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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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 tonnes

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

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

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?

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/HRMSild Staghorn sculpin (

2.1.1 reta

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

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 feeding16 fish cvyt

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

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

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 M]6.12 0 < < < < < < < < < < < < 39j 7.8 0 TD -0.1 0 Tc 0.0509 nj 19.081 TD -0.0184 Tc 0 Tw (Time)

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 l dir -0.0aD -0.0Tc 0 Tw Di.6 0 l

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**.

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

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

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

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

3.2.3

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

The observed

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

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

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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

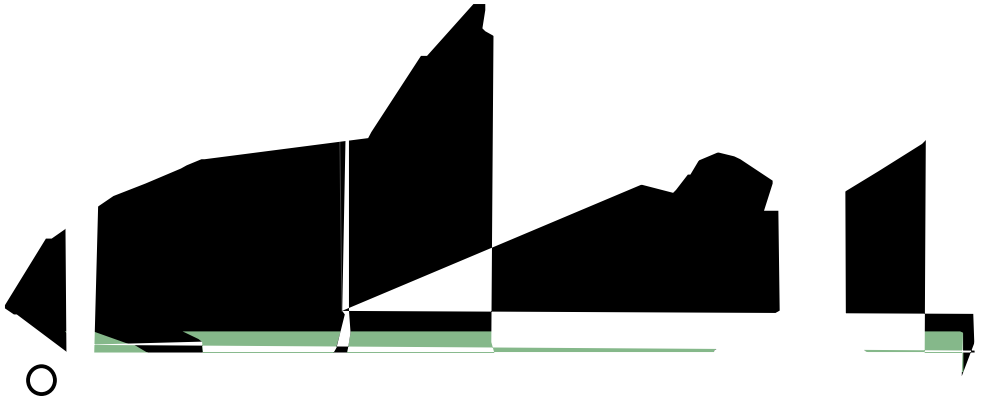


Figure
Dietary
elimina

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**

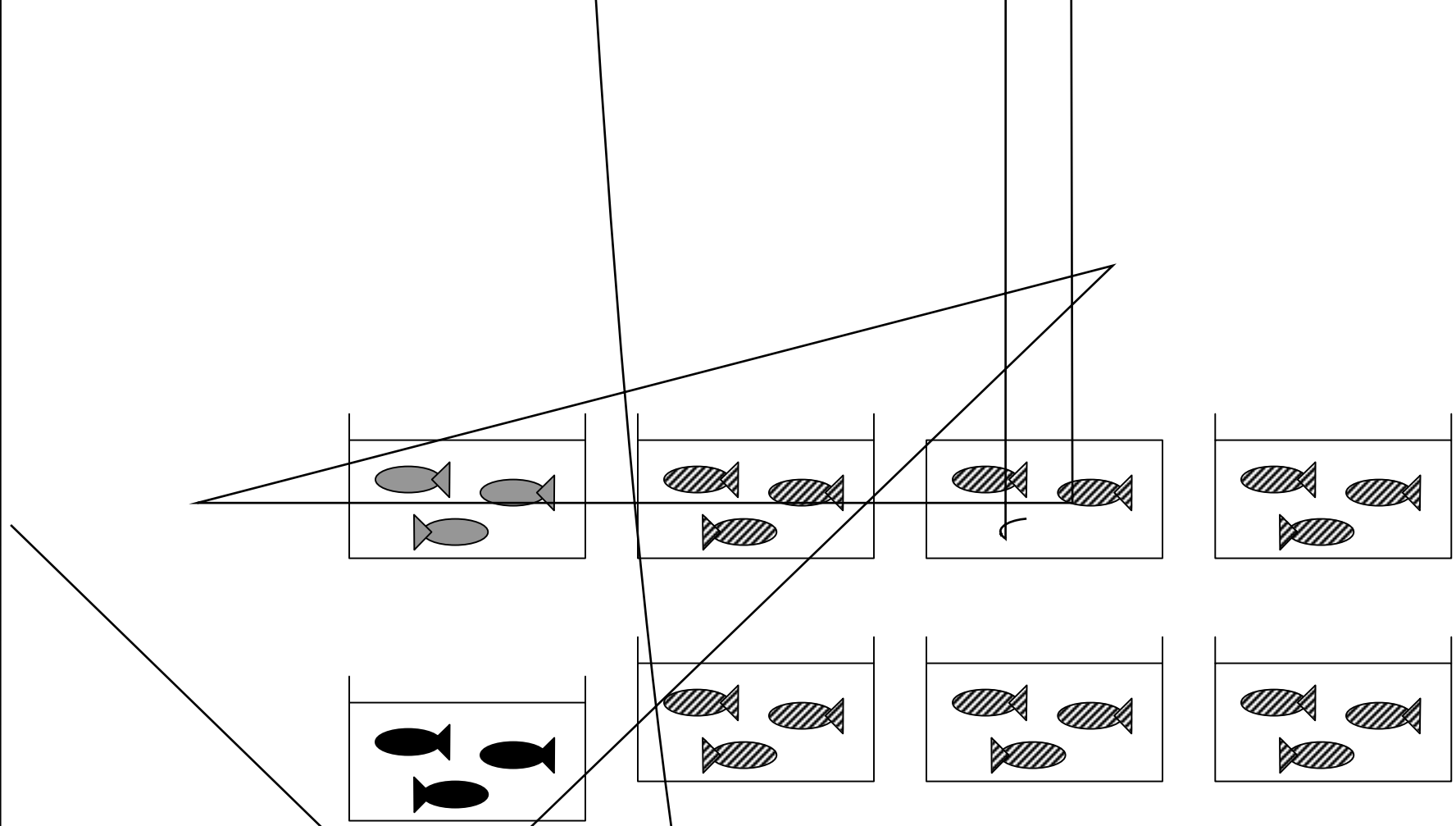


Figure 5. Experimental set-up. Experimental fish received PCB & DPE spiked experimental food for 14 days (the uptake phase), & control food for a further 14 days (the elimination phase). (Only 6 of 10 experimental tanks are shown here). Day 0 and Day 30 fish controlled for background contamination and 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)8.08 580.08 43.58.76 0 TD -0584.0E

