Supplementary Information

Occurrence of contaminants of emerging concern in mussels (*Mytilus* spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent

Nathan G. Dodder[a,*], Keith A. Maruya[a], P. Lee Ferguson[b], Richard Grace[c], Susan Klosterhaus[d,1], Mark J. La Guardia[e], Gunnar G. Lauenstein[f,2], Juan Ramirez[g]

[a] Southern California Coastal Water Research Project, Costa Mesa, CA 92626, United States
[b] Department of Civil & Environmental Engineering, Duke University, Durham, NC 27708, United States
[c] AXYS Analytical Services, Sidney, BC V8L 5X2, Canada
[d] San Francisco Estuary Institute, Richmond, CA 94804, United States
[e] Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062, United States
[f] National Oceanic and Atmospheric Administration, National Centers for Coastal Ocean Science, Silver Spring, MD 20910, United States
[g] TDI-BI/B&B Laboratories, College Station, TX 77845, United States

*Corresponding author email: nathand@sccwrp.org
[1] Present address: Cradle to Cradle Products Innovation Institute, San Francisco, CA 94108, United States
[2] Present email (retired): gglauenstein@gmail.com

Contents

Analytical Methods

Alkylphenols

Target analytes: 4-nonylphenol (NP), 4-n-octylphenol (OP), 4-nonylphenol monoethoxylate (NP1EO) and 4-nonylphenyl diethoxylate (NP2EO).

After dispersion of 2 grams of tissue in water and addition of isotopically labeled surrogate standards, the sample was extracted into isooctane by steam distillation. The extract was cleaned up by solid phase extraction (SPE) using disposable cartridges containing aminopropyl sorbent. The SPE eluate was prepared in methanol, spiked with recovery standards, and analyzed by LC-MS/MS. The final extract volume was 1 mL.

Analysis of was performed on a Waters 2795 high performance liquid chromatograph (HPLC) coupled to a Quattro Ultima MS/MS (Micromass, Manchester, UK). The LC/MS/MS was run in MRM (Multiple Reaction Monitoring) mode and quantification was performed by recording the peak areas of the applicable parent ion/daughter ion transitions. QuanLynx software was used to process raw data into concentrations. Each sample extract was analyzed in two separate LC/MS/MS runs, one run in the -ESI (for NP and OP), and the other run in +ESI (for NP1EO and NP2EO).

Chromatographic separation was achieved using a Waters Xterra C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size column maintained at 40 °C. For the -ESI run the source temperature was 120 °C, desolvation temperature was 350 °C, ion spray capillary voltage 3.5 kV, hexapole voltage 21.8 V, and desolvation gas (N_2) flow rate 400 L hr^{-1}. For the +ESI run the source temperature was 120 °C, desolvation temperature 300 °C, ion spray capillary voltage 3.5 kV and hexapole voltage 21.8 V, and desolvation gas (N_2) flow rate 400 L hr^{-1}.

-ESI Analytes: Ions and Quantification References

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
4-NP	0.558	219	133	$^{13}C_6$-4-NP
4-n-OP	0.492	205.2	106	$^{13}C_6$-4-NP

+ESI Analytes: Ions and Quantification References

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
NP1EO	0.511	282.2	127	$^{13}C_6$-NP1EO
NP2EO	0.443	326.3	183	$^{13}C_6$-NP1EO

Current Use Pesticides

Tissue samples (5 grams wet weight) were spiked with a suite of labeled internal standards (see Table below) and soxhlet extracted with dichloromethane. The extract was concentrated and cleaned up by gel permeation chromatography using an Envirosep ABC 60 column, followed by solid phase extraction using a 1 gram aminopropyl-bonded silica cartridge (NH2,Varian), and then adsorption chromatography on 10% deactivated silica. The extract was concentrated to 500 µL and spiked with an aliquot of labeled injection internal standards in preparation for analysis by GC/HRMS.

GC/HRMS analysis of the analytes was performed using a HP 6890 gas chromatograph coupled to a Waters Autospec Ultima high-resolution mass spectrometer equipped with a equipped with manufacturer's software. A DB-17 MS column (30 m, 0.25 mm i.d., 0.25 µm film thickness) was coupled directly to the HRMS source. The HRMS was operated at a static (8000) mass resolution (10% valley) in the electron ionization (EI) mode using multiple ion detection (MID) and acquiring two characteristic ions for each target analyte and surrogate standard. Selected PFK ions were used as a reference for mass lock. A CTC GC-Pal autosampler was used to provide a splitless/split injection sequence. Quantification ions, confirmation ions, and quantification references for each analyte are listed in the table below. Mean reporting limits are shown for each analyte.

