Analysis of Ethoxylated Surfactants and their Degradation Products in Ambient Water, Wastewater, and Stormwater of San Francisco Bay, CA

by
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April 28, 2023

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Executive Summary

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Ethoxylated surfactants are non-ionic surfactants that are commonly used as cleaning and care products. The main classes of ethoxylated surfactants include alcohol ethoxylates (AEOs) and alkylphenol ethoxylates (APEOs) (Jardark et al., 2016). Ethoxylated surfactants are present in complex mixtures because their hydrophilic ethylene oxide chain length can be of varying lengths and their hydrophobic alkyl chains can have linear or branched isomers. As use and manufacturing of these compounds increases, they become more ubiquitous in the aquatic environment which raises concerns regarding their toxicity. Short chain ethoxylated surfactants and alkylphenols (APs) are the most abundant degradation products of these compounds and are a primary focus of monitoring efforts since they are known to be more toxic in aquatic environments. APEOs are known to degrade through shortening of the ethylene oxide chain and eventual production of short chain APEOs and APs (Ying et al., 2002). APEOs are endocrine disruptors, they act on the estrogen receptors. Various studies have noted toxicity after exposure to APEOs (Acir and Guenther, 2018, Kassotis et al., 2018, Soto et al., 1991, and TenEyck and Markee, 2006). Due to the degradation to more toxic products, the use of alternatives such as AEOs has increased. AEOs degrade faster and to less toxic and hydrophobic compounds, however studies have discovered toxicity resulting from exposure to AEOs in aquatic organisms (Cardellini and Ometto, 2000). Because of their widespread use and toxicity concerns, detection of ethoxylated surfactants in the environment is crucial to understand potential management strategies.

Ethoxylated surfactants have been a difficult group of compounds to analyze and manage because of the complexity of their mixtures, many of the compounds lack analytical standards, and they span a wide range of hydrophobicity which requires different analytical methods to extract and analyze. A previous study (Lindborg et al., 2023) aimed at analyzing a variety of ethoxylated surfactants in ambient water, wastewater, and stormwater in San Francisco Bay area to determine concentrations and predict sources of contamination. The present study updated the analytical method from that study to include alkylphenols and the mono-, di-, and tri-ethoxymers of nonylphenol ethoxylate (NPEO) and octylphenol ethoxylate (OPEO) surfactants, as well as the surfactants analyzed previously ($C_{11\text{-}14}$EO, C_{16}EOs, $NP_{3\text{-}15}$EO and $OP_{3\text{-}15}$EO).

Samples from Lindborg et al. (2023) and additional stormwater samples were reanalyzed using the updated method. 20 ambient Bay water, 16 wastewater, and 29 stormwater samples (n = 65, includes field blanks and duplicates) were analyzed. We used mixed mode high performance liquid chromatography coupled to orbitrap high resolution mass spectrometry to quantitate the alkylphenol and alcohol ethoxylates.

Total polyethoxylated surfactant concentrations were compared amongst the different matrices. Wastewater and stormwater sites had the highest concentrations of ethoxylated surfactants. In comparison with previous studies, we found higher concentrations of ethoxylated surfactants in wastewater (Sanderson et al., 2013 and Lara-Martín et al., 2014). We found both

higher and lower concentrations of NPEOs in stormwater compared to other studies (Rule et al., 2006 and Lindborg et al., 2023). Wastewater sites were dominated by AEOs, whereas stormwater sites were dominated by APEOs, mainly NPEOs. Ambient Bay water sites had orders of magnitude lower concentrations compared to wastewater and stormwater. We detected ethoxylated surfactants at all Bay water sites, compared to only two sites in Lindborg et al. (2023). Sites with the highest concentrations of ethoxylated surfactants were similar between this study and Lindborg et al. (2023).

In comparing long chain APEOs and short chains APEOs and APs, all sites were dominated by long chain APEOs, specifically NPEOs. Wastewater and stormwater sites contain mostly branched $C_{11}EO$, $C_{12}EO$, and $C_{13}EO$ compared to linear, whereas Bay water sites are dominated by linear $C_{12}EO$, followed by branched $C_{12}EO$ and $C_{13}EO$ and linear $C_{11}EO$. Ratios of long chain to short chain APEOs and ratios of linear to branched AEO isomers were assessed for an understanding of potential degradation between different matrices. Our results indicate that there is less degradation in the Bay compared to wastewater.

This study provided a thorough assessment of ethoxylated surfactants in an urban estuary. Future studies aimed at detection of monoethoxylate APEOs and refining determination of sources to the Bay would provide a more detailed understanding of sources of contamination and potential management strategies.

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Analysis of Ethoxylated Surfactants and their Degradation Products in Ambient Water, Wastewater, and Stormwater of San Francisco Bay, CA

Sammy DiLoreto

Abstract

Non-ionic surfactants such as ethoxylated surfactants are commonly used as cleaning and care products which leads to their ubiquity in the aquatic environment. As use and manufacturing of these compounds increases, concerns regarding their toxicity grows. Short chain ethoxylated surfactants and alkylphenols are the most abundant degradation products of these compounds and are a primary focus of monitoring efforts since they are known to be more toxic in aquatic environments. A previous study analyzed ethoxylated surfactants in ambient water, wastewater, and stormwater in San Francisco Bay, CA area to determine concentrations and predict sources of contamination. The present study updated the analytical method from that study to include alkylphenols and the mono-, di-, and tri-ethoxymers of nonylphenol ethoxylate (NPEO) and octylphenol ethoxylate (OPEO) surfactants, as well as the surfactants analyzed previously ($C_{11\text{-}14}$EO, C_{16}EOs, $NP_{3\text{-}15}$EO and $OP_{3\text{-}15}$EO). We used mixed mode high performance liquid chromatography coupled to orbitrap high resolution mass spectrometry to quantitate the alkylphenol and alcohol ethoxylates. Wastewater and stormwater sites had the highest concentrations of ethoxylated surfactants. Ambient Bay water sites had orders of magnitude lower concentrations compared to wastewater and stormwater. Wastewater sites were dominated by AEOs, whereas stormwater sites were dominated by APEOs, mainly NPEOs. Ratios of long chain to short chain NPEOs and ratios of linear to branched AEO isomers indicate that there is less degradation in the Bay compared to wastewater. In this study we assessed ethoxylated surfactants in an urban estuary. Future studies aimed at refining the determination of sources to the Bay would provide a more detailed understanding of sources of contamination and potential management strategies.

1. Introduction

1.1 Physical chemical properties of Ethoxylated Surfactants

Surfactants are one of the most commonly detected and undesirable pollutants found in the environment, particularly the aquatic environment. Surfactants are used as cleaning and care products such as shampoos, detergents, etc. This use leads to discharge of highly contaminated wastewaters into various aquatic environments. There are a variety of different kinds of surfactants including anionic, cationic, non-ionic, and zwitterion. The most common class of non-ionic surfactants are the ethoxylated surfactants, which include alcohol ethoxylates (AEOs) and alkylphenol ethoxylates (APEOs) (Jardark et al., 2016).

Ethoxylated surfactants contain a hydrophobic alkyl chain and hydrophilic ethylene oxide chain (EO) which leads to the formation of complex mixtures with linear and branched alkyl isomers and varying EO chain lengths. Alkylphenols are synthesized by a catalyzed reaction of an olefin with phenol, cresols, or xylenols. They are then ethoxylated by a condensation reaction with ethylene oxide using a basic catalyst (Maguire, 1999). Alcohol ethoxylates (AEOs) are produced by the reaction of alcohols with ethylene oxide. AEOs can be linear or branched and the manufacturing of these varies. Linear AEOs are produced from linear alcohols derived from vegetable or animal sources (Talmage, 1994). Figure 1 shows the structure of AEOs and APEOs where n is the number of units in the alkyl chain and m is the number of units in the EO chain (Jardark et al., 2016).

