Uptake of Contaminants of Emerging Concern by the Bivalves *Anodonta californiensis* and *Corbicula fluminea*

Niveen S. Ismail[1,2], *Claudia E. Müller*[1,2], *Rachel R. Morgan*[1,2], *Richard G. Luthy*[1,2*]
[1] Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305, United States
[2] ReNUWIt Engineering Research Center

Corresponding Author
*Phone: 650-721-2615; fax: 650-725-9720; e-mail: luthy@stanford.edu
Number of pages: 11
Number of tables: 7
Number of figures: 5

Sample Extraction Details:

Tissue, Algae/(pseudo)feces, and Filter Samples:

Samples were freeze dried and homogenized then spiked with 10 µl of a 2 µg/ml surrogate standard mix of triclocarban $^{13}C_6$, ibuprofen-d_3, clofibric acid-d_4, diuron-d_6, propranolol-d_7, and tris(2-chloroethyl)phosphate-d_{12}. A blank sample containing methanol and the internal standard was also prepared with each batch of samples extracted. Samples were extracted by adding 2 ml methanol, vortexing, sonicating for 10 min and shaking for a minimum of 2 hours. After shaking, the samples were centrifuged for 20 minutes at 4700 rpm. The supernatant was transferred to a pre-cleaned 15 ml centrifuge tube and the extraction was repeated two times. The extract was then concentrated to 1ml under nitrogen gas. As the extracts contained lipids and other matrices, they were subjected to a solid phase extraction (SPE) step for clean-up. In preparation for use with hydrophilic-lipophilic balance (HLB cartridges, Waters Corp, Millford, MA) SPE, 14 ml of water was added to the concentrated extract for tissue and filter samples.

For algae and (pseudo)feces samples containing chlorophyll, an additional liquid-liquid extraction with hexane was completed prior to use with HLB cartridges. Chlorophyll a containing extracts were also concentrated to 1 ml with nitrogen then 0.2 ml of water and 1ml of hexane were added. After shaking for 2 hours the samples were centrifuged at 4700 rpm for 20 minutes and the hexane layer containing chlorophyll a was discarded and 14 ml of MilliQ water was added to the remaining methanol fraction.

A solid phase extraction vacuum manifold was utilized with the 500 mg HLB cartridges. The HLB cartridges were preconditioned with 5 ml MTBE, 5ml methanol, and 5 ml MilliQ water. Samples were loaded by gravity only and the sample container was washed with 5ml of MilliQ water, which was also added to the cartridges. The cartridges were then dried under vacuum for 1 hour. After drying, the cartridges were eluted by gravity with 6 ml of 50/50 (v/v) MTBE/methanol followed by 7 ml of methanol. The 7 ml methanol was used to wash the sample container to remove any contaminant sorbed to the wall then used for cartridge elution then used in the SPE cartridges. The vacuum was used to recover any remaining solvent.

The extracts were then vortexed and concentrated to 0.5 ml under nitrogen. An additional 0.2 ml of methanol was added to the concentrated samples followed by 0.3ml of water. Then 5 µl of 500 ng/ml of internal standard TCC-d_7 was added. The samples were vortexed and transferred to 1.5 ml autosampler vials for analysis.

Water Samples:

Water samples did not undergo preliminary extraction steps and were extracted directly via HLB cartridges as described above.

LC-MS/MS analysis:

Samples were analyzed after extraction and cleanup utilizing an LC-MS/MS. A Shimadzu LC-10ADVP system (Columbia, MD) and AB Sciex API 3000 mass spectrometer (Framingham, MA) were utilized. Analytical procedure was dependent on analysis in positive mode or negative mode for the mass spectrometer. A Targa C18 column (40 x 2.1mm, 5 µm particles) fitted with a C18 guard column (Haipeek Targa C18, 20 x 2.1m, 5 µm) was utilized (Higgins Analytical, Mountain View, CA) and a sample volume of 10 µL was injected onto the C18 column for all samples.