Analytes, Ions, and Quantification References for Pesticide Analytes

Analyte Name	Mean Reporting Limit ng/g (wet)	Quantification Ion	Confirmation Ion	Quantification Reference
Tecnazene	0.112	258.8761	260.8732	^{13}C-HCB
Desethylatrazine	0.171	172.039	174.036	^{13}C-Atrazine
Terbufos	0.447	232.9696	NA	^{13}C-PCB-52
Quintozene	0.117	236.8413	238.8384	^{13}C-HCB
Diazinon-Oxon	0.944	273.1004	288.1239	d^{10}-Diazinon
Diazinon	1.13	276.0698	304.1011	d_{10}-Diazinon
Simazine	2.07	201.0781	203.0752	^{13}C-Atrazine
Atrazine	3.82	215.0938	217.0908	^{13}C-Atrazine
Fonofos	0.0910	246.0302	247.0336	^{13}C-Fonofos
Dimethoate	7.41	228.9996	NA	^{13}C-PCB-52
Chlorothalonil	0.0611	263.8816	265.8786	^{13}C-PCB-52
Chlorpyriphos-Methyl	0.0900	285.9261	287.9232	^{13}C-PCB-52
Parathion-Methyl	5.01	263.0017	264.0051	^{13}C-PCB-52
Pirimiphos-Methyl	0.285	276.0572	290.0728	^{13}C-PCB-52
Metribuzin	1.47	198.0701	199.0735	^{13}C-PCB-52
Dacthal	0.0383	298.8836	300.8807	^{13}C-PCB-52
Octachlorostyrene	0.161	270.8443	272.8413	^{13}C-Aldrin
Chlorpyriphos	0.185	313.9574	315.9545	^{13}C-PCB-52
Fenitrothion	0.797	260.0146	277.0174	^{13}C-PCB-52
Malathion	2.59	283.9942	285.002	^{13}C-PCB-52
Parathion-Ethyl	0.830	291.033	292.0364	^{13}C-PCB-52
Chlorpyriphos-Oxon	0.475	269.949	271.9462	^{13}C-PCB-52
Cyanazine	7.81	240.089	242.0861	^{13}C-PCB-52
Perthane	7.42	223.1487	224.152	^{13}C-PCB-52
Ethion	0.421	232.9695	NA	^{13}C-PCB-52
Hexazinone	3.37	171.0882	172.0916	^{13}C-PCB-52
Phosmet	1.27	160.0399	161.0432	^{13}C-PCB-52
Azinphos-Methyl	3.81	160.0511	161.0544	d_6-Azinphos-Methyl
Total-Permethrins	1.38	183.081	184.0843	^{13}C-Permethrins-Peak_1+2
Total-Cypermethrins	1.14	163.0081	165.0052	^{13}C-Permethrins-Peak_1+2

NOTE: Permethrins-Peak_1 is the cis isomer and Permethrins-Peak_2 is the trans isomer

Pharmaceuticals and Personal Care Products

The analysis required extraction at two different pH conditions: at pH 10 for analysis of fourteen analytes (List 4, see below); and at pH 2.0 for the analysis of the other analytes (Lists 1, 3, and 5; there is no List 2). To minimize matrix

effects, sample sizes were restricted to 2.5 grams (wet weight) for the acidic extraction and 1 gram (wet weight) for the basic extraction, with equal adjustments to batch QC sample sizes. For both acidic and basic extractions, the tissue sample was spiked with a suite of labeled internal standards and extracted first with acetonitrile and then twice with either aqueous pH 2 buffer for the acidic extraction or with aqueous NH_4OH (pH 10) for the basic extraction. The acetonitrile and buffer extracts from the acidic extraction were combined, treated with Na_4EDTA and cleaned up by Solid Phase Extraction (SPE) on an Oasis HLB cartridge. Each cartridge was eluted with methanol and acetone, adjusted to a final volume of 4 mL, spiked with labeled injection internal standards, and analyzed by +ESI LC/MS/MS for List 1 and List 5 compounds, and by -ESI LC/MS/MS for List 3 compounds. The acetonitrile and buffer extracts from the basic extraction were combined and cleaned up by SPE on an Oasis cartridge. Each cartridge was eluted with methanol and acidified methanol, adjusted to a final volume of 4 mL, spiked with labeled injection internal standards, and analyzed by +ESI LC/MS/MS for List 4 compounds.

Analysis was performed on a Waters 2795 high performance liquid chromatograph (HPLC) coupled to a Quattro Ultima MS/MS (Micromass, Manchester, UK). The LC/MS/MS was run in MRM mode and quantification was performed by recording the peak areas of the applicable parent ion/daughter ion transitions. QuanLynx software was used to process raw data into concentrations.

For the analysis of List 1 and 5 compounds chromatographic separation was achieved using a Waters Xterra C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size column maintained at 40 °C. The mobile phase consisted of a gradient from 95% Solvent A: 5% Solvent B to 100 % Solvent B where Solvent A is 0.1% formic acid and 0.1% ammonium formate in water and Solvent B is 1:1 acetonitrile: methanol. The LC/MSMS/MS system was operated in the positive ion electrospray MRM Mode. The source temperature was 120 °C, desolvation temperature 350 °C, ion spray capillary voltage 3.5 kV, hexapole voltage was 21.8 V, and desolvation gas (N_2) flow rate 400 L hr^{-1}.

For the analysis of List 3 compounds chromatographic separation was achieved using a Waters Xterra C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size column maintained at 40 °C. The mobile phase consisted of a gradient from 60% Solvent A: 40% Solvent B to 100 % Solvent B where Solvent A is 0.1% ammonium acetate and 0.1% acetic acid in water and Solvent B is 1:1 acetonitrile: methanol. The LC/MS/MS system for operated in the negative ion electrospray MRM mode. Source temperature was 100 °C, desolvation temperature 400 °C, ion spray capillary voltage 3.5 kV, hexapole voltage was 21.8 V, and desolvation gas (N_2) flow rate 300 L hr^{-1}.

For the analysis of List 4 compounds chromatographic separation was achieved using a Waters Atlantis HILIC, 10.0 cm, 2.1 mm i.d., 3.0 µm particle size column maintained at 40 °C. The mobile phase consisted of a gradient from 5% Solvent A: 95% Solvent B to 30 % Solvent A: 70% Solvent B where Solvent A is 0.1% acetic acid/ammonium acetate buffer in water and Solvent B is acetonitrile. The LC/MS/MS system was operated in the positive ion electrospray MRM mode. Source temperature was 120 °C, desolvation temperature 400 °C, , ion spray capillary voltage 3.5 kV, hexapole voltage 21.8 V, and desolvation gas (N_2) flow rate 400 L hr^{-1}.