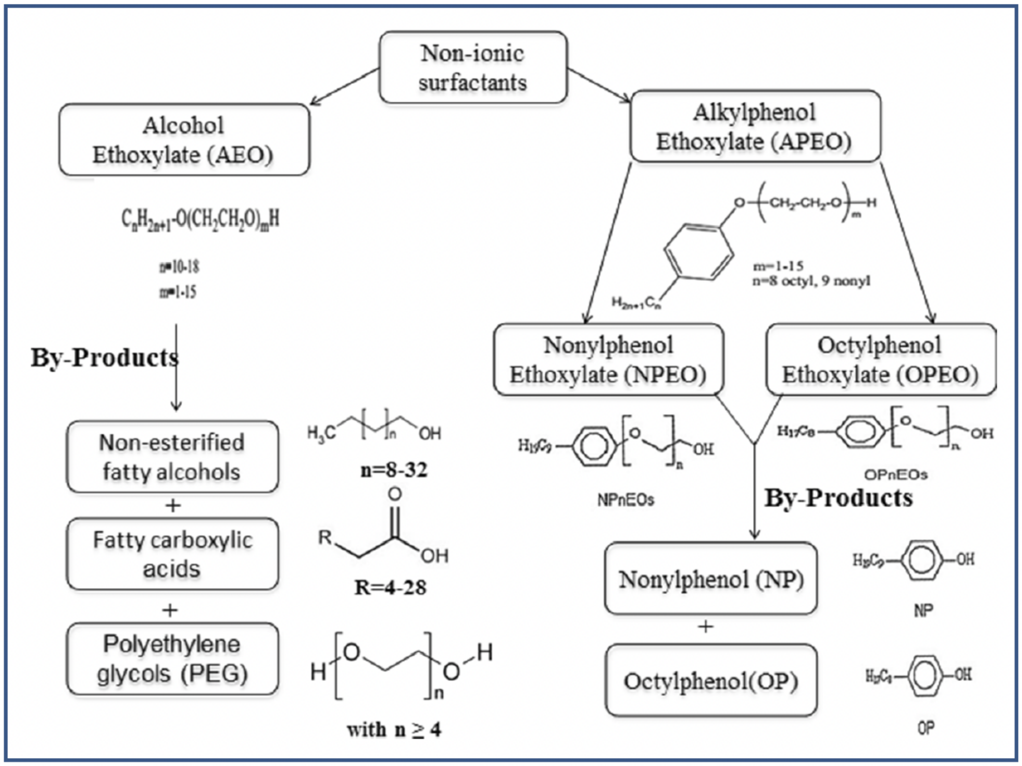


Figure 1. Structures and chemical formulas for non-ionic surfactants (Jardark et al., 2016)

The two most commonly studied and used APEOs are nonylphenol ethoxylate (NPEO) with a 9-carbon alkyl chain and octylphenol ethoxylate (OPEO) with an 8-carbon alkyl chain. In the U.S., production of NPEOs is hundreds of millions of pounds per year and they represent up to 85% of APEOs used (EPA, 2010). The use of APEOs has been restricted since 1984 because long-chain NPEOs and OPEOs can degrade to more toxic and hydrophobic products. APEOs degrade through shortening of the EO chain and production of alkylphenoxy ethoxy acetic acid and alkylphenoxy acetic acid through oxidation of the EO chain (Figure 2). The most common intermediates are alkylphenols (nonylphenol and octylphenol), short chain APEO (dominantly AP_2EO), and a series of ether carboxylates (Ying et al., 2002). Complete deethoxylation to form alkylphenols has been observed but only under anaerobic conditions. Half-lives ranging from four to 183 days in water have been seen in various conditions in field and laboratory studies for the degradation of NPEOs to shorter chains NPEOs and NP. These studies suggest that in many water systems including ambient water, wastewater, and stormwater, APEOs can degrade to their more hydrophobic and toxic metabolites (Ying et al., 2002).

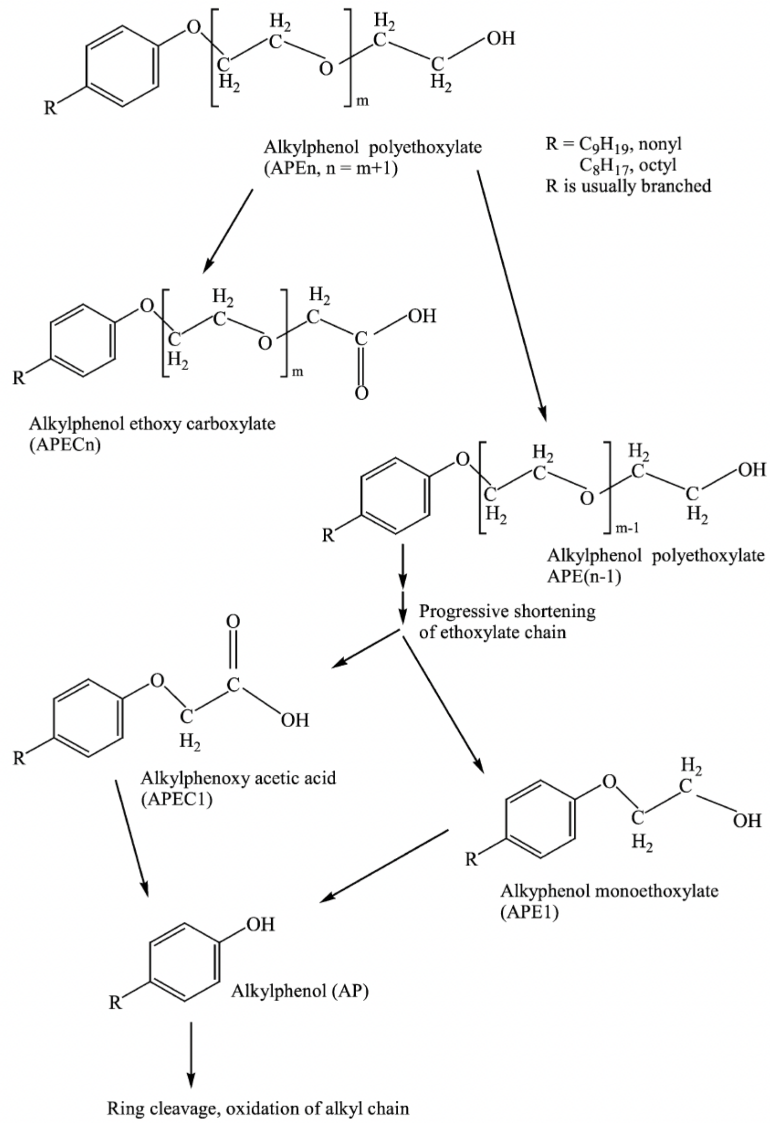


Figure 2. Degradation processes for alkylphenol ethoxylates (Ying et al., 2002)

Since the restriction of APEOs, alternatives such as AEOs have begun to be more widely used. In 2002, the use of AEOs was about 275,000 tons per year in European household detergents. AEOs biodegrade more rapidly and are considered to be less toxic than APEOs (DeArmond and DiGoregorio, 2013). Biodegradation of linear AEOs occurs primarily through hydrolysis of the ether linkage next to the alkyl chain followed by oxidation of the alkyl chain with releasing of two carbon units as acetyl groups and a slower oxidation of the ethylene oxide chain by two carbon units. These chains can be degraded to carbon dioxide and water. For branched AEOs, oxidation occurs at the terminal end of the ethylene oxide chain (Talmage, 1994). Due to the complexity of these mixtures of compounds, toxicity of the degradation products, and widespread use, the detection of ethoxylated surfactants in the environment is important to understand potential impacts and develop management strategies.

1.2 Detection in the Environment

1.2.1 Ambient Water

The use and production of APEOs and AEOs leads to detection in various aquatic environments including ambient water, wastewater, and stormwater. The detection of APEOs and their degradation products in surface waters is mainly due to discharge of effluents from wastewater treatment plants. Levels reported from various studies for NP range from <LOD to 644 μg/L, for $NP_{1-2}EO$ <LOD to 20 μg/L, for NP_3EO <LOD to 25.7 μg/L, and for OP <LOD to 0.47 μg/L (Ying et al., 2002). Ferguson et al. (2001) detected APEO metabolites in Jamaica Bay, Long Island, NY. Short chain NPEOs were detected at concentrations of 0.22-1.05 μg/L which were higher than concentrations of short chain OPEO (concentrations of 0.007-0.04 μg/L). They found a strong correlation between a sewage tracer and APEO metabolite concentrations which confirmed wastewater as a source of contaminants (Ferguson et al., 2001).

Lalonde and Garron (2020) conducted a study to determine the concentrations of NP, OP, and $NP_{1-2}EO$ in a Canadian freshwater environment. They detected concentrations ranging from 0.0013 to 0.48 μg/L, with NP having the widest range of concentrations. In this study they categorized various upstream activities of the sampling sites and these activities included textile mill, mixed use, urban, municipal wastewater treatment plant (MWWTP), and a reference. All compounds were detected at higher concentrations at MWWTP and urban sampling sites. In comparison to previous studies, they found a decrease in NP concentrations, likely due to decrease in use of NP. They detected $NP_{1-2}EO$ at some sites that have not been detected in the past due to limits of detection. Overall, this study detected large decreases in APEO concentrations in Canadian freshwater compared to previous studies which suggests that restrictions on use of these compound will reduce the presence in the environment (Lalonde and Garron, 2020). Lindborg et al. (2023) found $C_{12}EO$ and $C_{14}EO$ in San Francisco Bay with sum concentrations of 0.49 μg/L and 0.34 μg/L, respectively. However, AEOs overall were found in lower concentrations in Bay water than in other urban estuary studies (Lindborg et al., 2023).