For positive mode, liquid chromatography was performed using aqueous formic acid (0.04 mM) and methanol binary gradient at a flow rate of 0.3 mL/min. Initial methanol concentration was 10% then ramped up to 98% over 8 min, held at 98% for 1 min and reverted to 10% at 9.5 min followed by a 4.5 min hold. For negative ESI mode, liquid chromatography was performed using aqueous ammonium acetate (2mM) and methanol binary gradient at a flow rate of 0.3 mL/min. Initial methanol concentration was 5% then ramped up to 98% over 8 min, held at 98% for 1 min and reverted to 5% at 9.5 min followed by a 4.5 min hold. Table S3 lists the primary and secondary transitions and the limit of quantification (LOQ) for each compound. The LOQ was calculated based on the lowest value in the calibration curve with an accuracy of between 80-120%. The LOQ values presented in Table S3 for aqueous values apply for water, (pseudo)feces, and algae samples.

Table S1. Test Chemicals and their properties

Name	Class	Log K_{ow}	pKa	Log D_{ow} (pH 6.8)
Clofibric Acid	Pharmaceutical/Herbicide	2.7	3.2	-0.9
Diuron	Herbicide	2.68	N/A	2.68
Ibuprofen	Pharmaceutical	2.9	4.5	0.6
Propranolol	Pharmaceutical	3.48	9.5	0.8
TCC	Biocide	4.9	N/A	4.9
TCEP	Flame-Retardant	1.44	N/A	1.44
TDCPP	Flame Retardant	3.65	N/A	3.65

Table S2. Parameters for flow through experiment for *C. fluminea*

Number of bivalves	49
Duration: Exposure/Depuration	28 days/6 days
Water volume in Tank	18 L
Hydraulic Residence Time (days)	1
Water sample intervals/location	Daily/Control + Bivalve Inlet/Outlet+ Source Tank
Bivalve sampling	Every 3 days
Number of bivalves at each sample point	3

Table S3. Limit of Quantification (LOQ) and MS parameters for tested analytes

Analyte	Primary Transition	Secondary Transition	ESI Mode	LOQ Aqueous (ng/L)	LOQ Tissue (ng/g_{dw})
TCC	313 ->160	313->126	Negative	8.5	5.3
Clofibric Acid	213->127	213->85	Negative	8.5	5.3
Ibuprofen	205->159	205->161	Negative	8.5	5.3
Propranolol	260->116	260->183	Positive	8.5	5.3
TCEP	285->99	287->99	Positive	8.5	5.3
TDCPP	433->99	431->99	Positive	8.5	5.3
Diuron	233->72	233->46	Positive	17.0	10.7

Table S4. Surrogate recoveries for tested analytes

Analyte	Labeled Standard	% Recovery		
		Particulate	Water	Tissue
TCC	TCC-$^{13}C_6$	48	56	72
Propranolol	Propranolol-d_7	67	58	72
Clofibric Acid	Clofibric acid-d_4	89	106	95
Ibuprofen	Ibuprofen-d_3	76	93	98
Diuron	Diuron-d_6	42	56	65
TDCPP	TCEP-d_{12}	27	53	52
TCEP	TCEP-d_{12}	38	55	52

Table S5. Percent recovery determined by spike recovery relative to internal standard for tested analytes

Analyte	% Relative Recovery ± Std. Deviation (n=6)		
	Particulate	Water	Tissue
TCC	79±3	98±1	98±3
Propranolol	86±3	97±2	96±2
Clofibric Acid	98±2	95±6	97±5
Ibuprofen	92±3	88±4	97±7
Diuron	87±10	92±6	88±11
TDCPP	76±13	91±11	71±9
TCEP	75±4	89±17	72±3

Table S6. Repeatability for tested analytes reported as relative standard deviation