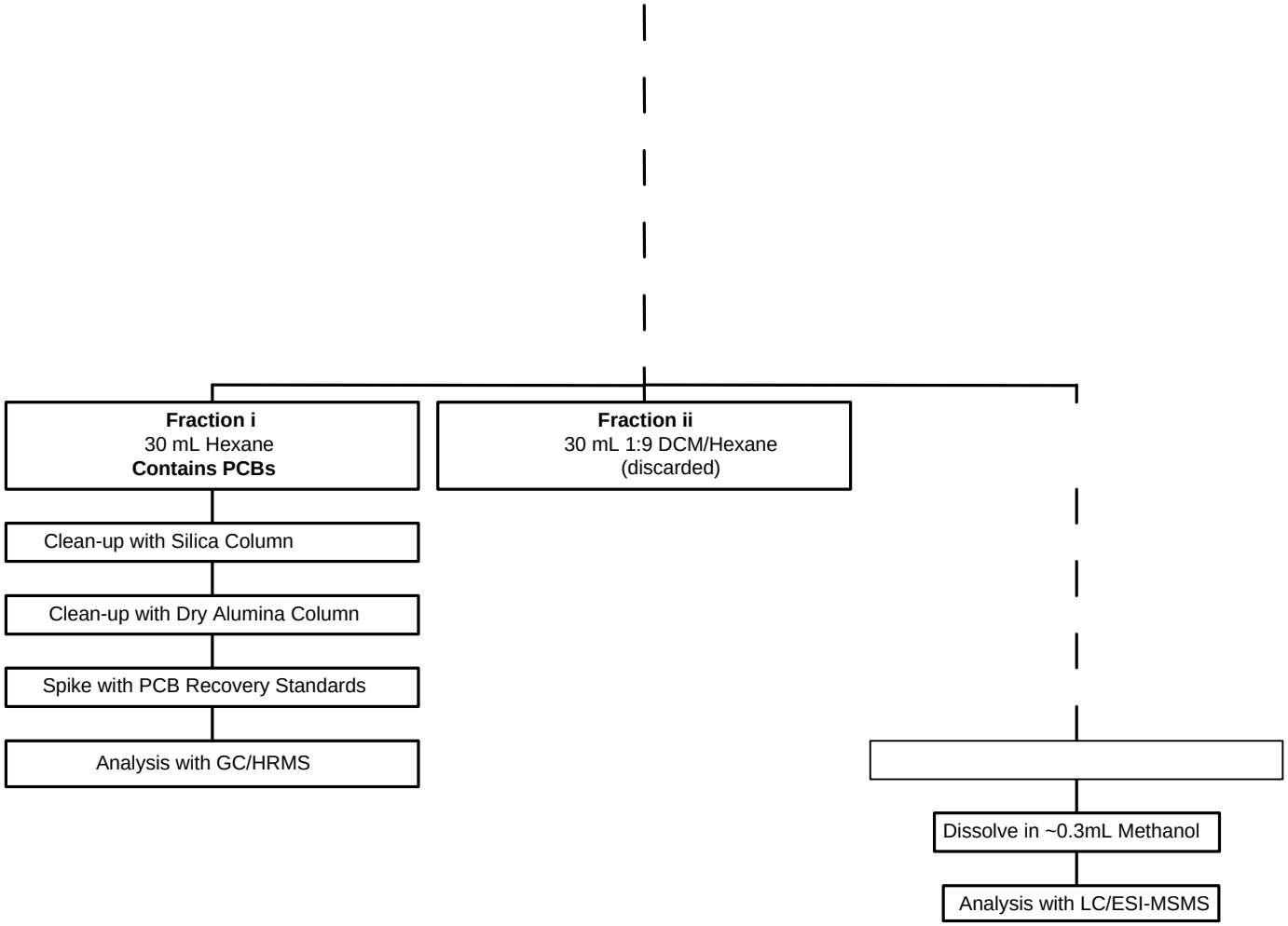


Figure 6. DPE & PCB extraction, cleanup & analysis in food and biota samples. Details are reported in (Lin, Ikonomou et al. 2003)

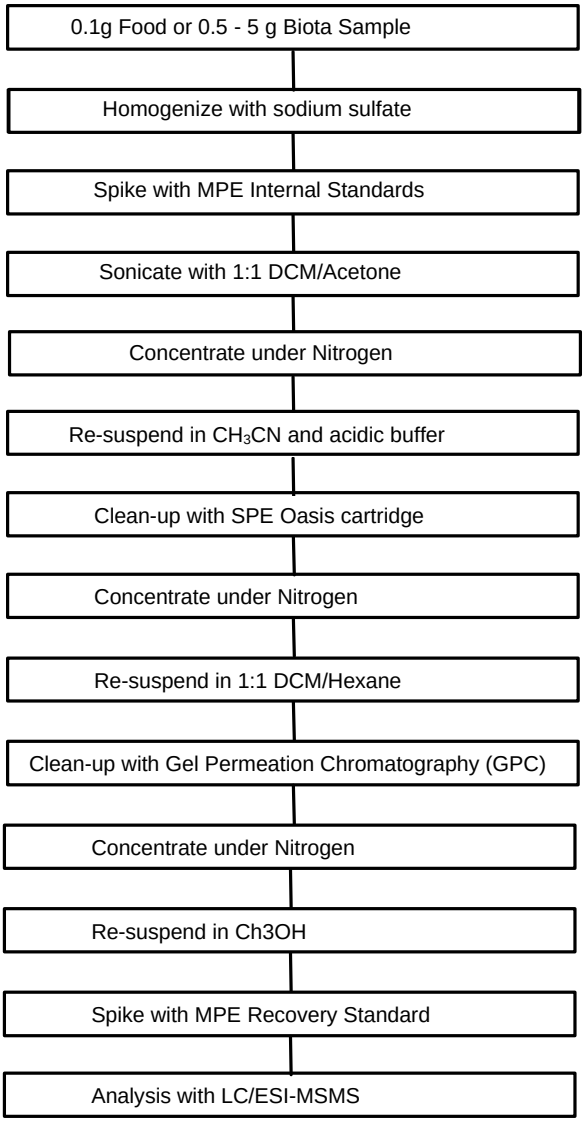


Figure 7. Summary of MPE extraction, cleanup and analysis in food and biota samples. Details will be reported in (Ikonomou, Hoover et al. 2003)

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

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

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	0.5y Tw (0.0033.12 0 TD 07j 2

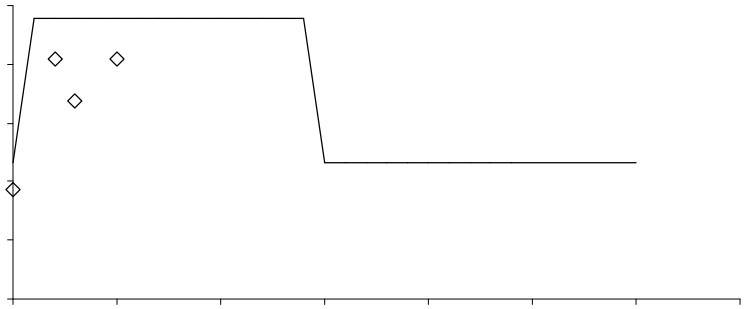
Table 12. Estimated biomagnification factors (BMFs) for PCBs and DPEs in sculpin liver and muscle. BMF muscle = C_m/C_d, and BMF liver = C_L/C_d (using lipid normalized concentrations). BMFs are substantially smaller for DPEs than for PCBs. BMFs < 1 indicate that compounds do not biomagnify in Staghorn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

