Special Study Proposal: Characterization of Pharmaceutical Contamination in Ambient Bay Water, Margin Sediment, and Wastewater

Summary: Pharmaceutical pollution is widely detected in the Bay, and earlier pilot studies indicate key pharmaceutical contaminants can approach levels of concern for wildlife. This study will monitor ambient Bay water and margin sediment for pharmaceutical pollution, providing data essential to a current evaluation of the potential risks of ~150 pharmaceutical contaminants via the RMP's Tiered CEC Risk and Management Framework. In addition, this study will monitor treated wastewater for pharmaceuticals, providing information useful for studying the loading rates and fate of pharmaceuticals discharged to the Bay.

Estimated Cost: $91,375

Oversight Group: ECWG

Proposed by: Rebecca Sutton (SFEI)

PROPOSED DELIVERABLES AND TIMELINE

Deliverable	***Due Date***
Task 1. Project Management (write and manage sub-contracts, track budgets)	Winter 2015 – Spring 2017
Task 2. Develop detailed sampling plan	Spring 2016
Task 3. Field Sampling	Summer 2016
Task 4. Lab analysis	Fall 2016
Task 5. QA/QC and data management	Winter 2016
Task 6. Final report	3/31/2017

Background

Pharmaceuticals are detected frequently in U.S. waterways, creating concern for their potential to impact wildlife as well as humans. Laboratory studies indicate fish exposed to antidepressant medications at environmentally relevant doses exhibit behavioral changes that affect survival and reproduction (e.g., Weinberger and Klaper 2014; Brodin et al. 2013). Antibiotic medications, designed specifically to kill organisms, may disrupt bacterial communities and essential functions (e.g., Näslund et al. 2008), impart broader antibiotic resistance (e.g., Rizzo et al. 2013), and are often toxic to algal species (e.g., Ferrari et al. 2004). Other pharmaceutical compounds have significant endocrine disrupting effects on aquatic species (e.g., Kolodziej et al. 2013). Pharmaceuticals typically enter the wastestream through excretion and flushing of unused medicines, suggesting the primary pathway for Bay contamination is via treated wastewater.

An increasing focus on proper pharmaceutical prescription, use, and disposal is occurring at federal, state, and local levels, and suggests the need to evaluate the level of concern associated with pharmaceutical pollution in the Bay. Current policy actions are largely motivated by concerns other than pollution (e.g., antibiotic resistance in infectious bacteria, drug abuse and accidental poisoning), meaning reduced Bay contamination may be an incidental result. Recent management actions include:

- Obama administration's National Action Plan for Combating Antibiotic-Resistant Bacteria, released March 2015, which lists activities such as "implementation of healthcare policies and antibiotic stewardship programs that improve patient outcomes, and efforts to minimize the development of resistance by ensuring that each patient receives the right antibiotic at the right time at the right dose for the right duration."
- Increased emphasis on drug takeback programs that prevent down-the-drain disposal:
    - Locally, the Alameda County ordinance requiring drug manufacturers fund stewardship and disposal costs has survived legal challenges to date;
    - San Francisco has just passed a similar stewardship program, and Marin may be next;
    - A 2014 bill to create a similar program statewide (SB 1014) passed the State Senate but died in the Assembly;
    - The federal DEA made significant changes to disposal rules to aid voluntary drug takeback programs.

Given this growing policy focus on pharmaceuticals, it would be appropriate at this time for the RMP to gather new data to evaluate the level of concern that should be associated with the presence of these contaminants in the Bay. Findings could suggest the need for targeted management actions, or could suggest existing activities are sufficient to protect wildlife from harm.