Analyte	% Relative Standard Deviation (n=10)
TCC	9
Propranolol	2
Clofibric Acid	11
Ibuprofen	13
Diuron	11
TDCPP	6
TCEP	7

Table S7. Mean mass recovery values (%) and standard deviations from mass balance experiments (n=6)

Analyte	Water		Tissue		(Pseudo)feces	
	Anodonta	*Corbicula*	*Anodonta*	*Corbicula*	*Anodonta*	*Corbicula*
TCC	11±2%	8±2%	36±2%	28±2%	22±8%	28±1%
Propranolol	39±1%	43±3%	34±4%	18±1%	5±1%	2±1%
TDCPP	39±13%	42±18%	2±3%	5±3%	9±3%	8±6%
Diuron	40±10%	48±7%	0%	2±2%	24±7%	28±8%
Clofibric Acid	62±11%	74±14%	3±4%	13±10%	3±2%	3±3%
TCEP	82±6%	80±4%	2±2%	5±5%	2±2%	7±3%
Ibuprofen	88±7%	73±19%	3±2%	0%	0%	0%

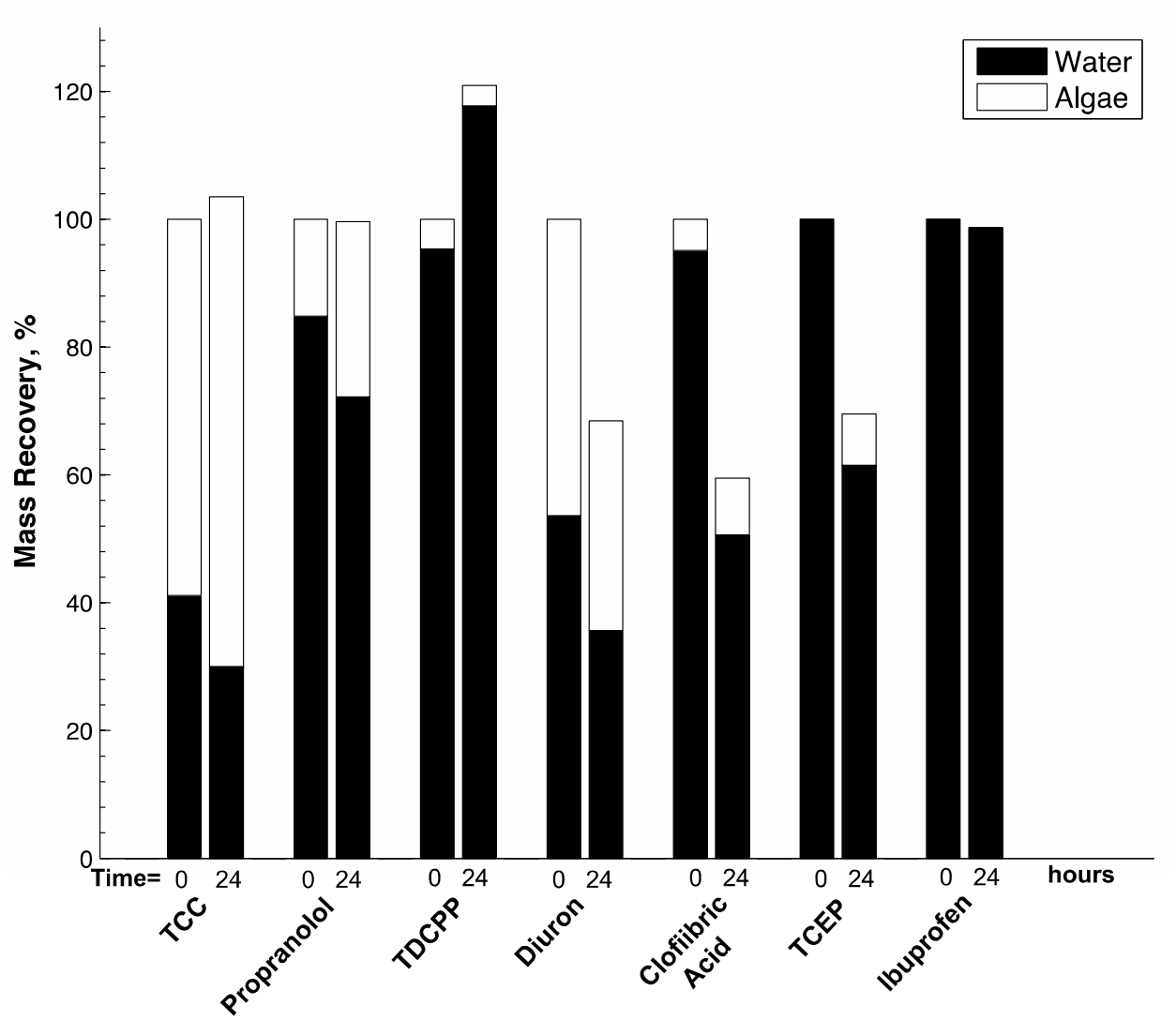

Figure S1. Distribution of analytes (initial spike concentration of 250 ng/L) between water and algae in control beakers at the start (time=0 hours) and end (t=24 hours) of batch experiment

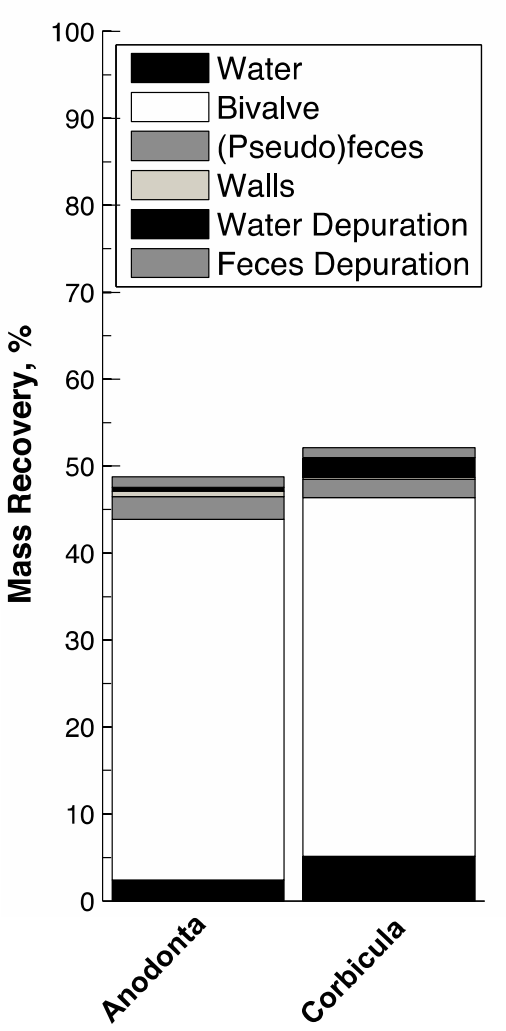

Figure S2. Average mass distribution of TCC spiked in water without particulate matter after 24-hour batch exposure followed by 72 hours of depuration for *A. californiensis* and *C. fluminea*.