Calibration of instrument response was achieved using a 7-point calibration curve covering at least 3 orders of magnitude of concentration for each analyte. All the results demonstrated that coefficients of determination (r^2) were greater than or equal to 0.99.

PPCP Analytes, Reporting Limits, Ions and Quantification References:

List 1 – Acid Extraction, Positive Electrospray Ionization

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
Sulfanilamide	5.95	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	6.48	152.2	110.0	$^{13}C_2$, ^{15}N-Acetaminophen
Sulfadiazine	0.595	251.2	156.1	$^{13}C_6$-Sulfamethazine
Sulfathiazole	0.595	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	0.367	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	5.95	195.0	138.0	$^{13}C_3$-Caffeine
Trimethoprim	0.638	291.2	230.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	0.307	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamethazine	1.12	279.0	156.0	$^{13}C_6$-Sulfamethazine
Ofloxacin	0.596	362.2	318.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Ormetoprim	0.238	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	6.45	320.0	302.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Thiabendazole	0.595	202.1	175.1	d_6-Thiabendazole
Sulfachloropyridazine	0.595	285.0	156.0	$^{13}C_6$-Sulfamethazine
Lomefloxacin	1.42	352.2	308.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Enrofloxacin	1.19	360.2	316.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfamethoxazole	0.324	254.0	156.0	$^{13}C_6$-Sulfamethoxazole
Sarafloxacin	5.95	386.1	299.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Clinafloxacin	4.73	366.3	348.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Digoxigenin	11.8	391.2	355.2	$^{13}C_3$-Trimethoprim
Oxolinic Acid	0.756	262.1	244.0	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	0.138	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Azithromycin	0.866	749.9	591.6	$^{13}C_3$-Trimethoprim
Penicillin G	1.19	367.1	159.9	$^{13}C_3$-Trimethoprim
Diphenhydramine	0.238	256.2	167.0	$^{13}C_3$-Trimethoprim
Flumequine	0.673	262.0	173.7	$^{13}C_3$-Trimethoprim
Penicillin V	1.25	383.2	159.9	$^{13}C_3$-Trimethoprim
Diltiazem	0.125	415.5	178.0	$^{13}C_3$-Trimethoprim
Carbamazepine	0.595	237.4	194.2	d_{10}-Carbamazepine
Erythromycin	0.119	734.4	158	$^{13}C_2$-Erythromycin - H_2O
Oxacillin	1.19	434.1	160.2	$^{13}C_3$-Trimethoprim
Tylosin	2.38	916.6	772.5	$^{13}C_6$-Sulfamethazine
Digoxin	2.39	798.5	651.3	$^{13}C_3$-Trimethoprim
Dehydronifedipine	0.238	345.1	284.1	$^{13}C_3$-Trimethoprim
Cloxacillin	1.19	468.1	160.1	$^{13}C_3$-Trimethoprim
Fluoxetine	0.595	310.1	148.0	d_5-Fluoxetine
Clarithromycin	0.595	748.9	158.2	$^{13}C_6$-Sulfamethazine
Roxithromycin	0.119	837.6	679.0	$^{13}C_6$-Sulfamethazine

List 3 – Acid Extraction, Negative Electrospray Ionization

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
Furosemide	15.9	329.0	204.7	^{13}C-d_3-Naproxen
Glipizide	2.39	444.2	319.0	d11-Glipizide
Naproxen	1.23	228.9	168.6	^{13}C-d_3-Naproxen
Bisphenol A	199	227.0	211.9	d6-Bisphenol A

Warfarin	0.596	307.0	161.0	d_5-Warfarin
Glyburide	1.73	492.1	169.8	d3-Glyburide
Ibuprofen	5.96	205.1	161.1	$^{13}C_3$-Ibuprofen
Gemfibrozil	0.666	249.0	121.0	d_6-Gemfibrozil

List 4 – Base Extraction, Positive Electrospray Ionization

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
Atorvastatin	1.51	559.3	440.0	d5-Enalapril
Cotinine	1.51	177.0	98.0	d3-Cotinine
Cimetidine	2.32	253.1	159.0	d3-Cimetidine
Triamterene	0.490	254.1	236.9	d4-Clonidine
Enalapril	0.303	377.2	233.9	d5-Enalapril
Oxycodone	1.64	316.2	240.9	d6-Oxycodone
Clonidine	1.54	230.0	43.9	d4-Clonidine
Amphetamine	2.69	136.1	90.8	d5-Amphetamine
Albuterol	0.338	240.0	148.0	d_3-Albuterol
Codeine	3.80	300.2	214.9	d6-Codeine
Hydrocodone	2.00	300.2	198.8	d3-Hydrocodone
Ranitidine	0.934	315.0	175.9	d_3-Albuterol
Atenolol	0.741	267.2	144.7	d7-Atenolol
Metformin	3.33	130.1	60.1	d_6-Metformin