The RMP has assessed Bay pharmaceutical pollution in two previous special studies involving samples collected in 2006 (Harrold et al. 2009) and 2009-2010 (Klosterhaus et al. 2013a). The results of these monitoring efforts indicate that the following specific pharmaceutical compounds merit further monitoring:

Ciprofloxacin – Meets state guidance criteria for monitoring in sediment.[1] This widely prescribed antibiotic was detected in Bay sediment at concentrations up to 678 ng/g dry weight (Klosterhaus et al. 2013b). The highest measured concentration exceeds both a lowest observable effect concentration, or LOEC, for effects on bacterial community structure (100 ng/g dry weight) and a half maximal effective concentration, or EC_{50}, for pyrene degradation (400 ng/g dry weight; Näslund et al. 2008). Current levels of contamination may be a concern for both bacterial diversity and an essential ecosystem

[1] Recent state guidance regarding contaminants of emerging concern (CECs) in California's aquatic ecosystems outlines an objective means of prioritizing monitoring activities through calculation of monitoring trigger levels (MTLs) using available toxicity thresholds, appropriate safety factors, and measured or predicted environmental concentrations (Anderson et al. 2012; Dodder et al. 2015).

service these organisms may perform in Bay sediment.

Sulfamethoxazole – Intermittent detection above a toxicity threshold.[2] This antibiotic was detected in ambient Bay water at concentrations up to 1,060 ng/L (Klosterhaus et al. 2013b). A PNEC calculated using standard methods endorsed by the EMEA (2006), and using an assessment factor (AF) of 50 as directed by the European Chemicals Bureau (European Communities 2003), has been calculated as 118 ng/L by Grung et al. (2008). Intermittent detection above a PNEC is insufficient grounds to classify a contaminant as a moderate concern (Tier III) contaminant according to the RMP's Tiered CEC Risk and Management Framework, but suggests the need for further monitoring. Should exceedances prove to be more common than limited previous data suggest, reclassification as a moderate concern contaminant may be indicated.

Erythromycin – Intermittent detection above a toxicity threshold.[2] This antibiotic was detected in ambient Bay water at concentrations up to 41.6 ng/L (Klosterhaus et al. 2013b). The highest Bay measurement exceeds an algal PNEC of 22 ng/L (back-calculated from molar value provided by Gonzalez-Pleiter 2013). As for sulfamethoxazole, intermittent detection of erythromycin above a PNEC in previous pilot studies suggests the need for further monitoring to evaluate how frequently exceedances occur, and whether this contaminant merits classification as a moderate concern for the Bay.

Previous studies of pharmaceutical contamination in the Bay evaluated ~100 different contaminants; over 3,000 pharmaceuticals are currently registered for use in the U.S. (Howard and Muir 2011). Continuing method development provides the ability to target important pharmaceuticals classified by Howard and Muir (2011) as high priorities for environmental monitoring, such as:

- Bupropion hydrochloride (Wellbutrin XL; antidepressant; CAS 31677-93-7)
- Irbesartan (Avapro; blood pressure medication; CAS 138402-11-6)
- Trazadone (Oleptro; antidepressant; CAS 19794-93-5)

Analytical methods for these particular compounds are expected to be available in May 2015, as part of a new list of pharmaceutical targets offered by AXYS Analytical. Approximately 50 additional pharmaceuticals for which no Bay data exist can be measured using the full suite of AXYS pharmaceutical analyses.

Study Objectives and Applicable RMP Management Questions

This study will provide data essential to determining the level of concern associated with pharmaceutical pollution in the Bay. Currently available data suggest the need for further monitoring of three antibiotics: ciprofloxacin, sulfamethoxazole, and erithromycin. Should

[2] According to the RMP's Tiered CEC Risk and Management Framework, a Tier III or "moderate concern" chemical is typically one where there is "…frequent detection at concentrations greater than the PNEC or NOEC but less than EC_{10}, the effect concentration where 10% of the population exhibit a response, or another low level effects threshold…" Sutton et al. 2013).

new monitoring show levels of these pharmaceuticals frequently exceed toxicity thresholds, reclassification as moderate concern (Tier III) contaminants may be appropriate.

An expanding array of pharmaceutical targets available via AXYS Analytical also means the RMP can now collect data on new analytes that have been specifically identified by Howard and Muir (2011) as priority contaminants for environmental monitoring. In addition, up to 50 pharmaceutical analytes for which no Bay data are yet available can be assessed via the full suite of AXYS analyses.