Figure 8. Mean PCB & DPE concentrations



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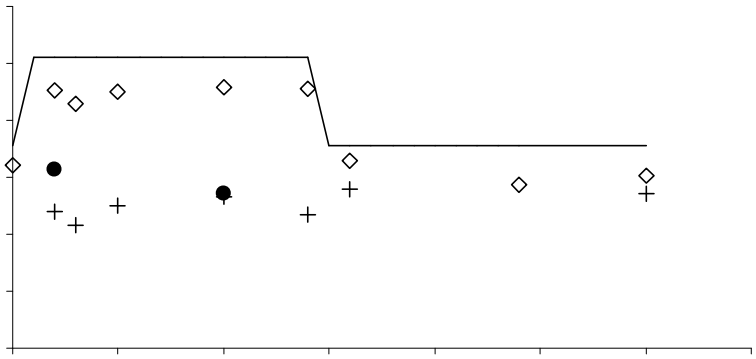

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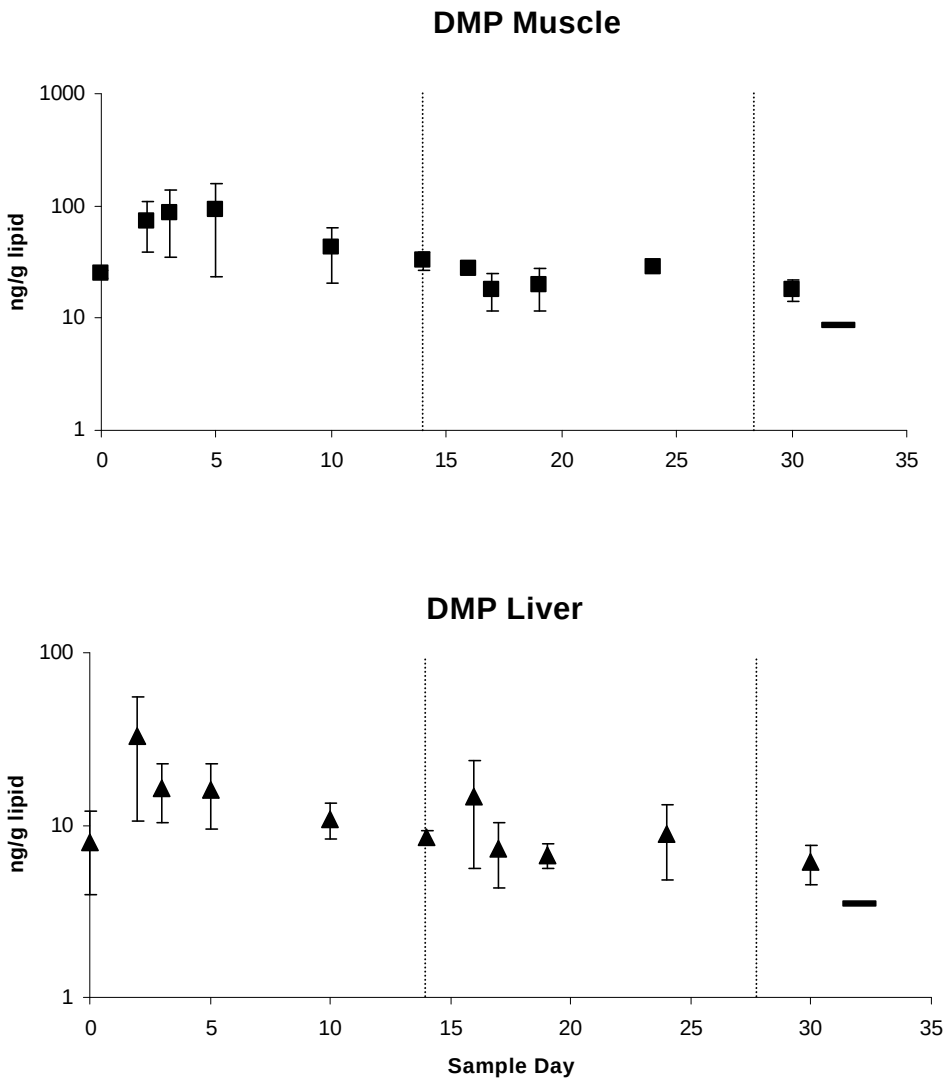
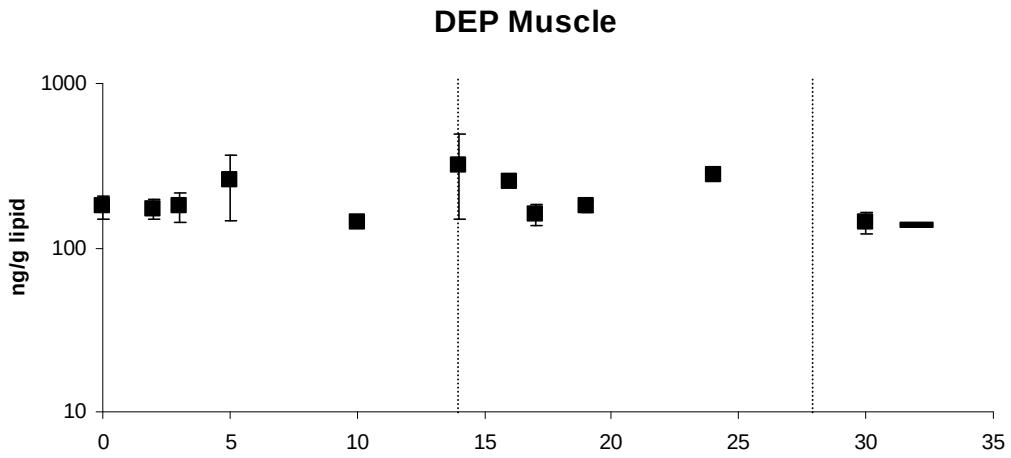
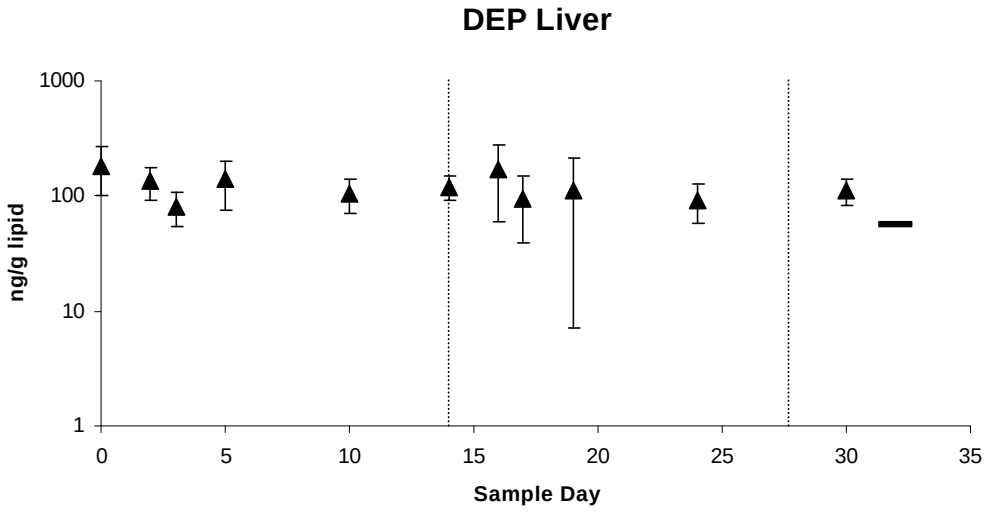