List 5 – Acid Extraction, Positive Electrospray Ionization

Target Analyte	Mean Reporting Limit ng/g (wet)	Parent Ion Mass	Daughter Ion Mass	Quantification Reference
Benzoylecgonine	0.121	290.1	167.8	d8-Benzoylecgonine
Metoprolol	0.655	268.2	190.7	d7-Metoprolol
Cocaine	0.0639	304.1	181.8	d3-Cocaine
Meprobamate	1.59	219.0	157.8	d7-Metoprolol
10-hydroxy-amitriptyline	0.0655	294.2	215.0	d7-Propranolol
Propranolol	0.794	260.2	115.8	d7-Propranolol
Prednisone	8.60	359.2	341.0	d7-Propranolol
Hydrocortisone	37.1	363.2	120.7	d4-Hydrocortisone
Prednisolone	2.77	361.2	343.0	d7-Propranolol
Promethazine	0.217	285.1	197.8	d4-Promethazine
Desmethyldiltiazem	0.0624	401.2	177.8	d4-Promethazine
Paroxetine	1.59	330.2	191.8	d6-Paroxetine
Norverapamil	0.0609	441.3	164.7	d7-Propranolol
Verapamil	0.0610	455.3	164.8	d6-Amitriptyline
Methylprednisolone	2.74	375.2	357.0	d3-Methylprednisolone
Propoxyphene	0.187	340.2	57.9	d5-Propoxyphene
Amitriptyline	0.144	278.2	232.8	d6-Amitriptyline
Benztropine	0.119	308.2	166.7	d3-Benztropine
Alprazolam	0.129	309.1	280.9	d5-Alprazolam
Amlodipine	0.685	409.1	237.8	d5-Norfluoxetine
Norfluoxetine	0.624	296.1	133.7	d5-Norfluoxetine
Sertraline	0.230	306.1	274.8	d7-Propranolol
Diazepam	0.194	285.1	192.8	d5-Diazepam
Fluocinonide	2.74	495.2	337.0	d5-Alprazolam

Trenbolone acetate	0.258	313.2	253.0	d5-Alprazolam
Fluticasone propionate	0.799	501.2	293.0	d7-Metoprolol

Polybrominated Diphenyl Ethers

The analysis of polybrominated diphenyl ethers (PBDEs) was performed using methods similar to those used in support of NOAAs National Mussel Watch Program (NOAA Technical Memorandum NOS NCCOS 30 and NOAA Technical Memorandum NOS ORCA 130). Samples were extracted using accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA). Approximately 10 to 15 g of wet tissue homogenate was dried with Hydromatrix (Agilent Technologies, Inc., New Castle, DE). A surrogate solution containing 4'-fluoro-2,3,3',4,5,6-hexabromodiphenyl (PBDE-160) ether and decabromodiphenyl ether ($^{13}C_{12}$) (PBDE-209) was spiked into the sample and extracted using 100% dichloromethane inside stainless-steel extraction cells held at elevated temperature and solvent pressure. The extracted compounds were collected in 60 mL glass vials and concentrated to a volume of 3 mL from which a 100 µL aliquot was taken to determine percent lipids. The remaining portion was purified through activated silica gel and alumina followed by gel permeation chromatography to minimize matrix interference. Extracts were then concentrated to a final volume of 0.5 mL of dichloromethane. An internal standard solution consisting of 4'-fluoro-2,3',4,6 tetrabromodiphenyl ether (PBDE-69) and polybrominated biphenyl-209 ($^{13}C_{12}$) was added to all samples prior to instrumental analysis.

PBDEs were analyzed using an Agilent 7890 trace GC coupled to a 5975 MSD in electron capture negative ionization (ECNI) mode. The GC column was an Agilent Technologies DB-XLB (15 m × 0.25 mm ID and 0.10 mm film thickness). The GC was operated in splitless mode using a PTV injection port with a carrier gas of helium at a flow rate of 1 mL/minute, and methane was used as the reactant gas with a flow rate of 2.0 mL/min. The temperature of the injection port was 40 °C (ramp to 300 °C) and the transfer line was at 290 °C. The initial oven temperature was 110 °C, the ramp rate was 7 °C/minute to a final oven temperature of 280 °C, and held for 20 minutes. Other than BDE-209, the quantification and confirmation ions for all analytes, surrogate standards, and internal standards were 79.0 *m/z* and 81.0 *m/z*. The quantification ion for the unlabeled BDE-209 was 496.0 *m/z,* and 486.0 *m/z* for the surrogate $^{13}C_{12}$-BDE-209.

Initially there was an apparent detection of PBDE congeners (BDE-1, -12, -32, -37,-77 and -190) not known to be present in the technical mixtures and not commonly reported in the literature. Subsequent full scan analysis in EI and ECNI modes either identified these compounds as suspected naturally occurring methoxy brominated diphenyl ethers (MeO-BDEs), or could not confirm the peak was a PBDE. Congeners not known to be present in the technical mixtures were therefore removed from the data set.

Other Flame Retardants

Analytes:
Hexabromocyclododecane (HBCD), gamma
Hexabromocyclododecane (HBCD), alpha
Hexabromocyclododecane (HBCD), beta
1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE)
2-Ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB)
2-Ethylhexyl-2,3,4,5-tetrabromophthalate (TBPH)
Tris(1-chloro-2-propyl)phosphate (TCPP)
Tris(1,3-dichloro-2-propyl)phosphate (TDCPP)
Tris(2-chloroethyl)phosphate (TCEP)

The analytical method described by La Guardia et al. (2012), was used for the analysis of alternative-BFRs (TBB, TBPH and BTBPE) and HBCD isomers (α-, β-, γ-HBCD). This method was modified to include chlorinated organophosphate