Comparison of contaminant levels in the pathway of WWTP effluent with Bay water and sediment levels can provide preliminary information as to pharmaceutical loadings and fate in the Bay. These comparisons may suggest that specific compounds are especially persistent in the environment and may require special attention, perhaps in the form of additional, targeted management actions.

Management questions to be addressed by monitoring pharmaceuticals in WWTP effluent and Bay water and sediment are the same as those of the overall RMP program, as shown in Table 1.

Table 1: Study objectives and questions relevant to RMP management questions

Management Question	Study Objective	Example Information Application
1) Are chemical concentrations in the Estuary at levels of potential concern and are associated impacts likely?	Monitor over 150 pharmaceuticals in Bay water and sediment. Compare measured concentrations to toxicity thresholds to determine levels of concern associated with each according to the Tiered CEC Risk Framework.	Do target pharmaceuticals have the potential to cause impacts to Bay wildlife? Do data indicate a need for management actions?
2) What are the concentrations and masses of contaminants in the Estuary and its segments? 2.1 Are there particular regions of concern?	Compare levels measured in different embayments.	Are expectations of higher levels of contamination in the Lower South Bay substantiated?
3) What are the sources, pathways, loadings, and processes leading to contaminant-related impacts in the Estuary? 3.1. Which sources, pathways, etc. contribute most to impacts?	Obtain information on pharmaceutical contamination in treated wastewater and ambient Bay water and margin sediment.	Are relative distributions of pharmaceutical contaminants in effluents versus Bay water and sediment consistent with our expectations for various contaminant processes?
4) Have the concentrations, masses, and associated impacts of contaminants in the Estuary increased or decreased? 4.1. What are the effects of management actions on concentrations and mass?	Review new results alongside available data from previous RMP studies for indications of trends in pharmaceutical contamination over time.	Are pharmaceuticals for which we have previous measurements found at increasing or decreasing levels in Bay media?
5) What are the projected concentrations, masses, and associated impacts of contaminants in the Estuary?	Review measured results alongside available projections of population growth and age as well as anticipated changes to pharmaceutical prescribing and other relevant actions.	Which anticipated changes or actions are likely to have the greatest impact on pharmaceutical pollution? Are additional/different actions needed?

This monitoring effort would most directly address questions 1, 2, and 3, characterizing pharmaceutical contamination and its potential for impacts at the current time. Inferences regarding past or future levels of contamination would involve digestion of the data within the context of changes to the Bay Area population (size and age distribution), patterns in prescribed medications, and wastewater treatment technologies, all of which may play a role in addressing questions 4 and 5. This additional research is not part of this proposal but could be completed as a second phase of this study.

In addition, the study will address the emerging contaminants priority question: What emerging contaminants have the potential to adversely impact beneficial uses of the Bay?

Approach

Effluent Sampling

Effluent samples provide essential information on the major pathway for pharmaceutical contaminants to enter the Bay. The state guidance on CECs directs agencies to include sampling WWTP effluent when screening for emerging contaminants (Dodder et al. 2015).

24-hour composite samples of WWTP effluent (up to 4 L HDPE) voluntarily provided by two to four high volume Bay Area dischargers will be characterized. Participants will include a WWTP employing secondary treatment, as well as one using more advanced measures. Sampling will occur in the summer of 2016, when inflow and infiltration are insignificant. A total of up to five samples will be analyzed, up to four effluent samples and a blank designed to capture airborne pharmaceuticals with the potential to contaminate samples.

One discharger has agreed to participate and contribute in-kind services to collect samples but is not specifically named here, as dischargers will have the option to keep their identities confidential in subsequent reporting of the data. Measurements for each discharger will be reported individually.

Ambient Bay Water Sampling

Bay water sample collection will take place in Central, South, and Lower South Bays in the summer of 2016. Previous study of Lower South Bay has revealed elevated levels of some pharmaceuticals (Harrold et al. 2009), a finding consistent with the greater influence of treated wastewater and reduced levels of dilution, particularly in the dry season.