Figure 9. DPE concentrations (ng/g lipid +/- 1 standard deviation) over time in muscle (top) and liver (bottom) The dashed vertical lines represent the end of the uptake phase (Day 14) and the end of the elimination phase (day 28). Water uptake control fish are plotted at Day 30. When applicable, matrix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





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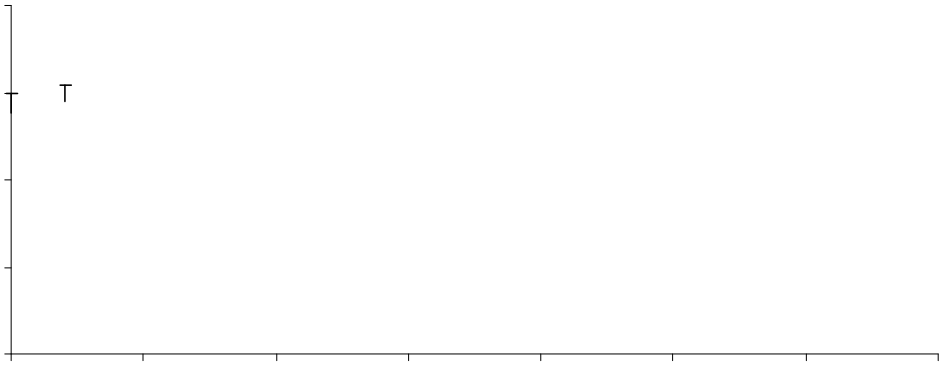

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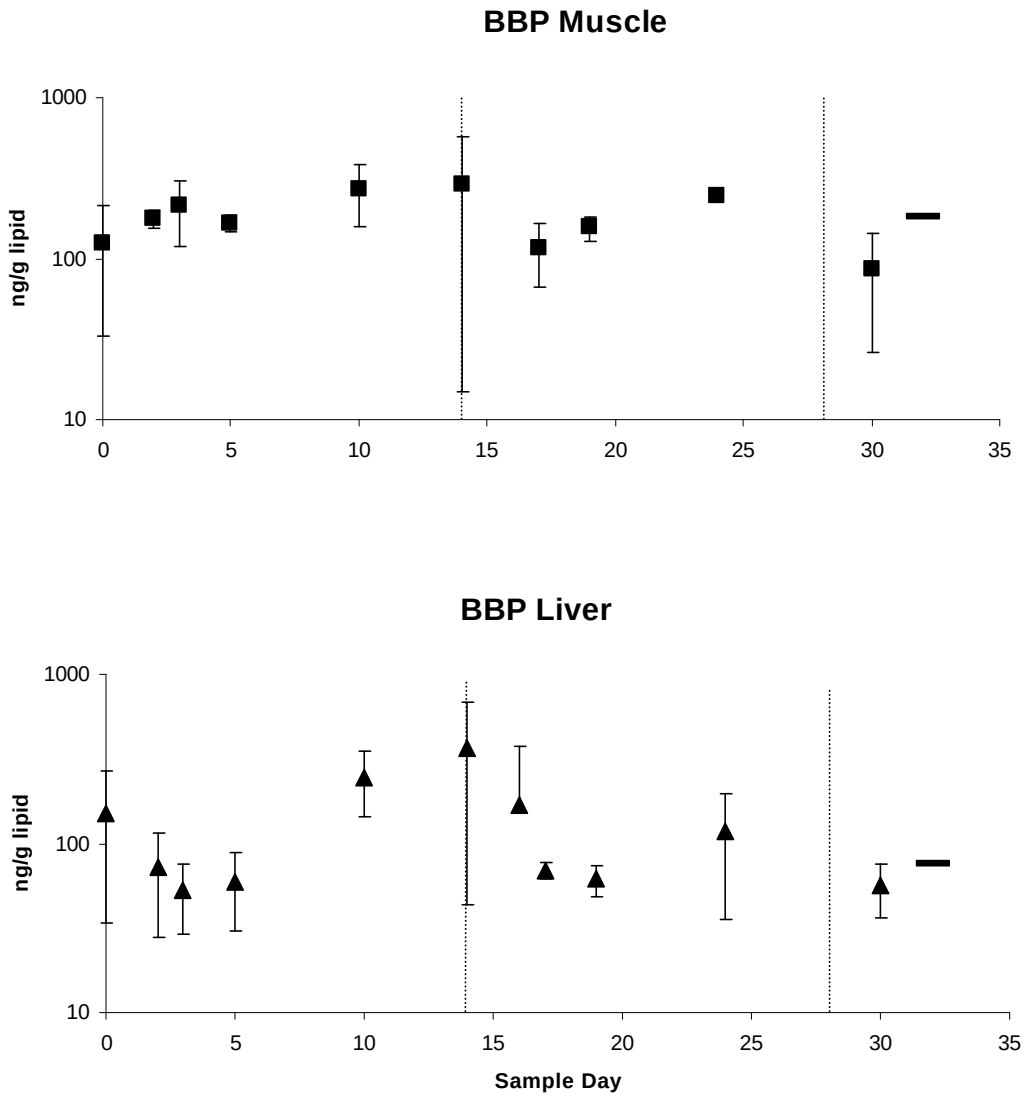