flame retardants (OPFRs) (i.e., TCEP, TCPP and TDCPP) by collection of a third solid phase extraction (SPE) eluent, containing OPFRs. This eluent was further purified by flourasil SPE and analyzed by gas chromatography mass spectrometry (GC/MS). Method validation was established by a performance-based QA/QC approach. This included method blanks, duplicates, matrix spike and matrix spike duplicates and surrogate recovery analysis. Briefly, samples were freezed-dried, then approximately 2 g tissues (dry weight) were subjected to accelerated solvent extraction (Dionex ASE 200, Sunnyvale, CA) with methylene chloride. Surrogate standards (200 ng of 2,3,4,4', 5,6-hexabromodiphenyl ether (BDE-166); Cambridge Isotope Laboratories, Inc., Andover, MA, 200 ng of ^{13}C-labeled α-HBCD; Wellington Laboratories, Ontario, Canada and 1000 ng of deuterated triphenyl phosphate (d15-TPP); Sigma-Aldrich, St. Louis, MO) were added to each sample prior to extraction. Extracts were purified by size exclusion chromatography (SEC, Envirosep-ABC, 350 x 21.1 mm. column; Phenomenex, Torrance, CA). Each post-SEC extract was solvent exchanged to hexane, reduced in volume and added to the top of a solid phase 2-g silica glass extraction column (Isolute, International Sorbent Tech.; Hengoed Mid Glamorgan, UK). Each column was eluted with 3.5-mL hexane (fraction one), followed by 6.5 mL of 60:40 hexane/DCM and then 8 mL DCM (fraction two) and 5 ml 50:50 acetone/DCM (fraction three). The second fraction, containing alt-BFRs and HBCD, was reduced in volume and solvent exchanged to hexane. Decachlorodiphenyl ether (DCDE; 100 ng; Ultra Scientific, North Kingstown, RI) was then added as the internal standard. The third fraction, containing OPFRs and were further purified to remove interfering polar organics. Extracts were reduced in volume, solvent exchanged to hexane added to the top of a 0.5-g florisil SPE column and eluted with 3.5 ml hexane (fraction four) followed by 5 ml ethyl acetate (fraction five). The fifth fraction was reduced, solvent exchanged to hexane and pentachlorobenzene (ptClB; 5000 ng; Ultra Scientific, North Kingstown, RI) was added as an internal standard.

Extracts (fraction-two) were analyzed for alt-BFRs by gas chromatography-electron capture negative ionization selective ion monitoring mass spectrometry (GC/ECNI-SIM-MS). Quantitation ions were *m/z* 79 ($[^{79}Br]^-$), 81($[^{81}Br]^-$), confirmation ions *m/z* 356, 463, 251 were monitored for TBB, TBPH, BTBPE, respectively. For HBCD analysis, extracts (fraction-two) were solvent exchanged to methanol and spiked with 100 ng of d18-labeled α-HBCD (Wellington Laboratories, Ontario, Canada), as an internal standard. These were then analyzed for α-, β-, and γ- isomers by ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) negative ionization mode. Native isomers (α-, β-, and γ-HBCD) were identified by monitoring ion pairs produced from quadrupole scans (Q1/Q3): 640.9/78.9 and 640.9/80.9. For ^{13}C-α-HBCD ion pairs 652.1/78.9 and 652.1/80.9 and for d_{18}- α-HBCD, 657.7/78.9 and 657.7/80.9 were monitored for identification. The quantitation ion was 78.9 *m/z*.

OPFRs (fraction-five) extracts were analyzed by GC (CP-3000 Varion, Agilent Tech., Palo Alto, CA) with MS detection (Saturn 220 Varion, Agilent Tech.). Sample aliquots (1 μL) were introduced into the split/splitless injector, equipped with a glass liner (1 mm, ID), and separated on a 60-m DB-5MS (0.32 mm i.d., 0.25 μm, J&W Scientific, Agilent Tech.) column. The injector temperature was 320 °C, carrier gas (helium), flow 1.2 mL/min. Initial column oven temperature was 90 °C, held for 1 minute, then increased to 320 °C at 4 °C/minute, then held for 10 minutes. Total run time was 68.5 minutes. The MS was operated in the electron impact (EI) mode and ions were monitored by SIM. Ion source temperature was 220 °C and the GC transfer line was maintained at 320 °C. Quantitation ions for TCEP were *m/z* 249, 251, TCPP *m/z* 277, 279 and TDCPP *m/z* 379, 381. Internal and surrogate standards ptClBZ and d15-TPP were quantitated using *m/z* 250 and *m/z* 399, 341, respectively.

La Guardia, M.J., Hale, R.C., Harvey, E., Mainor, T.M., Ciparis, S., 2012. In Situ Accumulation of HBCD, PBDEs, and Several Alternative Flame-Retardants in the Bivalve (Corbicula fluminea) and Gastropod (Elimia proxima). Environ. Sci. Technol. 46, 5798–5805.

Perfluorinated Compounds

Homogenized mussel tissue samples were analyzed according to the ion-pairing method described elsewhere (Kannan, et al, 2001). Approximately 0.4 g of mussel sample was transferred to a 15-mL polypropylene (PP) tube, and 5 ng each of internal standards ($^{13}C_4$-PFOS, $^{13}C_4$-PFOA, $^{13}C_2$-PFNA, and $^{13}C_2$-PFDA), 1 mL of 0.5 M tetrabutylammonium hydrogensulfate, 2 mL of sodium carbonate buffer and 5 mL of methyl tert-butyl ether (MTBE) were added. The mixture was shaken at 250 rpm at room temperature for 1 h, centrifuged, and the MTBE layer was transferred into another tube. The extraction was repeated twice with another 3 mL of MTBE. The MTBE extract was combined and evaporated to near dryness under a gentle stream of nitrogen and then reconstituted with 1 mL of methanol. The sample was vortexed for 30 sec and filtered through a 0.2-µm nylon filter into an autosampler vial.