Grab samples of ambient Bay water (up to 4 L HDPE) will be collected at up to nine Bay sites. A field duplicate will also be collected at one site; a blank collected at a wastewater facility will be used to assess the likelihood of contamination with airborne pharmaceuticals (e.g., asthma medications). To collect samples, SFEI staff will collaborate with existing sampling cruises conducted by other agencies; initial exploration of these opportunities is already underway. As such, equipment and rental costs are likely to be low.

Bay Margin Sediment Sampling

Sediment sample collection will occur in margin locations near treated wastewater discharges associated with participating WWTPs. Samples (up to 4 L HDPE) will be conducted at up to four margin sites in the summer of 2016. A field duplicate will also be collected, for a total of five samples.

Analytical Methods

Samples will be analyzed by AXYS Analytical (Sidney, BC, Canada) for pharmaceuticals in Lists 1-7 (Lists 1-6, AXYS Method MLA-075, currently available; List 7, AXYS Method MLA-104, to be released May 2015) using liquid chromatography tandem mass spectrometry (LC-MS/MS). AXYS Analytical was selected to provide analytical services for this study because they have unique qualifications for analyzing pharmaceuticals in environmental media. They test for more different pharmaceutical compounds than any other commercial laboratory in North America. Target analytes for List 7 in particular were selected following consultation with health and environmental agencies regarding pharmaceutical compounds of greatest potential concern for ecological health.

Analytes targeted via Lists 1-6 are provided in Table 2, along with initial information as to extraction and LC-MS/MS mode needed for each. Potential analytes for List 7 are provided in Table 3. This method is expected to be available in May 2015.

Previous studies in the Bay have utilized Lists 1, 3, 4, and 5 only.

Table 2. Pharmaceutical analytes in Lists 1-6 (AXYS Analytical). Superscripts indicate analytes for which only estimates of concentration are available.

List 1 - Acid Extraction in Positive Ionization	***List 4 - Basic Extraction in Positive Ionization***
Acetaminophen	Albuterol
Azithromycin	Amphetamine
Caffeine	Atenolol
Carbadox	Atorvastatin
Carbamazapine	Cimetidine
Cefotaxime	Clonidine
Ciprofloxacin	Codeine
Clarithromycin	Cotinine
Clinafloxacin	Enalapril
Cloxacillin [1]	Hydrocodone
Dehydronifedipine	Metformin
Digoxigenin	Oxycodone
Digoxin	Ranitidine
Diltiazem	Triamterene
	List 5 - Acid Extraction in Positive Ionization
1,7-Dimethylxanthine	
Diphenhydramine	Alprazolam
Enrofloxacin	Amitriptyline
Erythromycin-H20	Amlodipine
Flumequine	Benzoylecgonine
Fluoxetine	Benztropine
Lincomycin	Betamethasone
Lomefloxacin	Cocaine
Miconazole	DEET
Norfloxacin	Desmethyldiltiazem
Norgestimate	Diazepam
Ofloxacin	Fluocinonide
Ormetoprim	Fluticasone propionate
Oxacillin [1]	Hydrocortisone
Oxolinic acid	10-hydroxy-amitriptyline
Penicillin G [1]	Meprobamate
Penicillin V	Methylprednisolone
Roxithromycin	Metoprolol
Sarafloxacin	Norfluoxetine
Sulfachloropyridazine	Norverapamil
Sulfadiazine	Paroxetine
Sulfadimethoxine	Prednisolone
Sulfamerazine	Prednisone
Sulfamethazine	Promethazine

Sulfamethizole
Sulfamethoxazole
Sulfanilamide
Sulfathiazole
Thiabendazole
Trimethoprim
Tylosin
Virginiamycin

List 2 - Tetracyclines in Positive Ionization

Anhydrochlortetracycline
Anhydrotetracycline
Chlortetracycline
Demeclocycline
Doxycycline
4-Epianhydrochlortetracycline
4-Epianhydrotetracycline
4-Epichlortetracycline
4-Epioxytetracycline
4-Epitetracycline
Isochlortetracycline [2]
Minocycline
Oxytetracycline
Tetracycline