1.2.2 Wastewater

APEOs and their degradation products are frequently detected in effluents of municipal sewage treatment plants. In the Ying et al. (2002) review, concentrations of NP, OP, and short chain NPEO in sewage varied widely from less than the limit of detection to over 300 μg/L. A study in Michigan found NP in effluent at concentrations of 0.017 to 37 μg/L, OP at concentrations of <LOD to 37 μg/L, and NPEOs at concentrations of <LOD to 332 μg/L. Sole et al. (2000) conducted a study in Northeast Spain and detected NP at levels ranging from 6 to 343 μg/L in effluent samples with levels as high as 644 μg/L in receiving waters. A more recent study aiming at developing a method to detect the level of contamination of various endocrine disrupting chemicals, including APEOs, in sewage found levels of total NP (NP and $NP_{1-12}EO$) to be 7.60 μg/L and levels of total OP (OP and $OP_{1-12}EO$) to be 0.83 μg/L in primary treatment. Final effluent levels for total NP and total OP indicated over an 80% decrease in concentration levels. Despite this decrease, the mixtures became enriched in the shorter chain ethoxylates and parent compounds as the treatment progressed as a result of the degradation of these APEOs (Vega-Morales et al., 2010).

A recent study in San Francisco Bay, CA found that an AEO, $C_{13}EO$, was the dominant ethoxylated surfactant in wastewater effluent with concentrations reaching 45.4 μg/L. Little information has been previously available for the presence of AEOs in wastewater effluent. Their study had generally lower concentrations than of the United States but higher than other areas similar to San Francisco Bay, CA. The high concentrations found are likely due to local factors or the transition from APEOs to AEOs (Lindborg et al., 2023).

1.2.3 Stormwater

In addition to surface water and wastewater effluent, stormwater can also be contaminated with ethoxylated surfactants. Little research has been done in detecting these surfactants in stormwater, but studies have shown NP and OP to be present in stormwater. Zgheib et al. (2010) conducted a study in Paris to determine contaminants present in stormwater and NP was detected in all samples with a median concentration of 0.75 μg/L. Previous studies in Germany, England, and Sweden found concentrations of NP in stormwater at 3.46 and 9.90 μg/L, 1.75 μg/L, and 12 μg/L, respectively (Zgheib et al., 2010). Lindborg et al. (2023) found concentrations of total ethoxylated surfactants in stormwater runoff to be 0.004 – 4.7 μg/L. NPEO and OPEO dominated surfactant concentrations at each stormwater sites, followed by $C_{13}EO$. Stormwater sites with high concentrations were associated with high levels of industrial and/or transportation land use. The presence of these surfactants in stormwater may be due to leaching from tires and concrete, from sealants and coatings applied to roadways, and emulsifiers in vehicle related products. Sites with low concentrations were associated with high proportions of agriculture and open space land use. This study also noticed differences in magnitude of detected concentrations between years suggesting that either frequency and intensity of storm events or increases in ethoxylated surfactant use (Lindborg et al., 2023). This finding will be important to monitor in future years. Due to vast detection in other aquatic environments, further research on the presence of ethoxylated surfactants in stormwater is needed.

1.3 Toxicity

The importance of studying the presence of ethoxylated surfactants in the environment is accompanied by understanding the toxicity of these compounds. Ethoxylated surfactants are persistence, bioaccumulative, and toxic (PBT), thus stressing the relevance of understanding their presence in aquatic environments. As previously mentioned, APEO metabolites such as NP, OP, and their short chain ethoxylates are formed through degradation in the environment and there is an increase in toxicity with a decrease in EO chain length (Servos, 1999). An Action Plan was developed by the U.S. EPA for NP and NPEOs due to widespread use and toxicity (EPA, 2010).

The main mode of toxicity for these APEO metabolites is endocrine disruption, specifically estrogenic effects. Acute toxicity has been seen in fish, invertebrates, and algae from exposure to NP and OP at concentrations of 17-3000 μg/L and chronic toxicity has been seen at 3.7-6 μg/L (Acir and Guenther, 2018). Estrogenic activity of NP in mammals was realized by Soto et al. (1991) and confirmed by various studies. Estrogenic activity has also been identified in fish for OP and short chain NPEOs. TenEyck and Markee (2006) assessed the toxicity of NP and $NP_{1\text{-}2}EO$ in freshwater species *Pimephales promelas* (fathead minnow) and *Ceriodaphnia dubia* (water flea). The LC_{50} values for NP and $NP_{1\text{-}2}EO$ in fathead minnows were 136, 218, and

323 ug/L, respectively. The LC_{50} values for NP and $NP_{1-2}EO$ in the water flea were 92.4, 328, and 716 ug/L, respectively. Concentrations similar to these LC_{50} values have been seen in the environment as discussed in the previous section. In this study, they also assessed interactions of these compounds and discovered that when present in mixtures there is an additive or synergistic effects of these contaminants (TenEyck and Markee, 2006). Ethoxylated surfactants are present in complex mixtures in the environment, thus stressing the importance of this finding.

AEOs are known to be less toxic than APEOs but studies have also assessed their toxicity in various organisms (DeArmond and DiGoregorio, 2013). Cardellini and Ometto (2000) studied the teratogenic and toxic effects in *Xenopus laevis* (African clawed frog) embryos and tadpoles. The 72-h LC_{50} was 4,590 μg/L for an AEO, and it exhibited alterations of mitochondria and narcotic effects. Although AEOs are seen to be less toxic than APEOs, their use is increasing with the phasing out of APEOs, so it is important to study their toxicity as well.

In addition to toxicity to aquatic organisms from exposure to APEOs and AEOs, a recent study assessed the disruption of human metabolic health related to use of common household products containing APEOs and AEOs. After exposure to increasing concentrations of ethoxylated surfactants, 3T3-L1 cells were assessed for triglyceride accumulation and preadipocyte proliferation. Triglyceride accumulation and preadipocyte proliferation were promoted at APEO and AEO concentrations ranging from 0.1 to 10 uM. Activity was related to the length of the ethoxylate chain. Particularly for NPEOs, increasing activity occurred for NP and short chain ethoxylates but decreased activity for longer chains. Their data suggests metabolic disruption at environmentally relevant concentrations and also confirms that AEOs exhibit toxicity as well as APEOs (Kassotis et al., 2018). It is crucial to prioritize monitoring and detection of ethoxylated surfactants in order to understand the scope of need for additional toxicity evaluations.

1.4 Past Research and Current Study

The current study analyzed ethoxylated surfactants in an urban estuary, San Francisco Bay, which receives wastewater and stormwater inputs. The Bay has been evaluated for various other contaminants as seen in previous research studies (Klosterhaus et al., 2013, Mendez et al., 2022, Shimabuku et al., 2022). Most of this previous work has been funded by San Francisco Estuary Institute (SFEI). SFEI's Regional Monitoring Program for Water Quality (RMP) evaluates contaminants in the Bay and provides that information to regulators and decision-makers to best manage the Bay. This study was conducted in collaboration with SFEI.

Ethoxylated surfactants are difficult to analyze due to the complexity of the mixtures. Most of these compounds do not have analytical standards and they have a wide range of hydrophobicity, which requires different extraction and analysis methods. These difficulties have limited researchers in the past to only analyzing some of these compounds in this class, particularly the short-chain NPEOs. However, these short-chain ethoxylates are difficult to identify with current analytical methods.

According to RMP's tiered risk and management action framework, alkylphenols and alkylphenol ethoxylates are considered to be of moderate concern, stating that there is data from the Bay that suggests a "high probability of a low-level effect on Bay wildlife." Their current data states that they are found at concentrations below most toxicity thresholds in Bay water, sediment, bivalves, fish, bird eggs. Potential impacts include larval barnacle settlement, synergistic effects with pyrethroids, and estrogenic activity (Miller et al., 2020). Due to this, it is important to determine the concentrations of these contaminants and other ethoxylated surfactants in the Bay to better understand management strategies.

Lindborg et al. (2023) analyzed a variety of ethoxylated surfactants in ambient Bay water, wastewater, and stormwater using UHPLC-MS/MS. Samples were analyzed for $C_{12\text{-}14}$EO, C_{16}EO, $C_{12(Br)}$EO, NPEO and OPEO. Short-chain NPEO and short-chain OPEO (including NP, OP, $NP_{1\text{-}2}$EO and $OP_{1\text{-}2}$EO) were either not analyzed or not quantified due to a limitation in the analytical method. The limitation in their analytical methods limited the ability to fully address concerns regarding these emerging contaminants.