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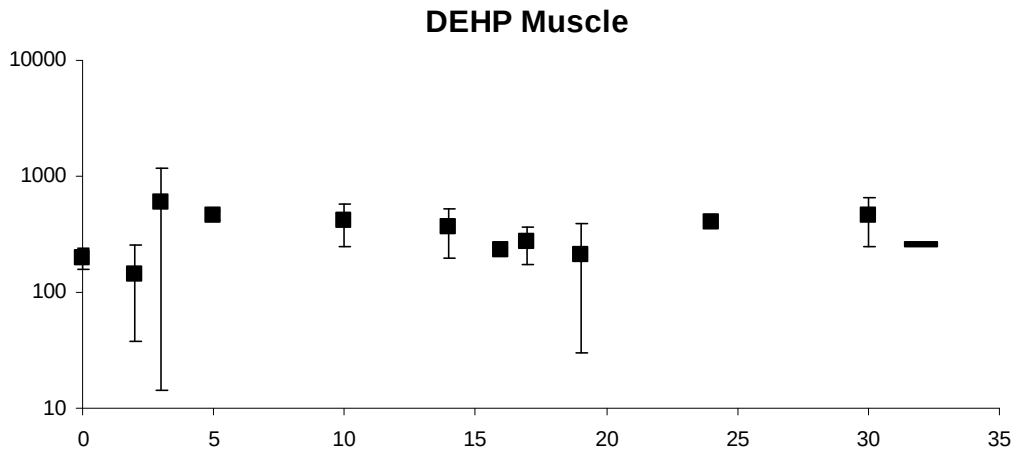

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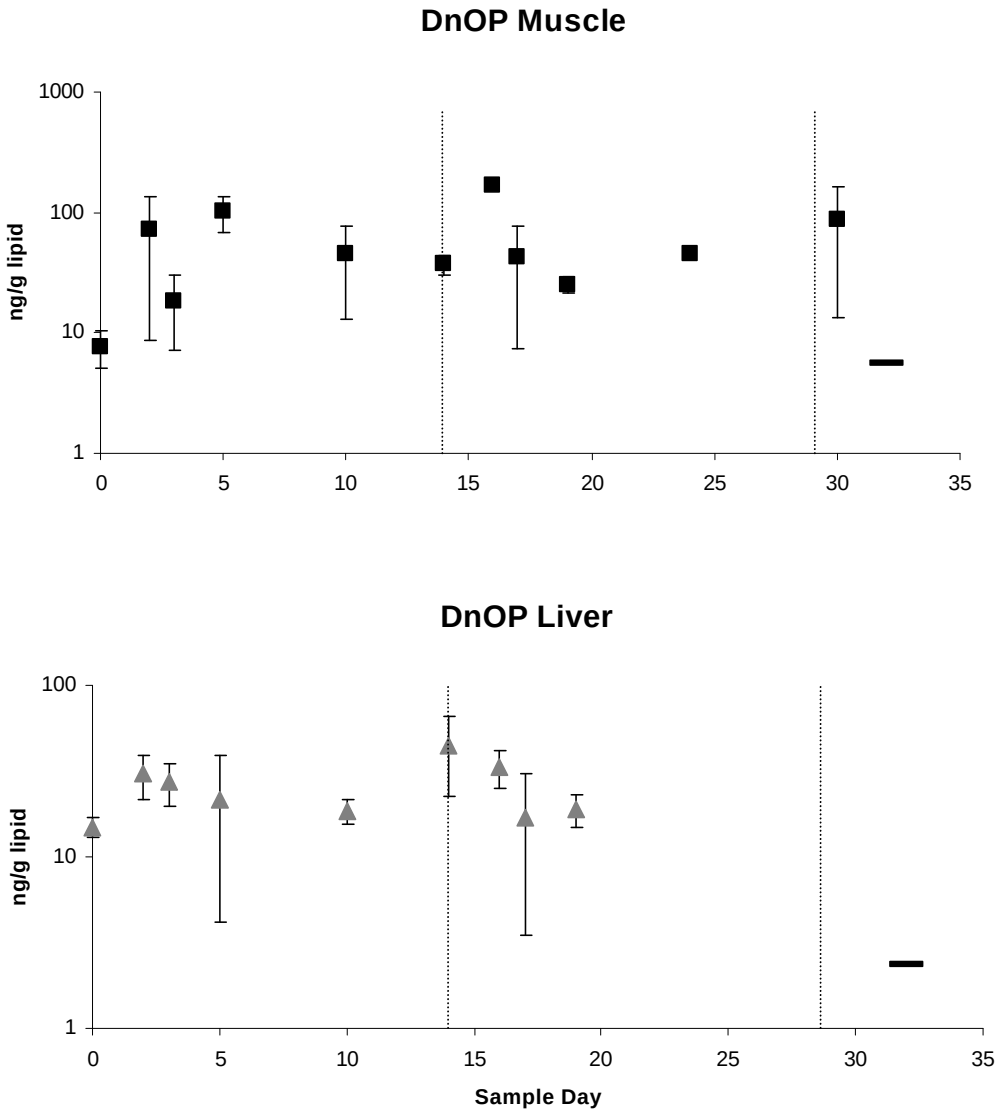

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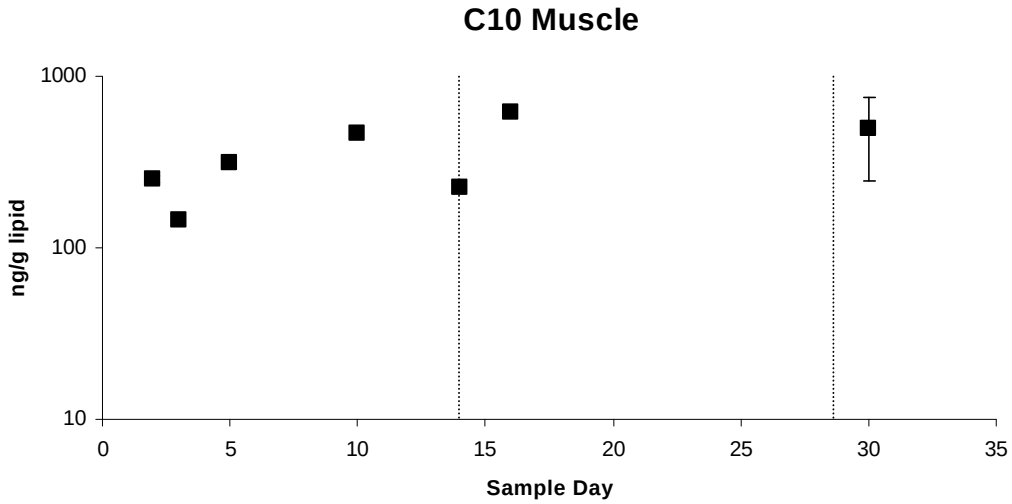


Figure 9 continued

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.