Analytes were detected and quantified using an Agilent 1100 series high-performance liquid chromatography (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). Ten microliters of the extract were injected onto a 100 x 2.1 mm (5 µm) Keystone Betasil C18 column. The mobile phase was 2 mM ammonium acetate/methanol starting at 10 % methanol, at a flow rate of 300 µL/min. The gradient increased to 100% methanol at 10 min and was held for 2 min, and then reversed back to 10% methanol. The MS/MS was operated in electrospray negative ion mode. Target compounds were determined by multiple reaction monitoring (MRM). The MRM transitions were 299/80 for PFBS, 399/80 for PFHS, 499/99 for PFOS, 599/99 for PFDS, 498/78 for PFOSA, 313/269 for PFHxA, 363/169 for PFHpA, 369/169 for PFOA, 463/219 for PFNA, 513/219 for PFDA, 563/169 for PFUnDA, and 613/169 for PFDoDA. Samples were injected twice to monitor sulfonates and carboxylates separately.

The quantitation was performed using isotopic dilution quantification method, with quadratic regression fit analysis weighted by 1/x of the extracted calibration curve. The limit of quantitation (LOQ) was determined as the lowest acceptable standard in the calibration curve; defined as a standard within ±30% of the theoretical value and that has a peak area twice as great as the analyte peak area in blanks. Reported LOQs were corrected by the dilution factor during the sample preparation for wet weight concentrations and dry weight percentage for dry weight concentrations.

Matrix spikes were performed for tissue samples. Known amounts of mixed PFC standards (10 ng each) were spiked into sample matrices before extraction and were passed through the entire analytical procedure. Recoveries for ^{13}C-labeled internal standards ranged from 67% to 75%. Matrix effect occurred during eletrospray ionization affected the recoveries. The corrected mean matrix spike recoveries for PFOS, PFOA, PFNA, and PFDA were 102%, 104%, 100%, and 101%, respectively. Reported concentrations of PFCs were corrected for the recoveries of internal standards. Blanks were analyzed by passing Milli-Q water and reagents through the whole analytical procedure. No analytes were detected in blank samples. A midpoint calibration standard was injected after every 10 samples to check for instrumental stability, response and drift. Calibration standards were injected daily before and after the analysis.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J.F., Hansen, K.J., Jones, P.D., Helle, E., Nyman, M., Giesy, J.P., 2001. Accumulation of perfluorooctane sulfonate in marine mammals. Environ. Sci. Technol. 35, 1593–1598.

Single-Walled Carbon Nanotubes.

SWNT were quantified in a subset of mussel samples (n=10) using both near infrared fluorescence spectroscopy (NIRF) and inductively-coupled plasma mass spectrometry (ICP-MS). Tissue samples (1.5 g wet tissue, n=3 replicates per sample) were suspended in 3 ml 2% sodium deoxycholate (SDC, a bile salt surfactant) and the SWNT were extracted via high power sonication (10 min at power output 19.1 Watts, Branson Sonifier450, mircotip). The sonication power output was determined following Taurozzi et al. (2010) under modified conditions (200 ml beaker and 100 ml deionized water). Following sonication, the samples were centrifuged at 17860 g for 10 min to remove the tissue material. The extract was analyzed using NIRF (NS1, Applied Nano fluorescence). NIRF spectra of the mussel extracts in SDC were referenced

against 2% SDC for quantification. Prior to analysis, the sensitivity and linearity of NIRF for detecting cobalt-molybdenum catalyst (CoMoCat) SWNT materials (type SG65 nanotubes from SouthWest Nanotechnologies) suspended in SDC were tested by analyzing serial dilutions of SDC-stabilized SG65 SWNT (1:5) prepared in triplicate. The detection limit for the analytical method was determined by performing standard addition experiments, where CoMoCat SWNT type 65 (Southwest Nanotechnology Inc) was spiked into a mussel extract sample. Because SWNT-derived, characteristic spectral features were observed at a SWNT concentration above 180 ng/mL, the absence of any spectra features in the non-spiked extracted mussel sample indicated that the SWNT concentration in the mussel extracts were below the detection limit of 180 ng/mL. This corresponded to a method detection limit of < 360 ng/g wet weight for the mussel tissue.

Mussel tissue extracts were also analyzed by ICP-MS (Agilent technologies 7700 ICP-MS series) for detection of cobalt (Co) and molybdenum (Mo), which are known metal catalysts used in SWNT production. The reporting limit for Mo and Co in the mussel samples was 0.1 ng/g wet weight.

Taurozzi, J.S., V.A. Hackley and M.R. Wiesner. 2010. CEINT/NIST Protocol for Preparation of Nanoparticle Dispersions from Powdered Material Using Ultrasonic Disruption. Version 1.

Tables and Figures

Table SI-1. ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_037_047SI_TableSI_1.xlsx.

Table SI-2. Tissue analysis data quality objectives for analyte classes other than alkylphenols. Analytes with values exceeding these thresholds were discarded (for that sample). RPD = relative percent difference.

Parameter	Other FR	PFC	PBDE	PPCP and CUP
Standard Recovery	70-130	25-130	50-130	25-175
Blank Concentration	At least twice the method blank concentration	At least twice the method blank concentration	At least twice the method blank concentration	At least twice the method blank concentration
MS/MSD Recovery	70-130	70-130	55-130	40-150
MS/MSD RPD	< 30	NA	< 30	< 50
Duplicate Sample RPD	< 30	NA	<30	< 50

Table SI-3. Tissue analysis data quality objectives for alkylphenols. Analytes with values exceeding these thresholds were discarded (for that sample). RPD = relative percent difference.

Parameter	NP	n-OP	NP1EO	NP2EO
Standard Recovery	40-130	40-130	30-130	30-130
Blank Concentration	At least twice the method blank concentration	At least twice the method blank concentration	At least twice the method blank concentration	At least twice the method blank concentration
MS/MSD Recovery	60-130	70-130	70-150	40-130
MS/MSD RPD	< 90	< 90	< 90	< 90
Duplicate Sample RPD	< 90	< 90	< 90	< 90

Table SI-4. Comparison (showing *p*-values) of tissue concentrations within the land use categories using a non-parametric Kruskal-Wallis hypothesis test and a non-parametric pairwise Wilcoxon test.

