1.04E+02
3	3.86E+02	6.65E+02	4.14E+02
5	5.72E+02	1.09E+03	5.67E+02
10			
14	5.97E+02	5.61E+02	6.93E+02
16	7.22E+00	1.29E+01	1.49E+01
17	1.02E+02	1.48E+02	1.22E+02
19			
24			
H20	8.44E-02	2.52E-01	6.42E-02
Mean uptake	**3.84E+02**	**5.79E+02**	**4.24E+02**

Table 41. Mean PCB intestine concentrations (ng/g) across sample days.

Sample Day	PCB 52	PCB 155	PCB 209
0	4.93E-02	3.79E-02	3.36E-02
2	1.54E+02	2.19E+02	5.19E+02
3	2.19E+02	3.33E+02	5.85E+02
5	1.42E+02	2.24E+02	6.21E+02
10	1.56E+02	1.85E+02	4.32E+02
14	5.93E+02	6.59E+02	8.93E+02
16	8.96E+01	1.14E+02	1.16E+02
17	4.21E+01	4.14E+01	6.99E+01
19	2.06E+02	1.25E+02	1.08E+02
24	4.42E+01	4.45E+01	2.73E+01
H20	2.61E-01	2.06E-02	3.48E-02
Mean uptake	**2.29E+02**	**2.98E+02**	**5.90E+02**

Table 42. Standard deviations (ng/g) for mean PCB intestine concentrations across sample days

Sample Day	PCB 52	PCB 155	PCB 209
0	2.60E-02	2.42E-03	1.06E-02
2	7.64E+01	1.01E+02	1.19E+02
3	3.27E+00	1.33E+01	1.30E+02
5	5.15E+01	8.49E+01	1.79E+02
10	1.13E+02	1.46E+02	3.15E+02
14	5.07E+02	4.16E+02	3.64E+01
16	8.67E+01	8.96E+01	3.93E+01
17	3.97E+01	3.32E+01	6.63E+01
19	2.43E+02	1.43E+02	1.20E+02
24	1.59E+01	7.66E+00	2.99E+00
H20			
Mean uptake	**2.27E+02**	**2.21E+02**	**2.21E+02**

Table 43. Mean PCB concentrations and standard deviations (ng/g) in the control food (Day 0 and Days 15-28) and the experimental food (Days 2-14)

Sample Day	PCB 52	PCB 155	PCB 209
Control	2.83E-01	3.76E-01	2.54E-01
SD	3.86E-02	3.07E-02	8.20E-02
Expt	4.66E+03	7.22E+03	5.67E+03
SD	3.86E-02	3.07E-02	8.20E-02