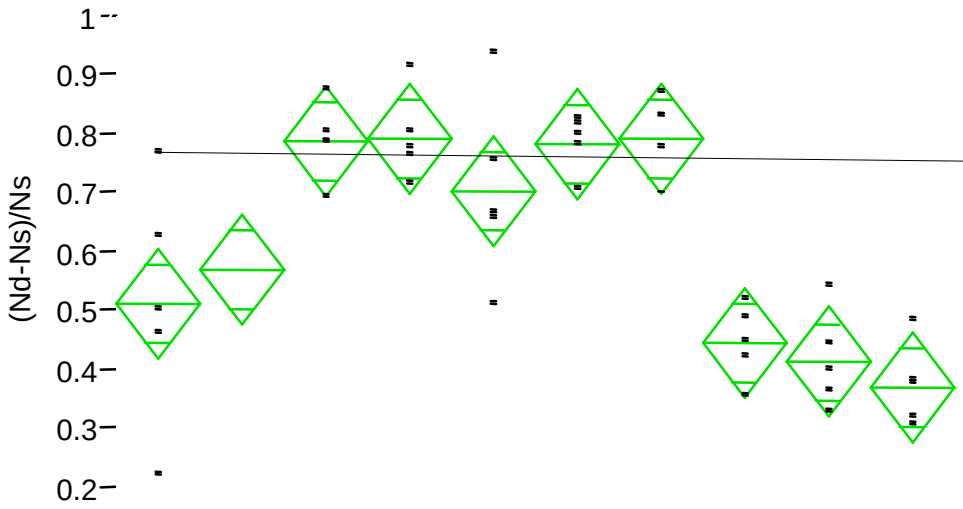


Figure 11. ANOVAs testing for differences in chemical loss along the GIT across all DPE and PCB congeners: A. Fraction of DPE lost in the stomach (Nd-Ns)/Nd. B. Fraction of DPE lost in the intestine (Ns-Ni)/Nd. This figure shows that more DPE than PCB is lost in the stomach (except BBP and C10) and in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

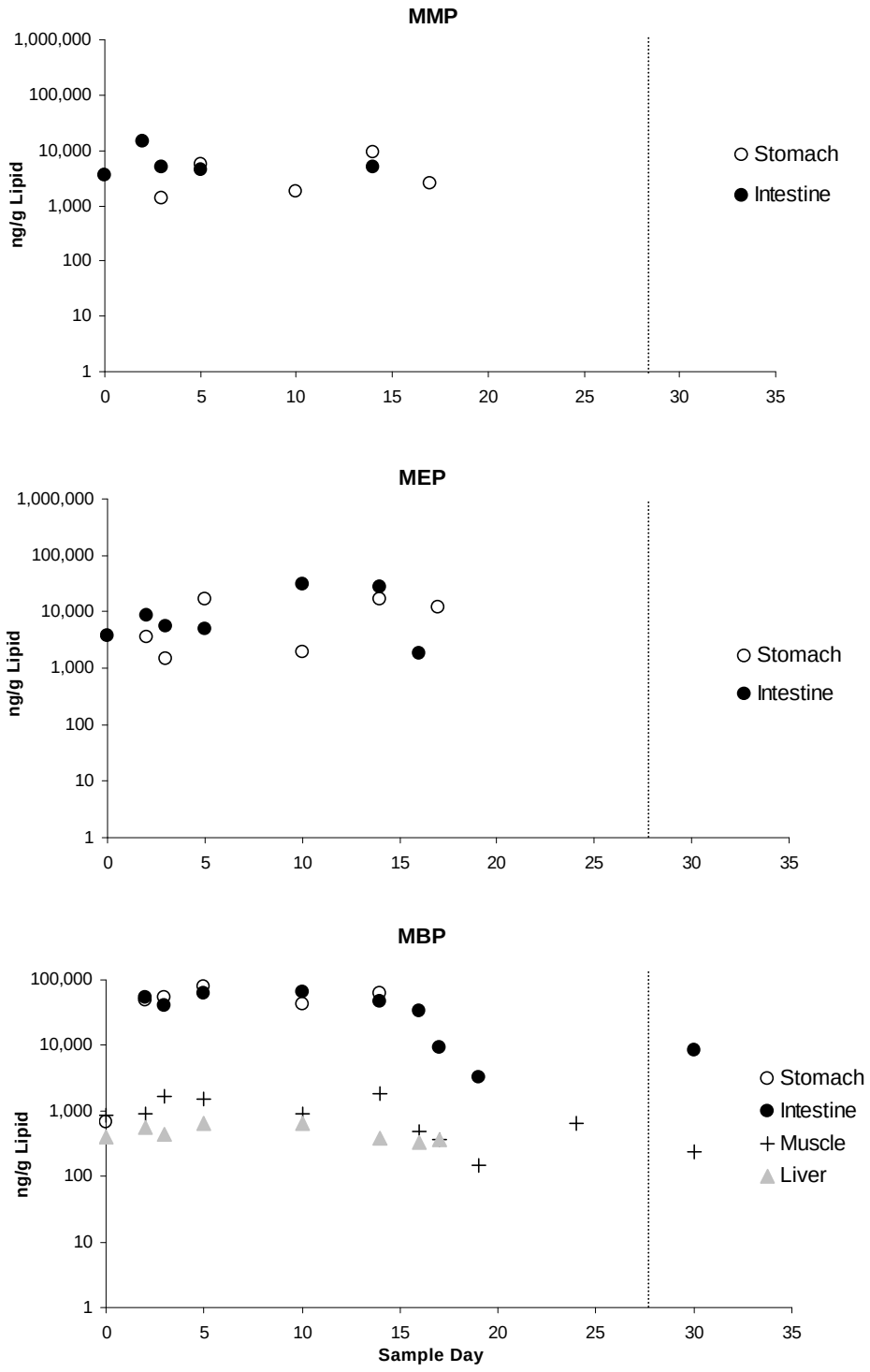
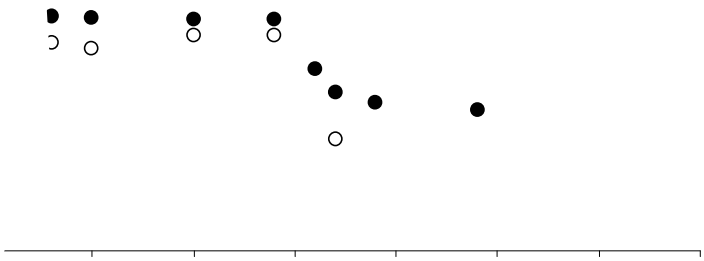


Figure 12. MPE concentrations (ng/g lipid) over time in all matrices. When applicable, matrix specific MRLs are plotted at day 32 (see Table 8)