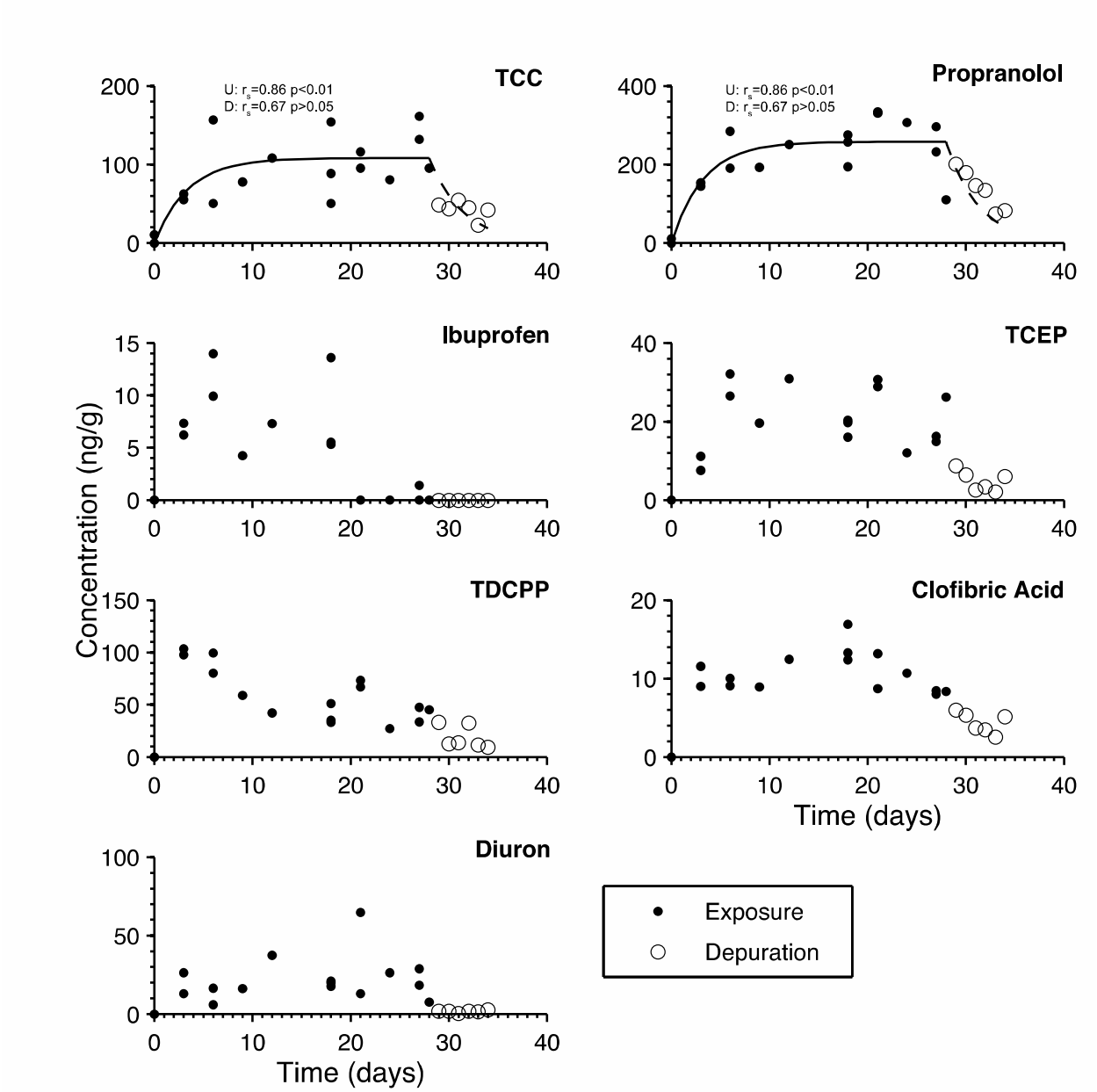

Figure S3. Uptake and depuration kinetics of 7 analytes in *C. fluminea*. The solid and dotted lines represent first order kinetics for uptake and depuration respectively and r_s values show the correlation between experimental and modeled data (U=Uptake and D=Depuration). Concentration is based on dry weight. Fit lines were omitted for compounds with r_s (spearman's rho) values less than 0.7.