List 3 - Acid Extraction in Negative Ionization

Bisphenol A
Furosemide
Gemfibrozil
Glipizide
Glyburide
Hydroclorothiazide
2-hydroxy-ibuprofen
Ibuprofen
Naproxen
Triclocarban
Triclosan
Warfarin
Propoxyphene
Propranolol
Sertraline
Simvastatin
Theophylline
Trenbolone
Trenbolone acetate
Valsartan
Verapamil

List 6 - Acid Extraction in Positive Ionization

Amsacrine
Azathioprine
Busulfan
Citalopram
Clotrimazole
Colchicine
Cyclophosphamide
Daunorubicin
Diatrizoic acid
Doxorubicin
Drospirenone
Etoposide
Iopamidol
Medroxyprogesterone acetate
Melphalan
Metronidazole
Moxifloxacin [3]
Oxazepam
Rosuvastatin
Tamoxifen
Teniposide
Venlafaxine
Zidovudine

Table 3. Possible pharmaceutical analytes in List 7 (AXYS Analytical), expected May 2015.

Bupropion hydrochloride (31677-93-7)
Cefazolin sodium (27164-46-1)
Cefprozil (92665-29-7)
Clopidogrel - clopidogrel carboxylic acid
Clopidogrel, Clopidogrel bisulfate (113665-84-2; 120202-66-6)
Eprosartan (13304-01-4)
Fenofibrate (49562-28-9)
Fenofibrate metabolite: Fenofibric acid
Gabapentin (60142-96-3)
Irbesartan (138402-11-6)
Lamotrigine (84057-84-1)
Lamotrigine metabolite: Lamotrigine 2-N-glucuronide
Mycophenolate Mofetil (128794-94-5)
Mycophenolate Mofetil metabolite: Mycophenolic acid
Pravastatin sodium (81131-70-6)
Quetiapine, Quetiapine fumurate (111974-69-7; 111974-72-2)
Quetiapine metabolite: Norquetiapine
Ramipril (87333-19-5)
Ramipril metabolite: ramiprilate
Telmisartan (144701-48-4)
Topiramate (97240-79-4)
Trazadone (19794-93-5)
Trazadone metabolite: m-chlorophenylpiperazine
Decoquinate (CAS# 18507-89-6)
Hygromycin B (CAS# 31282-04-9)
Nicarbazin (CAS# 330-95-0)
Melengestrol Acetate (CAS 2919-66-6)
Iopromide (CAS# 73334-07-3)
Tilmicosin

Budget

The following budget represents estimated costs for this proposed special study (Table 4). Efforts and costs can be scaled up or down by changing the types of analyses (e.g., Lists 1-7) and the number and type of samples.

Table 4. Pharmaceuticals Characterization: Proposed Budget.

Expense	Estimated Hours	Estimated Cost ($)
Labor		
Project Staff	220	30000
Senior Management Review	16	3200
Project Management	0*	
Contract Management	0*	
Data Technical Services		13000
GIS Services	12	975
Creative Services	20	1600
IT Services	0	
Communications	0	
Operations	0	
Subtotal		
Subcontracts		
Name of contractor		
AXYS		42000
Direct Costs		
Equipment		0
Travel		200
Printing		0
Shipping		400
Other		0
Grand Total		
		91375

*services included in the base RMP funding

Budget Justification

Field Costs

Field costs will be low as a result of strategic study design, as well as the collaborative nature of the Bay science and management community. Wastewater agencies that choose to participate in the study will receive sample collection kits with instructions to allow them to provide crucial in-kind services to collect and ship samples themselves, minimizing SFEI staff time needed for sample collection. We expect to find ready accommodation on pre-existing water sampling cruises conducted by other agencies, limiting the cost of ambient water sample collection to staff labor hours spent on the Bay. Sediment samples will be collected from readily accessible margin sites near WWTP discharges, and will not require additional funds apart from staff time and shipping.

Laboratory Costs

Analytical costs per sample for pharmaceuticals (Lists 1-7) are expected to be $2,300 per water or wastewater sample and $2,400 per sediment sample. For 13 water samples and 5 sediment samples (including duplicates and blanks), the analytical costs are expected to be $42,000.