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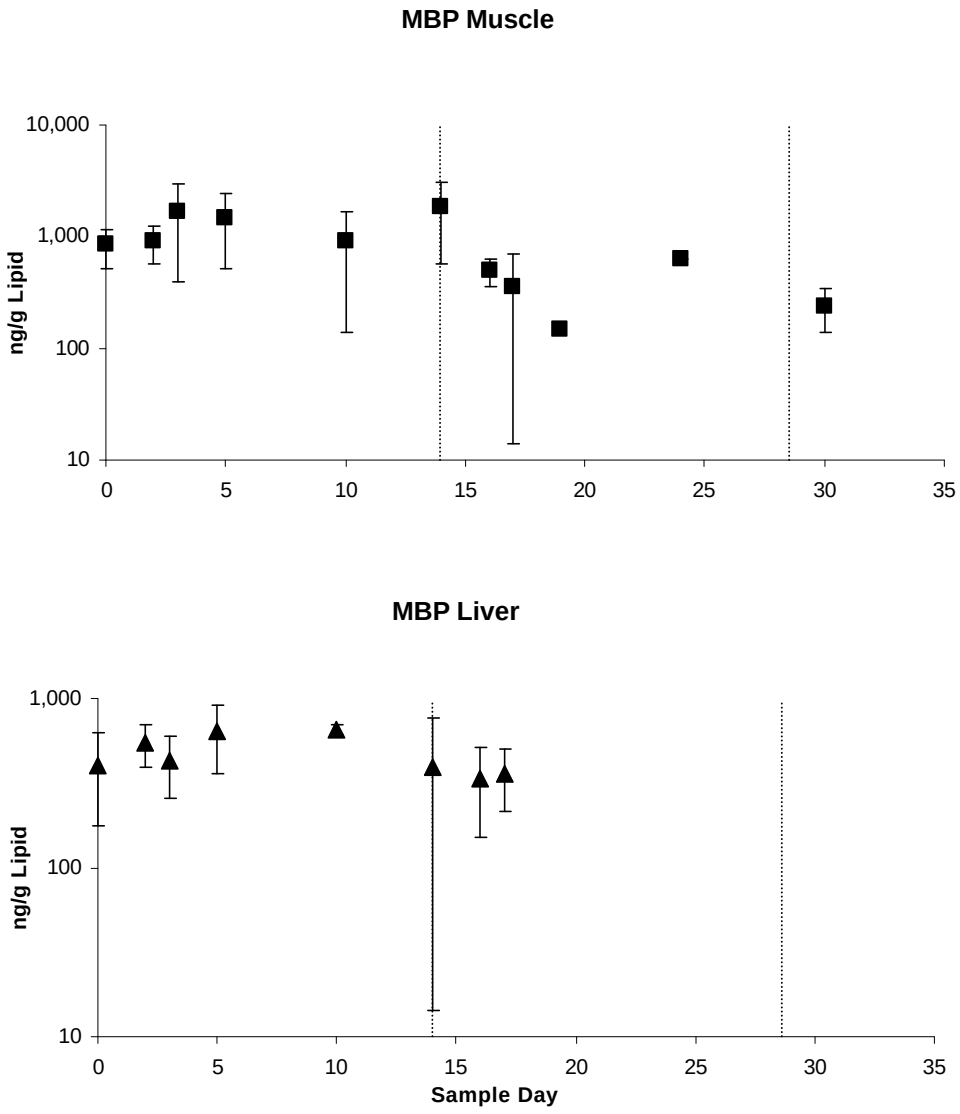
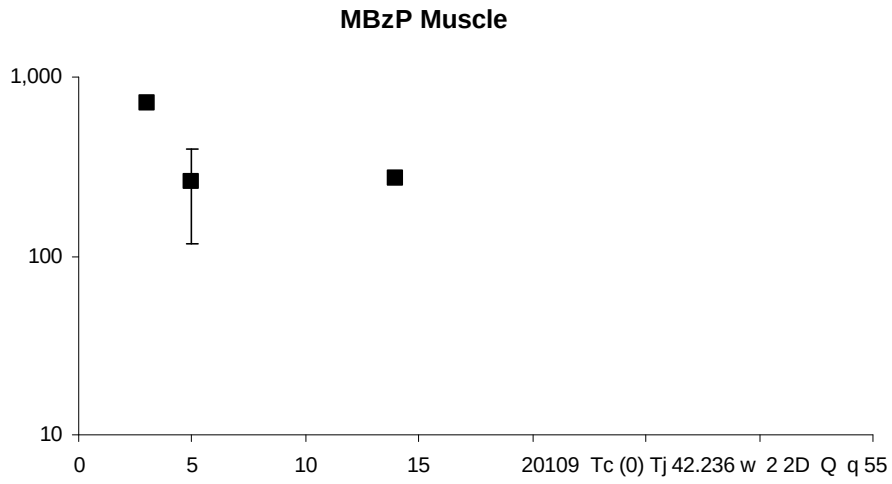


Figure 13. MPE concentrations (ng/g lipid +/- 1 standard deviation) over time in muscle (top) and liver (bottom) The vertical lines represent the end of the uptake phase (Day 14) and the end of the elimination phase (day 28). Water uptake control fish are plotted at Day 30. When applicable, matrix specific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



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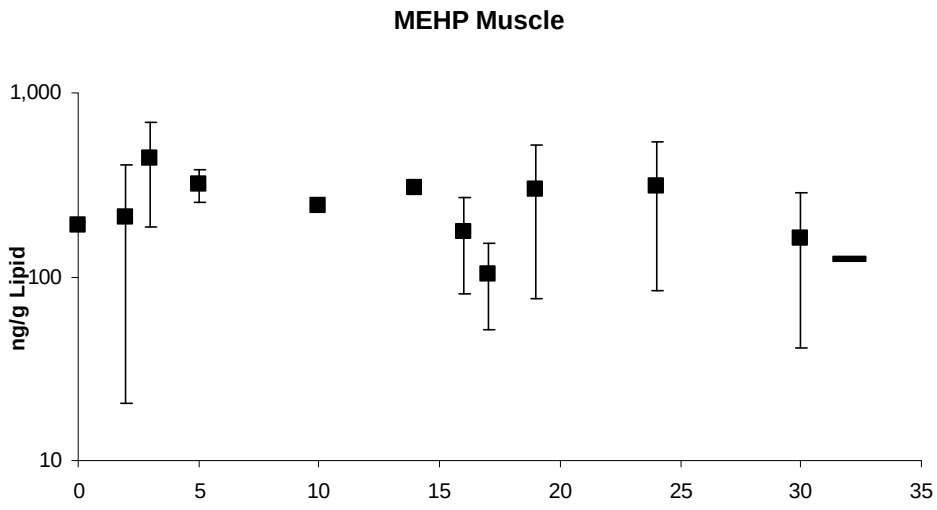