The objective of this master's project was to test an updated analytical method that would include short-chain NPEO and OPEO (NP, OP, $NP_{1\text{-}2}$EO and $OP_{1\text{-}2}$EO), in addition to the previously analyzed ethoxylated surfactants ($C_{11\text{-}14}$EO, C_{16}EOs, $NP_{3\text{-}15}$EO and $OP_{3\text{-}15}$EO). This provided a comprehensive analysis of ethoxylated surfactants in environmental samples. Previously analyzed samples were analyzed or re-analyzed and additional samples were collected and analyzed using this updated method.

2. Materials and Methods

2.1 Sample Collection

Sample collection for the previously analyzed samples from Lindborg et al. (2023) can be described as follows (Figure 3). Wastewater effluent samples were collected in August, September, and October 2019 from eight wastewater treatment facilities. These months were chosen because they are during the dry season, thus eliminating the influence of inflow and infiltration from precipitation on wastewater concentrations. The eight sites were selected to be representative of the effluent flows from the publicly owned treatment works (POTWs) to the Bay because they encompass approximately 60% of the total effluent flows to the Bay. These POTWs represent different geographic locations, wastewater treatment types, and population sizes. Effluent samples were 24-hour composites on either a flow-weighted or time-weighted basis using auto-samplers that are for regular use and maintenance for regulatory monitoring purposes.

Stormwater samples were collected between November 2018 and January 2020, as this is during the wet season. Sample sites were chosen based on the following criteria: 1) high urban land use in the watershed and proximity to roadways; 2) other land uses connected with possible contaminant sources; and 3) existing sample collection as part of other studies. 14 samples (including one field blank) were collected from 11 sites (Lindborg et al., 2023). Sample containers (2.5L glass bottles) were rinsed three times with site water prior to sampling. Pre-cleaned stainless-steel bailers were dipped into stormwater channels during a storm event and poured into sample containers. 15 additional stormwater samples (including 1 field blank) were collected in 2022 from 11 sites. These samples were collected and transported using the same method as described above.

Ambient Bay water grab samples, including field blanks and duplicates, were collected July to August 2019 from 19 sites. Site selection was randomized based on the RMP's sampling design to represent the ambient waters in each sub-embayment. Field blanks were collected at every site for POTWs, two sites for Bay water, and one site for stormwater by filling a sample container with 2 L LCMS-grade reagent water. Clean 2 L glass bottles were used for sample collection. Samples on ice were shipped overnight from SFEI (Richmond, CA) to Duke

University (Durham, NC). Two wastewater samples (blanks) and three Bay water samples (a sample, a duplicate, and a field blank) were broken during travel. Samples were extracted upon receiving.

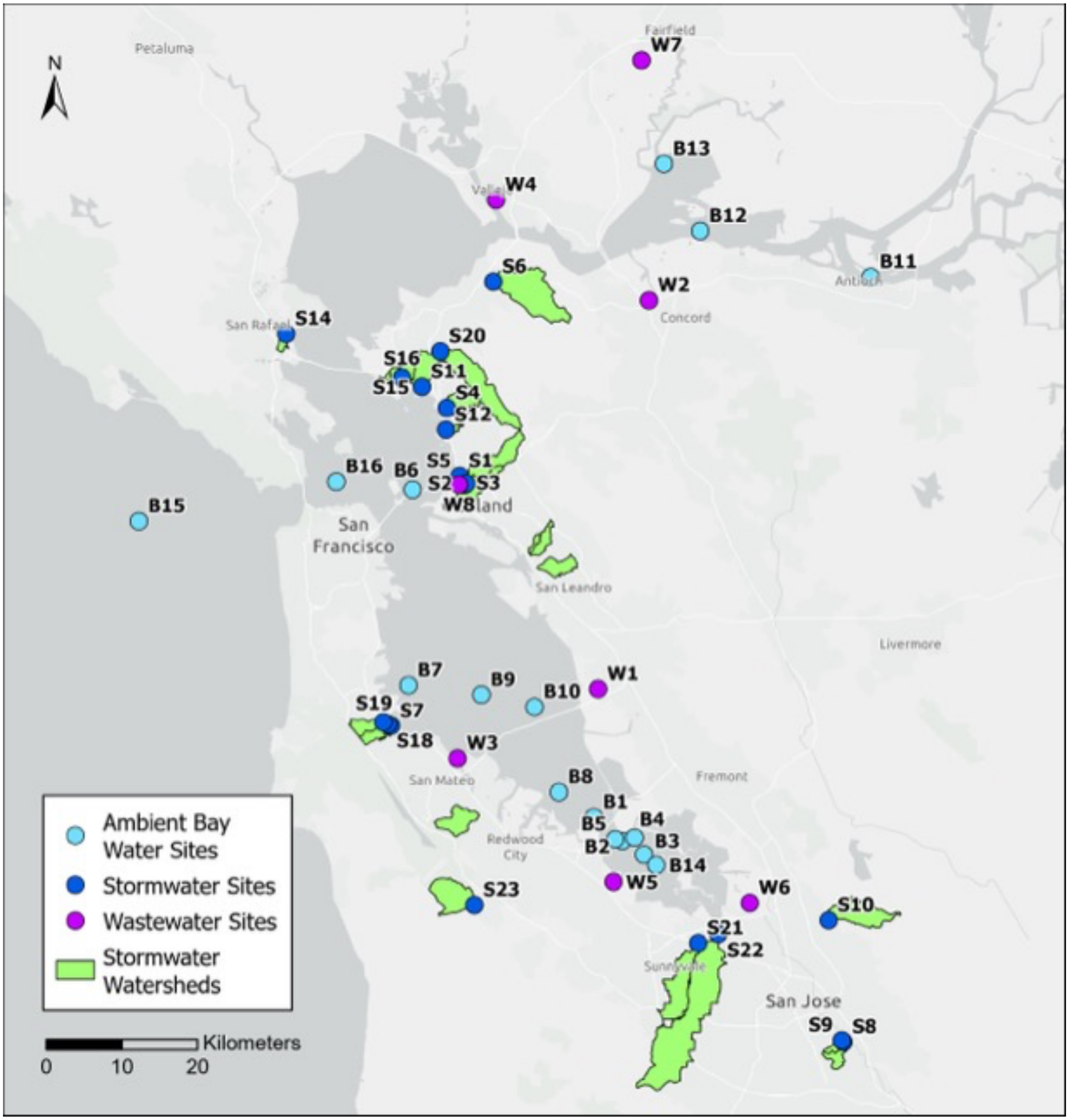


Figure 3. Ambient Bay water, stormwater, and wastewater sites (publicly owned treatment works (POTW)) analyzed. All site names have been assigned samples codes for anonymity. Duplicates have been averaged and field blanks are excluded.

2.2 Sample Preparation

A total of 20 Bay water samples, 16 wastewater, and 29 stormwater samples were processed (n=65, including field blanks and duplicates). Samples were prepared following the methods in Lindborg et al. (2023). 1 L aliquots of each ambient Bay water and stormwater sample were filtered (< 0.45µm GF/F, Whatman) for particle removal. An automated solid phase extraction (SPE) system (Dionex Autotrace 280) was used to perform solid-phase extraction on the samples with Waters Oasis HLB SPE cartridges (500 mg sorbent, eluted with 10% MTBE in methanol). The same method was followed for a 1 L laboratory blank. 250 mL aliquots of each wastewater sample were filtered using the same procedures. All samples were spiked prior to extraction with isotopically labeled surrogate standards (17β-estradiol-d4, 3,6-dichloro-2-methyl-d3-benzoic acid, atrazine-d5, carbaryl-d7, DEET-d7, imidacloprid-d4, iprodione-d5, mecroprop-d4, metalaxyl-d6, prometon-d14, propanolol-d7, thiophanate-methyl-d6, tramadol-d6; 10 µg/mL and spiked at 25 µL). The laboratory blank was also spiked to assess recovery.

The SPE extracts were concentrated using a SpeedVac centrifugal evaporator and reconstituted with HPLC starting mobile phase (1 mL volume, 5 mM ammonium acetate in 95%:5% Water:ACN). Wastewater samples were diluted 1:4. Solvent blanks containing 50%:50% methanol:water were also prepared for analysis. In the present study, 500 µL of each previously analyzed sample and additional samples were aliquoted into 2 mL vials and spiked with an internal standard mixture (4-(3,6dimethyl-3heptyl)phenol-ring-$^{13}C_6$, of 4-(3,6dimethyl-3heptyl)phenol-diethoxylate-ring-$^{13}C_6$, 4-tert-octylphenol-ring-$^{13}C_6$, and 4-tert-octylphenol-diethoxylate-ring-$^{13}C_6$; 1000 ng/mL and spiked at 50 µL). Hamilton syringes were utilized for all spiking and transfers to avoid sample contamination from laboratory equipment.