AP	
Kruskal Test	0.000503

Pairwise Test

	Agr	Low Dev	Mixed Dev
Low Dev	1		
Mixed Dev	0.0438	0.435	
Urban	0.0240	0.00414	0.316

PBDE	
Kruskal Test	3.84E-05

Pairwise Test

	Agr	Low Dev	Mixed Dev
Low Dev	0.577		
Mixed Dev	1	0.0331	
Urban	0.0357	0.000358	0.0149

PFC	
Kruskal Test	5.93E-05

Pairwise Test

	Agr	Low Dev	Mixed Dev
Low Dev	1		
Mixed Dev	1	0.218	
Urban	0.0374	9.64E-05	0.101

CUP	
Kruskal Test	0.0197

Pairwise Test

	Agr	Low Dev	Mixed Dev
Low Dev	0.127		
Mixed Dev	0.638	1.000	
Urban	1	0.0508	0.667

PPCP	
Kruskal Test	0.570

Pairwise Test

	Agr	Low Dev	Mixed Dev
Low Dev	1		
Mixed Dev	1	1	
Urban	1	1	0.997

AFR	
Kruskal Test	0.342

Pairwise Test

	Low Dev	Mixed Dev
Mixed Dev	0.996	
Urban	1	0.541

Table SI-5. Comparison showing *p*-values of tissue concentrations within the discharge categories using a non-parametric Kruskal-Wallis hypothesis test and a non-parametric pairwise Wilcoxon test.

AP	
Kruskal Test	0.00877

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	0.734	0.490	
STORM only	0.0367	0.130	1

PBDE	
Kruskal Test	1.00E-04

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	4.82E-02	1	
STORM only	5.14E-05	1	1

PFC	
Kruskal Test	3.66E-04

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	1	1.00	
STORM only	0.00130	0.330	0.169

CUP	
Kruskal Test	0.496

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	1	0.957	
STORM only	1	1	1

PPCP	
Kruskal Test	0.413

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	1	1	
STORM only	0.838	1	1

AFR	
Kruskal Test	0.00877

Pairwise Test

	none	POTW only	STORM + POTW
POTW only	1		
STORM + POTW	0.734	0.490	
STORM only	0.0367	0.130	1

Figure SI-1. Tissue concentration of individual contaminants in each of the land use categories (urban, mixed development, low development, and agricultural). Compounds detected at ≥ 3 stations are shown, and are ordered by the 90% quantile for each compound calculated across all land use categories. The box shows the interquartile range (IQR) and median. The whiskers flag potential outliers and extend to the furthest data point that is < 1.5 × IQR from the box. Outliers are data points beyond the whiskers and are shown as individual points. Abbreviations for contaminant names are as follows: NP1EO: 4-nonylphenol monoethoxylate, NP2EO: 4-nonylphenol diethoxylate, BDE: brominated diphenyl ether, PFDoDA: perfluorododecanoic acid, HBCD: hexabromocyclododecane, PFUnDA: perfluoroundecanoic acid.