Data Management Costs

Standard data management procedures and costs will be used for this project.

Reporting

Bay water and sediment data will be reported via RMP web tools (e.g., CEDEN). Results will be reported to the RMP committees in the form of a draft manuscript for publication in a peer-reviewed journal by 3/31/17.[3]

References

Anderson PD, Denslow ND, Drewes JE, Olivieri AW, Schlenk D, Scott GI, Snyder SA. 2012. Monitoring Strategies for Chemicals of Emerging Concern (CECs) in California's Aquatic Ecosystems. Costa Mesa, CA.

Brodin T, Fick J, Jonsson M, Klaminder J. 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339:814-815.

Dodder NG, Mehinto AC, Maruya KM. 2015. Monitoring of Constituents of Emerging Concern (CECs) in Aquatic Ecosystems – Pilot Study Guidance and QA/QC Guidance. SCCWRP Technical Report 854. Southern California Coastal Water Research Project, Costa

[3] This report will be distributed by email, instead of posting to the website, so as not to jeopardize potential journal publication.

Mesa, CA.
http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/854_CaliforniaCEC_PilotStudy_040315.pdf

EMEA (European Medicines Agency). 2006. European Medicines Agency Pre-Authorisation Evaluation of Medicines for Human Use (Doc. Ref. EMEA/CHMP/SWP/4447/00) Guideline on the environmental risk assessment of medicinal products for human use. Committee for Medicinal Products for Human Use (CHMP). 12pp.

European Commission. 2003. Technical Guidance Document on Risk Assessment—Commission Regulation (EC) No. 1488/94 on Risk Assessment for existing substances, 337pp.

Ferrari B, Mons R, Vollat B, Fraysse B, Paxeus N, Lo Giudice R, et al. 2004. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environmental Toxicology and Chemistry 23:1344-1354.

Gonzalez-Pleiter M, Gonzalo S, Rodea-Palomares I, Leganes F, Rosal R, Boltes K, et al. 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment. Water Research 47:2050-2064.

Grung M, Kallqvist T, Sakshaug S, Skurtveit S, Thomas KV. 2008. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. Ecotoxicol Environ Saf 71(2): 328-340.

Harrold KH, Yee D, Sedlak M, Klosterhaus S, Davis JA, Woudneh M, Riley P. 2009. Pharmaceuticals and Personal Care Products in Wastewater Treatment Plant Influent and Effluent and Surface Waters of Lower South San Francisco Bay. SFEI Contribution 549. San Francisco Estuary Institute, Oakland, CA. http://www.sfei.org/documents/2918

Klosterhaus SL, Grace R, Hamilton MC, Yee D. 2013a. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environment International 54, 92–9.

Klosterhaus S, Yee D, Sedlak M, Wong A, Sutton R. 2013. Contaminants of Emerging Concern in San Francisco Bay: A Summary of Occurrence Data and Identification of Data Gaps. RMP Contribution 698. San Francisco Estuary Institute, Richmond, CA. http://www.sfei.org/documents/cec-synthesis

Kolodziej EP, Qu S, Forsgren KL, Long SA, Gloer JB, Jones GD, et al. 2013. Identification and environmental implications of photo-transformation products of trenbolone acetate metabolites. Environmental Science & Technology 47:5031-5041.

Näslund J, Hedman JE, Agestrand C. 2008. Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. Aquatic Toxicology 90:223-227.

Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. The Science of the Total Environment 447:345-360.

Sutton R, Sedlak M, Davis J. 2013. Contaminants of Emerging Concern in San Francisco Bay: A Strategy for Future Investigations. SFEI Contribution 700. San Francisco Estuary Institute, Richmond, CA.
http://www.sfei.org/documents/contaminants-emerging-concern-san-francisco-bay-strategy-future-investigations

Weinberger J, 2nd, Klaper R. 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish *pimephales promelas* (fathead minnow). Aquatic Toxicology 151:77-83.