2.3 Sample Analysis

All samples (0.5 mL, in amber glass LC-MS vials) were analyzed by mixed mode high performance liquid chromatography coupled to high resolution mass spectrometry (ThermoFisher Orbitrap Fusion Lumos). This method was modified from methods published previously (Ferguson et al., 2001) to include a broader range of ethoxylated surfactants in addition to the alkylphenol ethoxylates. Samples were separated using Shodex (Japan) MSpak GF-310 4D gel filtration column (150 x 4.6 nm). This column is packed with cross-linked poly(vinyl alcohol) phase which is compatible with both normal-phase and reversed phase solvents (Ferguson et al., 2001). This phase is also suitable for size exclusion-based separation in the mass range of 200 to 1500, similar to the molecular mass range of NPEO ethoxymers (Ferguson et al., 2001). Sample separation occurred over a 27-minute multi-step gradient (mobile phases water [0.1mM ammonium acetate] and acetonitrile). The gradient was increased from 32% to 65% acetonitrile over the first 20 minutes; held at 65% acetonitrile for two minutes; then brought from 65% back to 32% over the last five minutes. The sodium salt was added to enhance and form sodium adducts to the NPEOs, OPEOs and AEOs.

NPEOs, [$^{13}C_6$]NPEOs, OPEOs, [$^{13}C_6$]OPEOs, and AEOs were detected in positive ion mode by selected ion monitoring of $(M+Na)^+$ ions. The polarity switched to negative ion mode after the NPEOs, OPEOs, and AEOs had eluted (around 18 min). Later eluting compounds, NP, [$^{13}C_6$]NP, OP, and [$^{13}C_6$]4-t-OP, were detected by selected ion monitoring of the $(M-H)^-$ ions (Ferguson et al., 2001). See Table 1 below for operating conditions. NP, [$^{13}C_6$]NP, OP, and

$[^{13}C_6]$4-t-OP were analyzed by MS2 whereas NPEOs, OPEOs, and AEOs were analyzed by MS1. The scan filters used for each compound can be found in Table 2 below.

Table 1. Instrument Parameters

HPLC Parameters			
Flow rate		0.3 mL/min	
Column Temperature		60°C	
Gradient Timetable			
	Time (min)	%A	%B
	0	32	68
	20	65	35
	22	65	35
	23	32	68
	27	32	68

Table 2. Scan filters

Compound	**Scan Filter**
$[^{13}C_6]$NP	FTMS - p ESI Full ms2 225.1956@hcd45.00 [42.0000-236.0000]
$[^{13}C_6]$4-t-OP	FTMS - p ESI Full ms2 211.1799@hcd45.00 [41.0000-222.0000]
$[^{13}C_6]NP_2EO$ $NP_{2-3}EO$	FTMS + p ESI SIM msx ms [197.0777-370.7952, 325.7690-326.7690, 331.7891-332.7891, 369.7952-370.7952]
$[^{13}C_6]OP_2EO$ $OP_{2-3}EO$	FTMS + p ESI SIM msx ms [197.0777-356.7795, 311.7533-312.7533, 317.7734-318.7734, 355.7795-356.7795]
NP	FTMS - p ESI Full ms2 219.1754@hcd45.00 [42.0000-230.0000]
NP_1EO	FTMS + p ESI SIM msx ms [197.0777-282.7428, 267.7271-268.7271, 281.7428-282.7428]
OP	FTMS - p ESI Full ms2 205.1598@hcd45.00 [41.0000-216.0000]
OP_1EO	FTMS + p ESI SIM msx ms [197.0777-282.7428, 267.7271-268.7271, 281.7428-282.7428]
$NP_{4-17}EO$ $OP_{4-16}EO$ $C_{11-14}EO$ $C_{16}EO$	FTMS + p ESI Full ms [197.0777-1600.0000]

2.4 Data processing

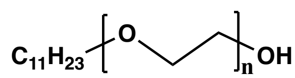
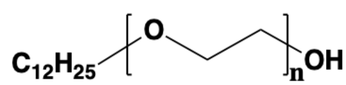
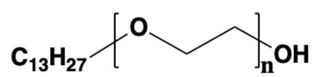
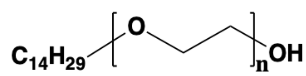
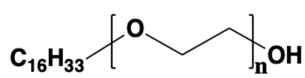
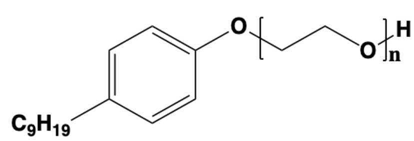
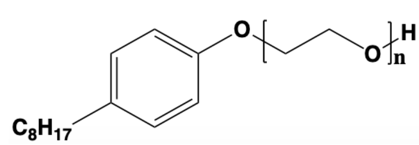
The compounds in all samples were quantified with reference to purified and characterized authentic standards for NPEO, OPEO, and the AEO series $C_{11-14}EO$, $C_{16}EO$ (Table 3). EO units 1-16 were analyzed for each series. Separation of commercial AEO surfactant mixtures by mixed-mode size exclusion chromatography with charged aerosol detection was employed to determine the component ethoxymers. Calibrated standards of AEOs were prepared using the data from the separation. Calibrated standards of APEOs were prepared with the same strategy but with separation of mixtures by mixed-mode size exclusion chromatography with UV-VIS detection.

A seven-point calibration curve was created, ranging from 0 to 900 ng/mL in 50%:%50 methanol:water and applied for all compounds except NP_1EO and OP_1EO due to inability to detect. An internal standard mixture (4-(3,6dimethyl-3heptyl)phenol-ring-$^{13}C_6$, of 4-(3,6dimethyl-3heptyl)phenol-diethoxylate-ring-$^{13}C_6$, 4-tert-octylphenol-ring-$^{13}C_6$, and 4-tert-octylphenol-diethoxylate-ring-$^{13}C_6$; 1000 ng/mL and spiked at 50 µL) was used as the internal standard in each sample. TraceFinder software was used to quantify the analytes [ThermoFisher, version 5.1].

10 stormwater samples and one wastewater sample were diluted 1:10, two stormwater samples and one wastewater sample were diluted 1:100 following initial quantitation due to calculated amounts outside of the linear range.

An instrument limit of detection (LOD) and limit of quantitation (LOQ) were determined for each individual ethoxymer of each ethoxylate series. The standard deviation of three lab blanks was calculated and multiplied by 3 for LOD and by 10 for LOQ. If one lab blank did not have a signal, standard deviation was calculated from the two lab blanks with signals. For ethoxymers with no detected signal in at least two lab blanks, the standard deviation of the lowest concentration calibration of three replicate calibration samples was used instead to calculate LOD and LOQ.

Table 3. Target alcohol and alkylphenol ethoxylated surfactants analyzed. Polyethoxymers n=1-16 were analyzed for each surfactant series. Adapted from Lindborg et al. (2023).

Ethoxylate Series	**Ethoxylate Category**	**Structure**	**Alkyl chain isomers**
$C_{11}EO$	alcohol		linear
$C_{12}EO$	alcohol		branched
$C_{13}EO$	alcohol		linear
$C_{14}EO$	alcohol		branched
$C_{16}EO$	alcohol		linear
NPEO	alkylphenol		branched
OPEO	alkylphenol		branched

3. Results and Discussion

3.1 Total Polyethoxylated Surfactant Concentrations

We determined quantitative concentrations of surfactant ethoxymers with EO units 2-15 in all alcohol ethoxylates, EO units 2-17 in NPEOs, and EO units 2-16 in OPEO, and nonylphenol and octylphenol. NPEO and OPEO monoethoxylates were not quantified due to sensitivity in the mass spectrometer. Concentrations at or above LOD were summed across ethoxymers for a total concentration of each ethoxylate series in each sample. Figure 4 shows concentration distributions for each ethoxylated surfactant among the different matrices.

One-way ANOVAs and generalized linear models were used to compare summed ethoxymer concentrations for each ethoxylate series across each matrix. Sum NPEOs were the only series to have no significant differences in concentration among matrices. Sum OPEO concentrations were higher in both stormwater and wastewater when compared to ambient Bay water (p-value < 0.01), but there were no significant differences between stormwater and wastewater. For all AEOs, concentrations were higher in wastewater compared to ambient Bay water and stormwater (at least p-value < 0.001). No differences were seen between Bay water and stormwater, suggesting similar profiles between the two matrices. Differences between wastewater and stormwater are expected because they likely have different sources. Differences in NP and OP concentrations between matrices were not assessed due limited samples with concentrations at or above LOD.