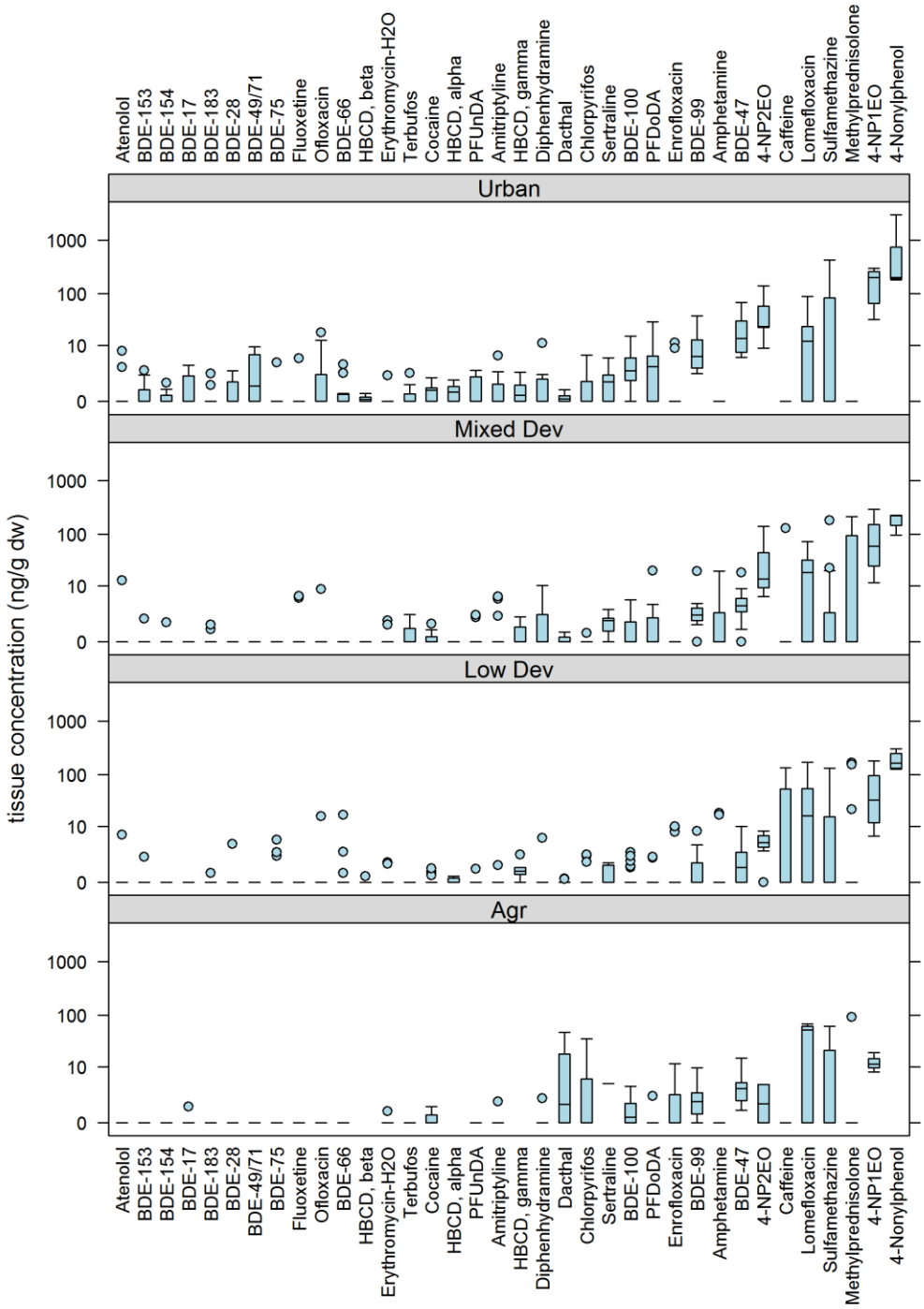

Figure SI-2. Total AP, PBDE, and PFC concentrations at each station. The y-axis is ordered by the mean concentration across the three classes.

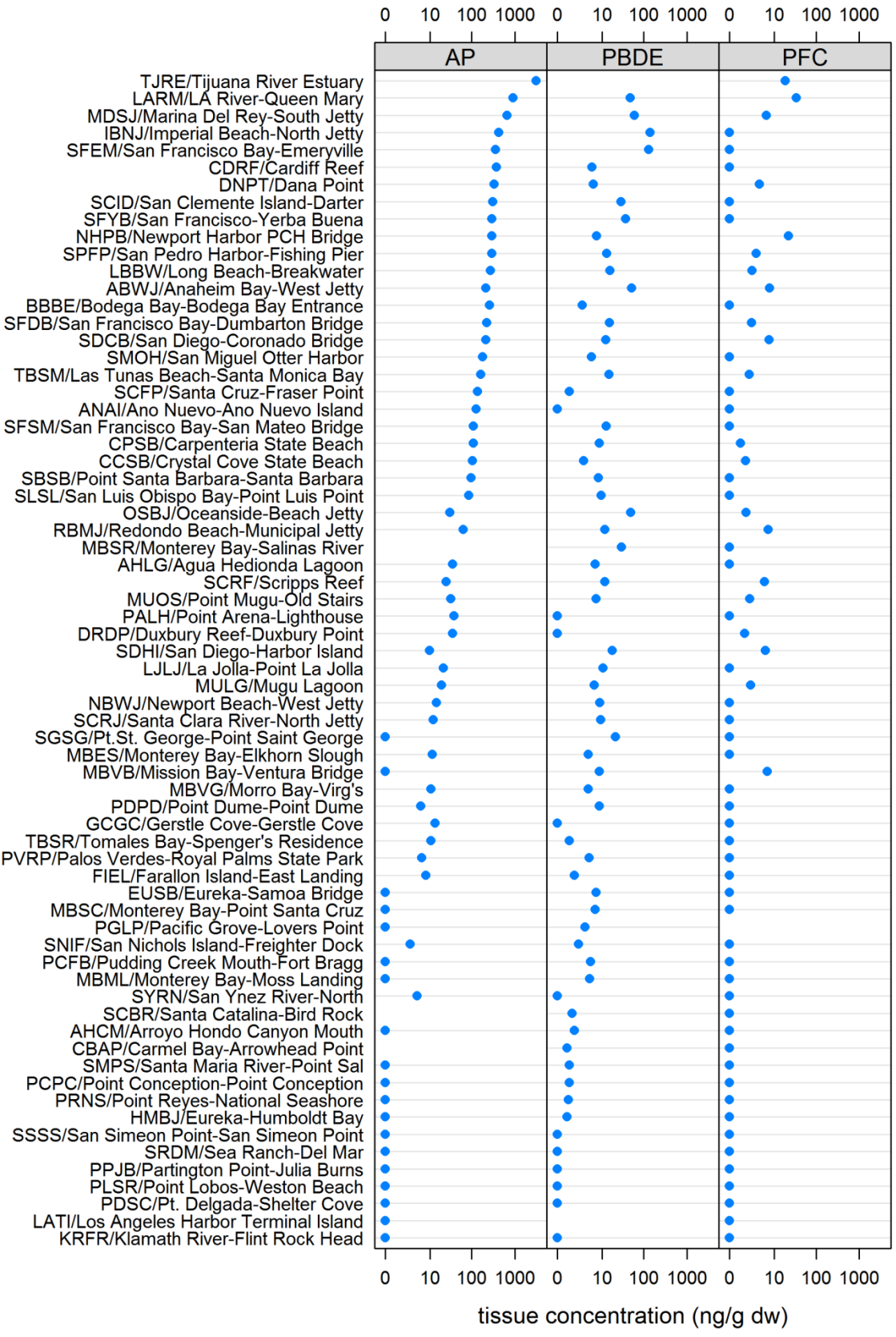

Figure SI-3. Tissue concentration of individual contaminants in each of the discharge categories. Compounds detected at ≥ 3 stations are shown, and are ordered by the 90% quantile for each compound calculated across all land use categories. A description of the box and whiskers, and abbreviations, is in Figure SI-1.