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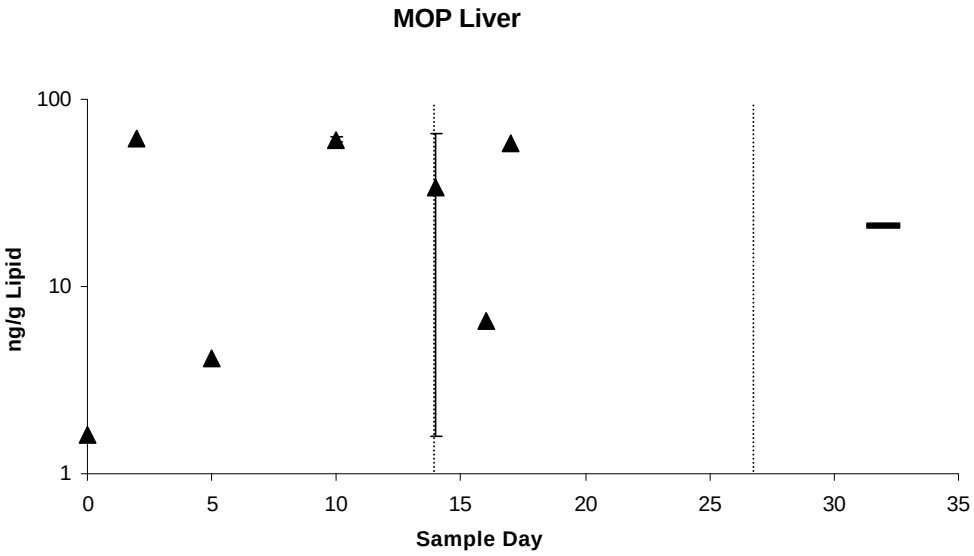

Figure 13 continued

Table 16. Fraction of neutral and ionized MPE found in the stomach and intestine,

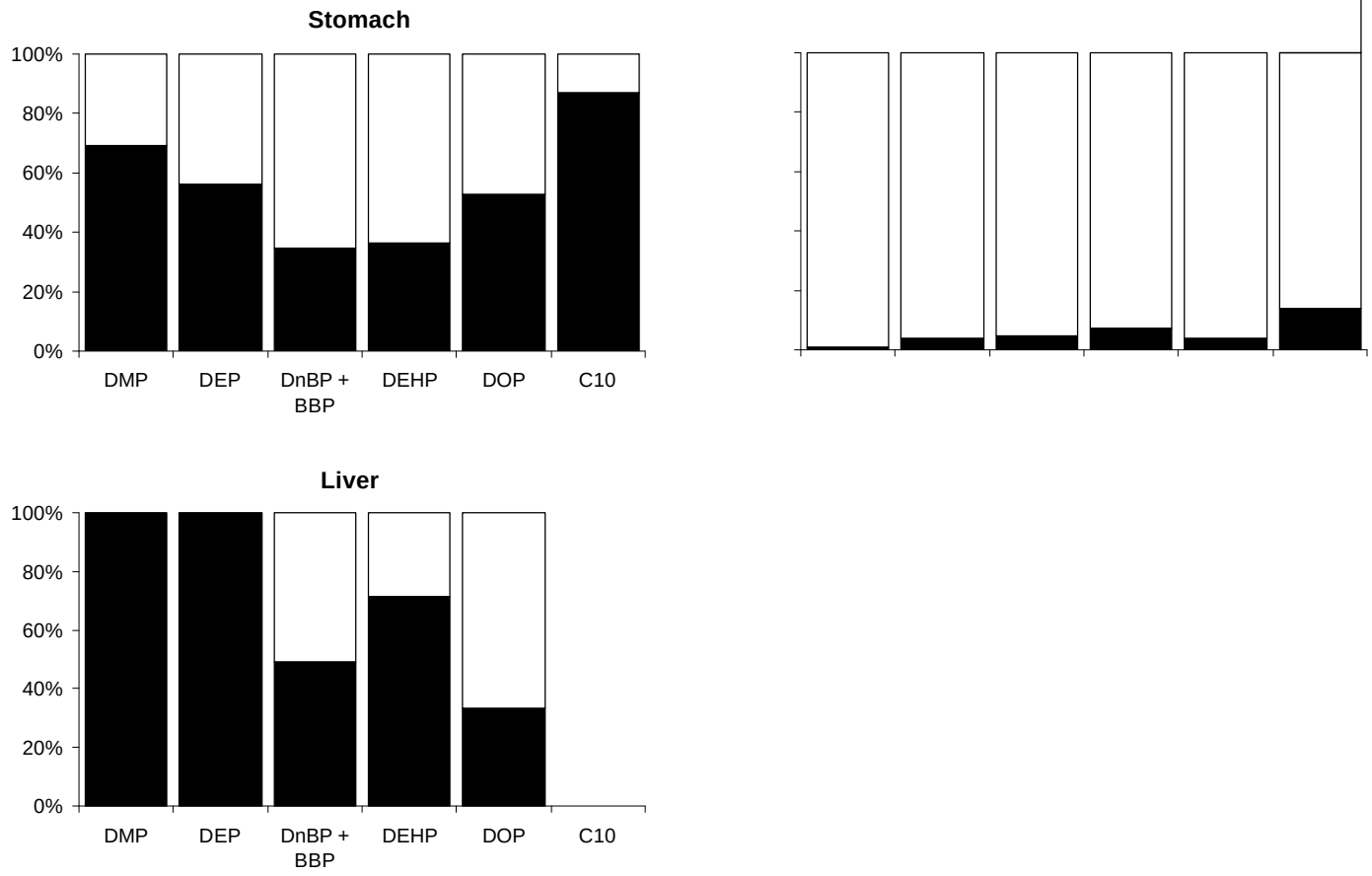

Figure 14. Fractions of total PE found as DPE and MPE in the stomach, intestine, liver and muscle of Staghorn sculpin (means across the uptake phase). DnBP and BBP are grouped together to avoid having to divide the observed MBP between these two parent compounds

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							

24.3.5643.56 0.16 TD 0 .T123 4.7248 re f BT 120.24 2 .t1.04 0 T584 0 T

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.

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

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

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		4.46	

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.

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 34. Mean MPE concentrations and standard deviations (ng/g) in the control food

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 1

Table 37. Mean PCB liver conc/.

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	2.44E+01	1.09E+02

e72.48 1.0.0089 Tw ( ) Tj 2619 h W0b h W0b h59.19E+02

10Tc 0 Tw (1.30E+02) Tj 42.72 0 TD 0 Tc -0.008 -0.020.0089 1.52 0c -0.0378 Tw (sl2D -0.0247 94.48 1.0.008

8 1.0.0089 Tw ( ) Tj 2672