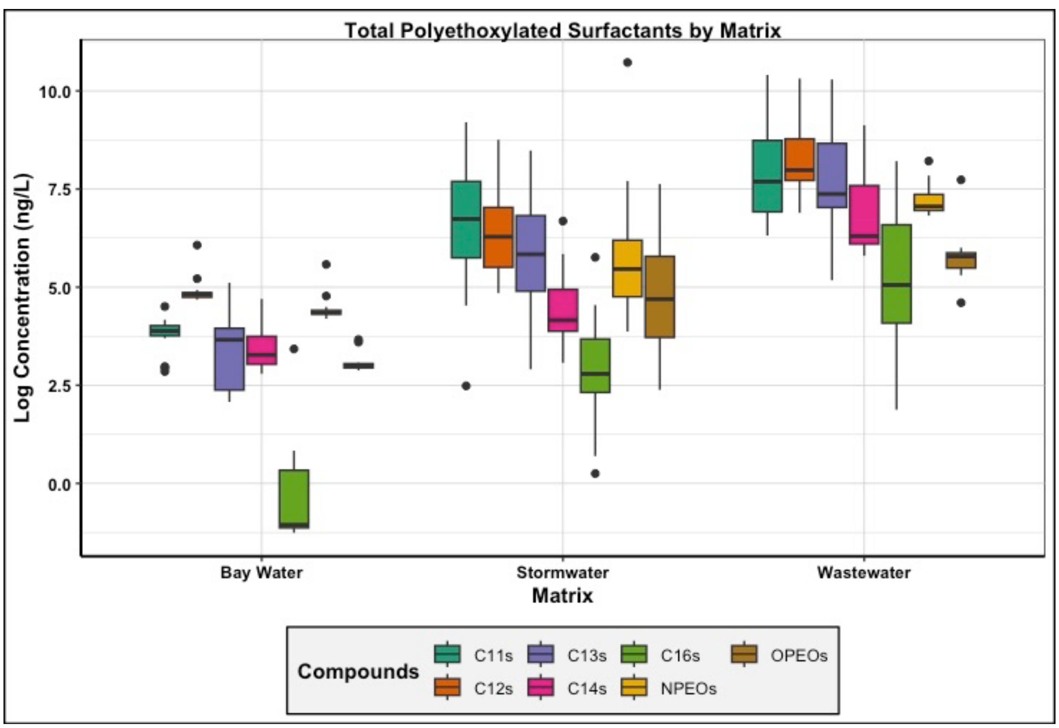


Figure 4. Log-transformed distribution of total polyethoxylated surfactant concentrations across different matrices. Concentrations at or above LOD were included in the sum total.

Figure 5 below is a map of distributions of total APEOs and AEOs by matrix. Each bubble is a pie chart comprised of concentrations of $C_{11}EO$, $C_{12}EO$, $C_{13}EO$, $C_{14}EO$, $C_{16}EO$, NPEOs, and OPEOs. The size of the bubble corresponds to the sum of all the APEOs and AEOs at that site. The range of total concentrations among all sites is 247.89 to 111,729.17 ng/L, see Table 4 for the range of each ethoxylate series. The highest total polyethoxylated surfactant concentrations were seen in wastewater (111,729.17 ng/L) and stormwater (52,977.95 ng/L). The top five sites with the largest total polyethoxylated surfactant concentrations are W1, S16, W8, S15, and W4.

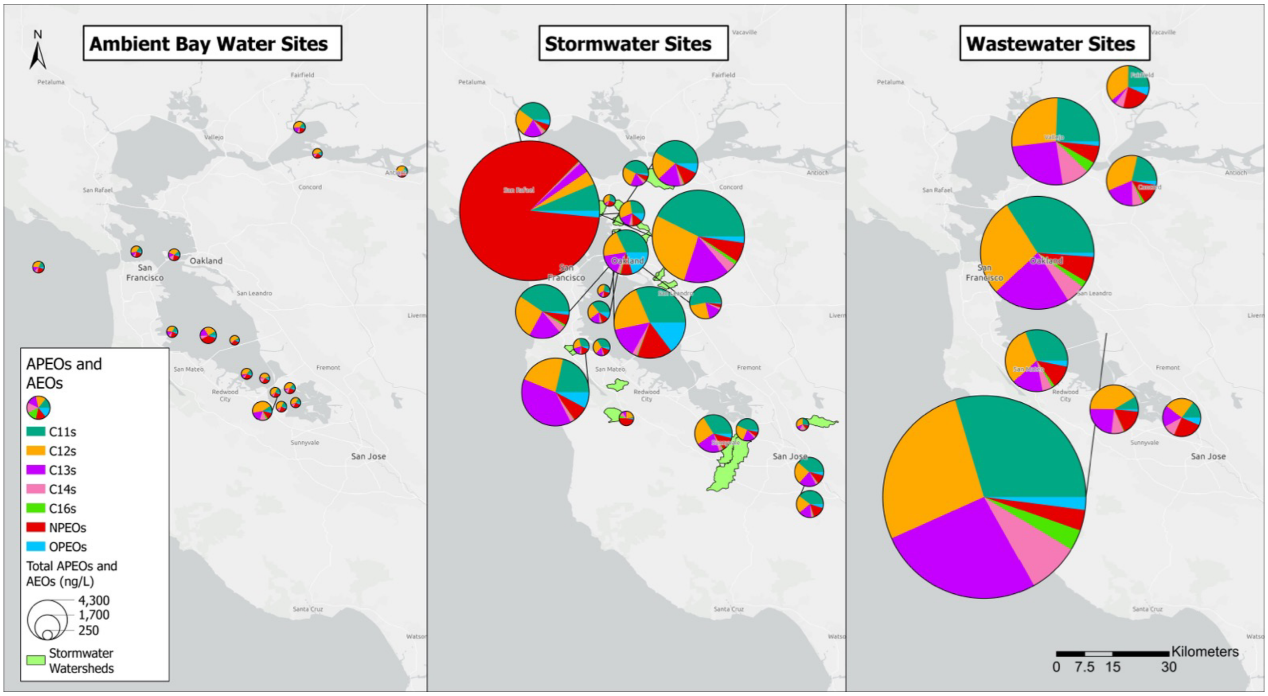


Figure 5. Profiles of APEOs and AEOs at each sampling site in the different matrices.

Table 4. APEO and AEO minimum and maximum concentrations in each matrix.

	$C_{11}EO$ (ng/L)		$C_{12}EO$ (ng/L)		$C_{13}EO$ (ng/L)		$C_{14}EO$ (ng/L)		$C_{16}EO$ (ng/L)		NPEOs (ng/L)		OPEOs (ng/L)	
	Min	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*
Bay Water (n = 16)	17	91	108	433	8	166	16	110	0.3	31	66	265	18	39
Stormwater (n = 23)	12	9,909	127	6,342	18	4,824	22	799	1.3	317	48	45,481	11	2,053
Wastewater (n = 8)	1217	33,080	984	30,261	177	29,550	330	9,184	30	3,679	918	3,691	201	2,285

3.1.1 Wastewater

APEOs and AEOs are present in wastewater effluent at publicly owned treatment works (POTWs) due to their widespread use and production (Camacho-Muñoz et al., 2014). Removal rates of ethoxylated surfactants from wastewater effluent are often between 95 and 98%, which would suggest that effluents have lower concentrations compared to influents (Lara-Martín et al., 2014). However, heavily loaded and inefficient wastewater treatment plants can have lower removal rates such as 50 to 90% (Staples et al., 2001). We found high concentrations of ethoxylated surfactants in effluent samples suggesting that either biodegradation at these POTWs is inefficient or influent wastewater contained large amounts of these surfactants.

In this study, the profiles of wastewater effluent samples were dominated by alcohol ethoxylates, particularly $C_{11}EO$ (29%), $C_{12}EO$ (28%), and $C_{13}EO$ (24%). Overall, AEOs were 91% (182,572 ng/L) of the profile for all POTW sites. Whereas NPEOs and OPEOs only made up about 8% (18,313 ng/L) of total ethoxylated surfactant concentrations at all POTWs, less than was detected in Lindborg et al. (2023). Increased use of AEOs as a result of restrictions on APEOs could explain this finding. Other studies found concentrations of AEO in wastewater effluent to range from 703 to 3,794 ng/L, much less than our range of about 3,000 to 100,000 ng/L (Sanderson et al., 2013). Another study found NPEOs and AEOs in wastewater effluent to be 314 and 167 ng/L, respectively, also much less than what we found in this study (Lara-Martín et al., 2014). Lindborg et al. (2023) detected higher concentrations of NPEOs in wastewater ranging from 95 to 36,300 ng/L. Overall, comparison with other studies shows that our wastewater effluents have higher polyethoxylated surfactant concentrations.

The three sites with the highest concentrations of ethoxylated surfactants are W1, W8, and W4. W1 and W4 were also the highest concentration sites in Lindborg et al. (2023). W1 is 95% AEOs, W8 is 91% AEOs, and W4 is 92% AEOs, all with dominant $C_{11\text{-}13}EO$. Concentrations reaching 33,079 ng/L for $C_{11}EO$, 30,260 ng/L for $C_{12}EO$, and 29,549 ng/L for $C_{13}EO$ at W1. $C_{14}EO$ and $C_{16}EO$ were present in lower concentrations at all three of these POTWs with maximum concentrations of 9,184 ng/L and 3,679 ng/L at W1, respectively. NPEOs and OPEOs at W1 were also low in proportion with concentrations of 3,691 ng/L and 2,285 ng/L, respectively. The POTW with the lowest concentration, W6, is also dominated by AEOs but with a higher proportion of NPEOs (25%) than other wastewater sites.