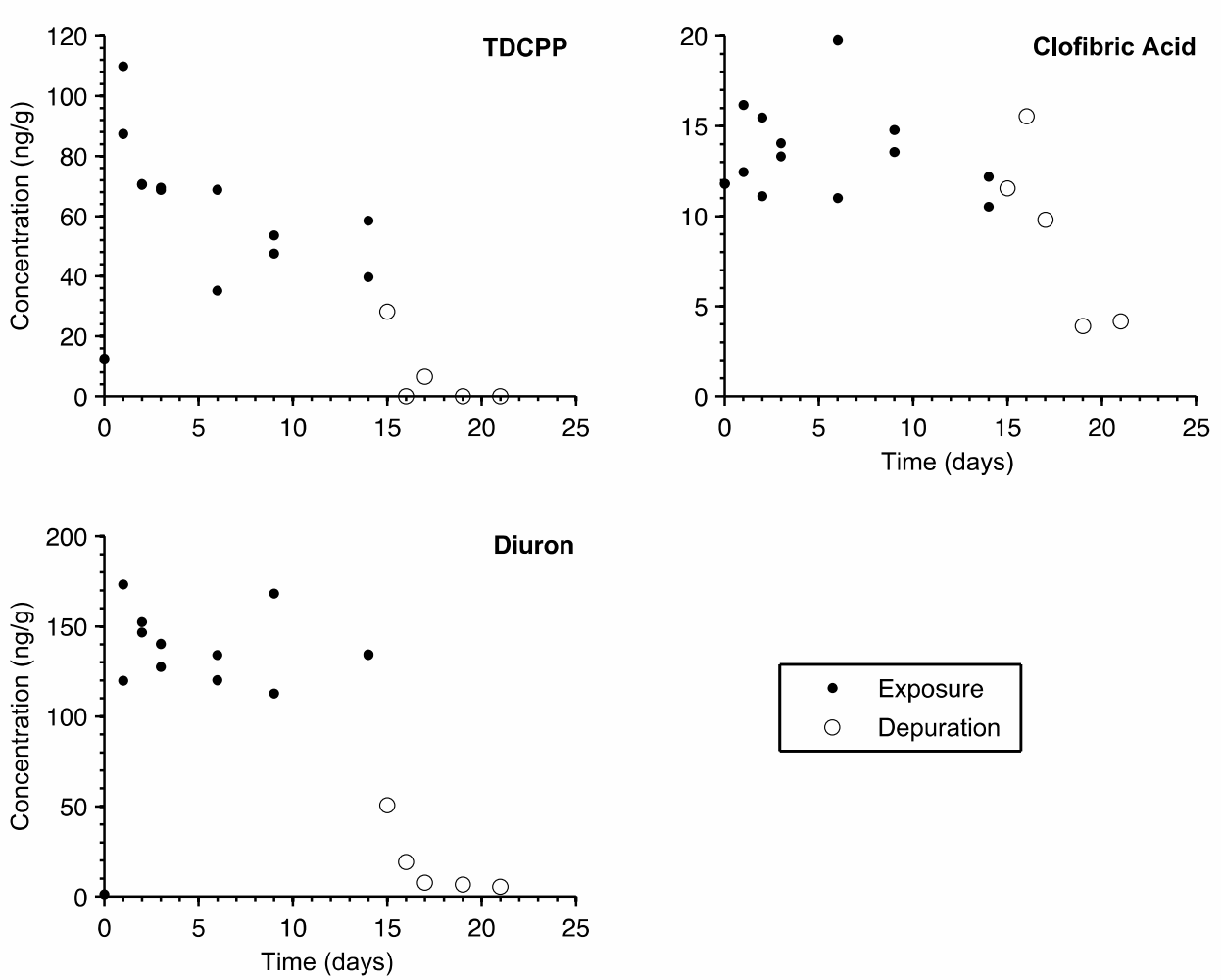

Figure S4. Uptake and depuration kinetics of 3 analytes in *A. californiensis*. Concentration is based on dry weight. Fit lines were omitted for compounds with r_s (spearman's rho) values less than 0.7.

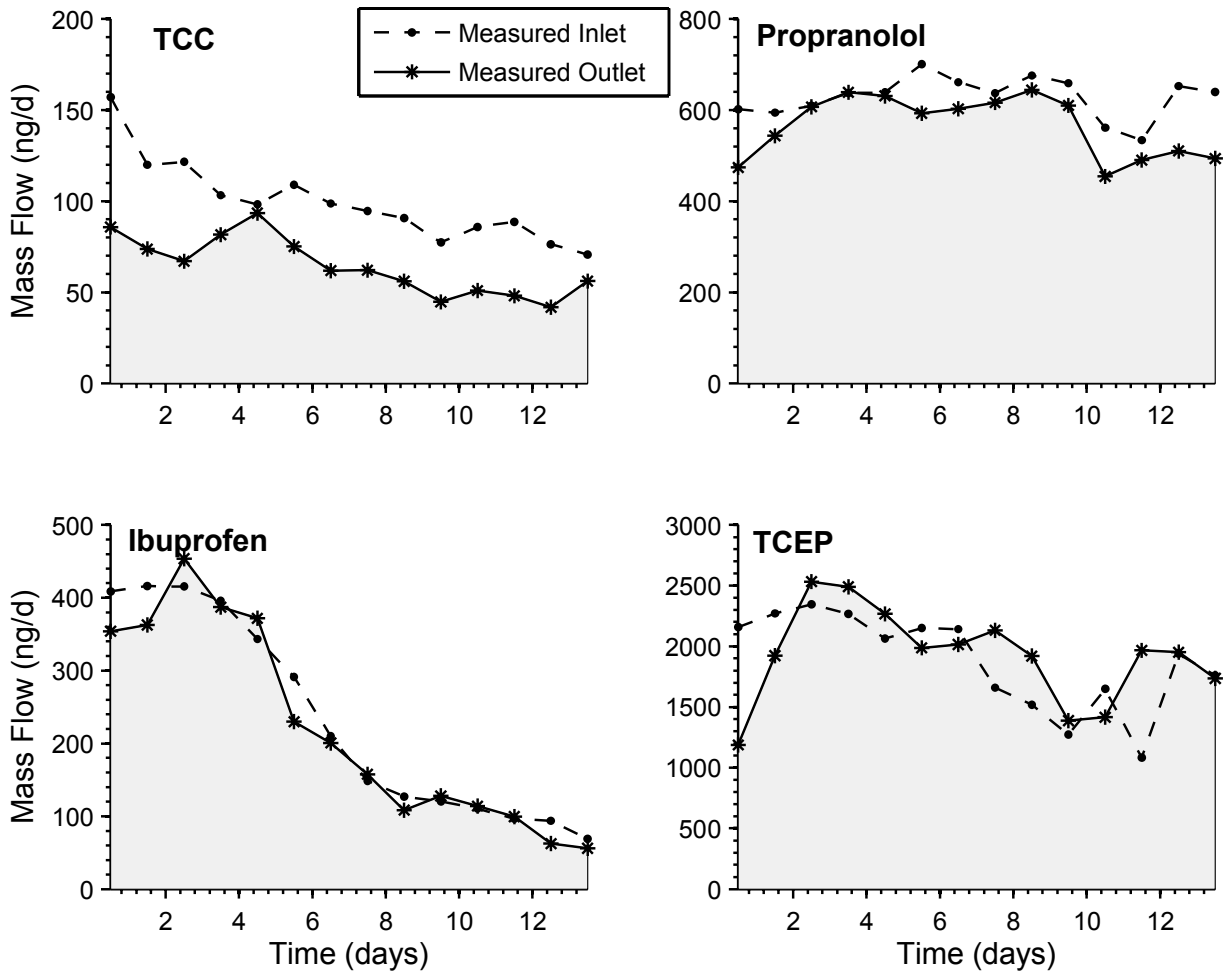

Figure S5. Mass flow comparison of water outlet and water inlet for the control tank. Reported inlet and outlet values are based on an average value over two days.