All wastewater sites have mostly residential/commercial influent compared to industrial. However, our top site, W1 receives 20% industrial influent compared to about 5% for other sites. The top three sites, W1, W8, and W4 receive wastewater from industries such as airports, automatic vehicle washing, breweries and wineries, electronic manufacturing, fabricated metal products, landfills (leachate), leather tanning and finishing, and textile and carpet finishing. Many of these industries use products such as detergents, car care products, and textiles, therefore explaining the high concentrations of AEOs at these POTWs.

These POTWs perform different treatment technologies to remove unwanted pollutants, however some treatments are more effective than others at removing AEOs and APEOs. W1 and W4 both use trickling filter/solids contact secondary treatment and no advanced tertiary treatment. Trickling filter treatment is known to be less effective than activated sludge at removing organics (Gross et al., 2004, Petrie et al., 2013). The inefficiency of trickling filters is due to short contact time between the organic contaminant and bacteria (Petrie et al., 2013). W8 uses high purity oxygen treatment but also no advanced tertiary treatment. The two POTW sites with the lowest polyethoxylated surfactant concentrations, W6 and W7, both use a tertiary treatment. Previous studies have seen that when tertiary treatments are applied, concentrations are lower than POTWs that only use secondary treatment (Gross et al., 2004). W6 uses activated sludge/biological nutrient removal as its treatment technology and activated sludge is known to be more effective because of its longer contact time between surfactant and bacteria (Petrie et al., 2013). W7 uses activated sludge/oxidation towers, with UV as its disinfectant. UV is known to remove APEO metabolites (Gross et al., 2004). As seen in various previous studies, treatment technology plays a role in the effectiveness of removal of AEOs and APEOs.

3.1.2 Stormwater

Contamination of urban stormwater has been shown to deteriorate the quality of receiving waters. Relationships between imperviousness and land use type have been evaluated for pollutants in the past, however there is limited knowledge of these relationships for pollutants such as APEOs and AEOs in urban runoff (Gasperi et al., 2013). Studies have shown that atmospheric contribution of APEOs to stormwater is low, confirming that local production from road, urban surfaces, and vehicle leaching are more likely common sources to stormwater (Gasperi et al., 2013). In this study, we found that NPEO (38%) and $C_{11}EO$ (25%) dominated the profile of stormwater samples. A maximum concentration of NPEO was seen at site S16 with 45,481 ng/L and maximum concentration of $C_{11}EO$ was at site S15 with 9,909 ng/L. APEOs and AEOs are both dominant in stormwater sites with about 58% AEOs and 42% APEOs. Rule et al. (2006) found concentrations of NPEOs in stormwater runoff ranging from 20,000 ng/L to 400,000 ng/L. Whereas Lindborg et al. (2023) detected a maximum concentration of 3,000 ng/L for NPEOs, much less than we detected.

S16, S15, and S12 had the highest concentrations of APEOs and AEOs for stormwater samples. S12 had the highest concentrations in Lindborg et al. (2023), however S16 and S15 were collected after that study took place. 86% of the profile at S16 is NPEOs with a total concentration of 45,481 ng/L. AEOs contribute to almost all the ethoxylated surfactants at S15 with $C_{11}EO$ and $C_{12}EO$ having the highest concentrations, 9,908 and 6,342 ng/L, respectively. S12 is split pretty evenly amongst all ethoxylated surfactants with 31% $C_{11}EO$, 22% $C_{12}EO$, 14% $C_{13}EO$, 16% NPEOs, 15% OPEOs, and the remainder $C_{14}EO$ and $C_{16}EO$. Lindborg et al. (2023) noted high concentrations of $C_{13}EO$ and relatively high concentrations of NPEOs and OPEOs, which is similar to our findings, however they did not quantify $C_{11}EO$. S20 had the lowest total concentrations of 382 ng/L. S10 had one of the lowest concentrations of ethoxylated surfactants in this study and it also had low concentrations in Lindborg et al. (2023).

Both S16 and S15 were labeled as an average to larger size storm with decent intensity. These sites have similar percentages of imperviousness, residential, commercial, industrial, transportation, open space, and urban (Table 5). They are 100% urban with no open space and about 70% imperviousness. S12 was a moderate storm but high intensity. S12 is also 100% urban with no open space but it has a higher percentage of industrial land use than both S16 and S15. APEOs and AEOs are present in concrete, tire rubber, coated metal surfaces, anti-corrosion paint for vehicles, and fuel and lubricant oil additives, therefore it makes sense that stormwater sampling sites with higher percentage of imperviousness, urban, and transportation would have higher concentrations. Sites with low ethoxylated surfactant concentrations like S20 and S10 have very low percentages of imperviousness, residential, commercial, and industrial land use, high proportions of open space and lower percentage of urban. S5 also has high proportions of urban and residential but low concentrations, this could be due to the storm size as it was smaller and less intense than other storms sampled at that location, which had higher total concentration (2,799 ng/L).

Table 5. Land use percentages of sites with the highest concentrations (S16, S15, and S12) and sites with the lowest concentrations (S5, S10, S20)

Site	Total (ng/L)	Imperviousness (%)	Residential (%)	Commercial (%)	Industrial (%)	Transportation (%)	Open Space (%)	Urban (%)
S16	52978	73	42	17	4	33	0	100
S15	23229	68	48	14	2	32	0	100
S12	13967	80	29	10	26	34	0	100
S5	430	46	58	6	3	24	5	95
S10	412	1	9	0	1	1	88	12
S20	382	11	12	3	0	4	72	28

Sites sampled in subsequent years, S11 in 2019 and S15 in 2020, have significant differences in total polyethoxylated surfactant concentrations. As mentioned above, S15 has one of the highest concentrations overall with a total of 23,229 ng/L whereas S11 has a total of 1,752 ng/L. AEO concentrations increased from S11 to S15, mostly C_{11}EO. However, NPEO and OPEO concentrations decreased. The storm event at S11 was considered average and the storm at S15 was average-larger but with decent intensity. The storm intensity and increased use of AEOs may explain this increase in concentrations and the decreased use of APEOs may be the reason for their decrease. S13 and S17 were also sampled in subsequent years (2020 and 2022, respectively), however, concentrations were all below LOD in S17 due to inability to detect the internal standard and quantify.

3.1.3 Ambient Bay Water

Both stormwater and wastewater can be sources of ethoxylated surfactants to surface waters. In this study, ambient Bay water concentrations were lower when compared to wastewater and stormwater which is consistent with other studies (Lindborg et al., 2023 and Sanderson et al., 2013). Lindborg et al. (2023) also found low concentrations in Bay water, however in their study, the majority of ethoxylated surfactants were only detected at two sites, in the current study we detected ethoxylated surfactants at all 16 sites. Sites with the highest concentrations were B2 and B9 with totals of 986 ng/L and 727 ng/L, many orders of magnitude lower than stormwater and wastewater concentrations. AEOs dominated these sites, mostly C_{12}EO, and NPEOs were also dominant in B9. These sites were also the highest concentration Bay water sites in Lindborg et al. (2023). Klečka et al. (2010) found concentrations of NPEOs in receiving waters of POTWs ranging from about 20 ng/L to 3000 ng/L which is much higher than we observed in the present study. Sanderson et al. (2013) found AEO concentrations in surface waters to be much lower than wastewater effluent. They detected concentrations of 76 to 921 ng/L of AEO, which is consistent with AEO concentrations we are seeing at our 16 Bay water sites (Sanderson et al., 2013).

B2 was mostly AEOs (84%) with C_{12}EO at 433 ng/L. B2 is located near many other Bay water sites and W5. W5 is also dominantly AEOs (82%) with a majority C_{12}EO and C_{13}EO with concentrations of 2,665 ng/L and 1,514 ng/L, respectively. B2 has 12% NPEOs and W5 has 17% NPEOs. The similarities of these profiles suggests that W5 could be a source to B2. B2 has much lower concentrations than W5 which is likely due to dilution. The closest stormwater site to B2 is S23 which has a profile of 50% AEOs and 50% APEOs, with C_{12}EO dominating the AEOs and NPEOs dominating the APEOs. The differences in profiles and distance from B2 suggest that S23 is likely not a source to B2's ethoxylated surfactant concentrations.

AEOs made up 58% of B9's profile, with the highest proportion being $C_{12}EO$ with a concentration of 184 ng/L. NPEOs were also dominant at B9 (36%) with a concentration of 265 ng/L. OPEOs were present in low proportions (~5%) at each site. B9 is near two other Bay water sites, two POTW sites, and three stormwater sites. Both POTW sites, W1 and W3, are dominantly AEOs (95% and 85%, respectively), with lower proportions of NPEOs (3% and 12%, respectively). S7, S18, and S19 are proximal to B9. These sites all have dominant AEOs (>75%) and less NPEOs (<18%). Site B7 which is located closer to these stormwater sites has a more similar profile with 72% AEOs and 22% NPEOs, suggesting that these sites are more likely a source for B7 rather than B9.

The ambient Bay water site with the lowest concentration was B12, with total polyethoxylated surfactant concentration of 248 ng/L. B12 was 65% AEOs (mostly $C_{12}EO$) and 28% NPEOs. B12 is located near W2 which was dominated by AEOs (84%), specifically $C_{12}EO$, $C_{11}EO$, and $C_{13}EO$ and 13% NPEOs. It is possible that W2 is a source to B12, but their profiles do still vary.

The comparison between APEO and AEO profiles of ambient Bay water sites with proximal wastewater and stormwater sites, suggests that wastewater effluent and stormwater runoff may not be the source contributing to the Bay's ethoxylated surfactant concentrations. More information and sampling would be needed to draw the conclusion that sites near Bay water sites are the main sources because there are spatial and temporal variables that could affect the profiles at these sites.

3.2 Long Chain and Short Chain APEOs

Various studies have looked at either short chain or long chain APEOs, but few studies have been able to detect both and compare difference in concentrations (Lindborg et al., 2023, Björklund et al., 2009, Camacho-Muñoz et al., 2014). For this reason, in the present study we aimed to understand the differences between long chain (EO units 4-17) and short chain APEOs (EO units 1-3) in wastewater, stormwater, and ambient Bay water to assess difference between the matrices. Figure 6 below is a map of distributions of APs and long and short chain APEOs by matrix. Each bubble is a pie chart comprised of concentrations of NP, OP and short chain and long chain NP and OP. The range of total AP and APEO concentrations among all sites is about 59 to 46,000 ng/L. The highest total polyethoxylated surfactant concentrations were seen in stormwater (46,323 ng/L), followed by wastewater (7,164 ng/L) (Table 6). The top five sites with the largest total AP and APEO concentrations were S16, W1, S12, W8, and S15. As discussed previously, stormwater sites were largely dominated by both AEOs and APEOs whereas wastewater sites were dominated by AEOs.

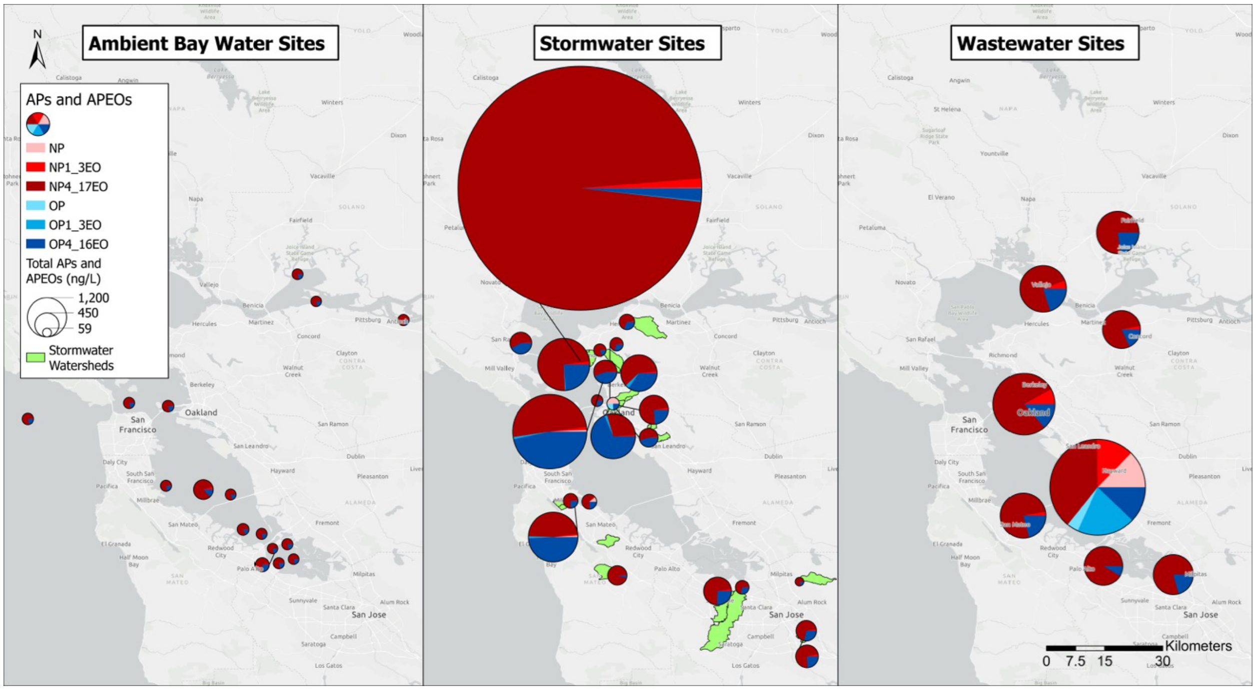


Figure 6. Profiles of APs and APEOs at each sampling site in the different matrices.

Table 6. APs and APEOs minimum and maximum concentrations in each matrix. *Below LOQ

	NP (ng/L)		**NP$_{2\text{-}3}$EO (ng/L)**		**NP$_{4\text{-}17}$EO (ng/L)**		**OP (ng/L)**		**OP$_{2\text{-}3}$EO (ng/L)**		**OP$_{4\text{-}17}$EO (ng/L)**	
	Min	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*
Bay Water (n = 16)	N/A	N/A	1.2	13	66	252	N/A	N/A	N/A	N/A	18	39
Stormwater (n = 23)	18*	62	2.0	579	48	44,902	N/A	30*	11	59	11	2,011
Wastewater (n = 8)	N/A	908	33	903	885	2788	N/A	281*	N/A	1418	100	887

The APEO profile across all wastewater sites is dominated by long chain NPEOs, followed by long chain OPEOs. NP and OP were only detected at W1 with concentrations of 907 ng/L and 281* ng/L, respectively. At W1, long chain NPEOs and short chain OPEOs make up more than half of the APEO profile. W4 and W8 had similar profiles of dominant long chain NPEOs and OPEOs. Gross et al. (2004) detected a range of concentrations of APs at wastewater treatments plants (3 to 5,200 ng/L). Our detected NP and OP concentrations fall within this range but are on the lower side. Short chain APEOs were detected at a range of 100 to 900 ng/L which is similar to what we found in this study for short chain NPEOs, however we detected higher concentrations of short chain OPEOs, particularly at W1 (Gross et al., 2004). Petrie et al. (2013) found higher concentrations of shorter chain NPEOs in wastewater effluent rather than longer chain, which is not consistent with our study. Camacho-Muñoz et al. (2014) detected NP in wastewater effluents at 5,800 ng/L and short chain NPEOs at 1,820 ng/L which is higher than what we found. APs and short chain APEOs were detected at lower concentrations in this study compared to previous studies.

Detection of NP and OP in stormwater runoff is expected because of their use in paints, concrete, building materials, asphalt, and vehicle parts (Gasperi et al., 2013). However, across all stormwater sites, long chain NPEOs are the majority (87%). Detected concentrations of NP at stormwater sites were below LOQ. S16 was almost entirely composed of long chain NPEOs (97%). S12 was dominated by both long chain NPEO (50%) and OPEO (47%). S15 was mostly long chain NPEO (74%) and some long chain OPEO (24%). Our detected NP and OP concentrations in stormwater are lower than other stormwater studies. Gasperi et al. (2013) found OP concentrations of 61 ng/L and NP at 359 ng/L. However, we found similar short chain OPEO concentrations (Gasperi et al., 2013). Björklund et al. (2009) detected concentrations of NP and short chain NP up to 1,200 ng/L and about 5,000 ng/L, respectively. Bressy et al. (2011) found median concentrations of NP and OP in stormwater to be 460 ng/L and 36 ng/L, respectively.

Across all ambient Bay water sites, long chain APEOs dominate. All sites were <LOD for NP, OP, and $OP_{2\text{-}3}EO$. B9 was dominated by long chain NPEOs (83%), followed by long chain OPEOs (13%), the remainder was short chain NPEOs (4%). B2 had similar profile to B9 but a higher percentage of long chain OPEOs (24%). Alkylphenols and short chain APs were detected in Santa Ana River, CA at concentrations much higher than we detected, less than 1000 ng/L and 10 to 300 ng/L, respectively (Gross et al., 2004).

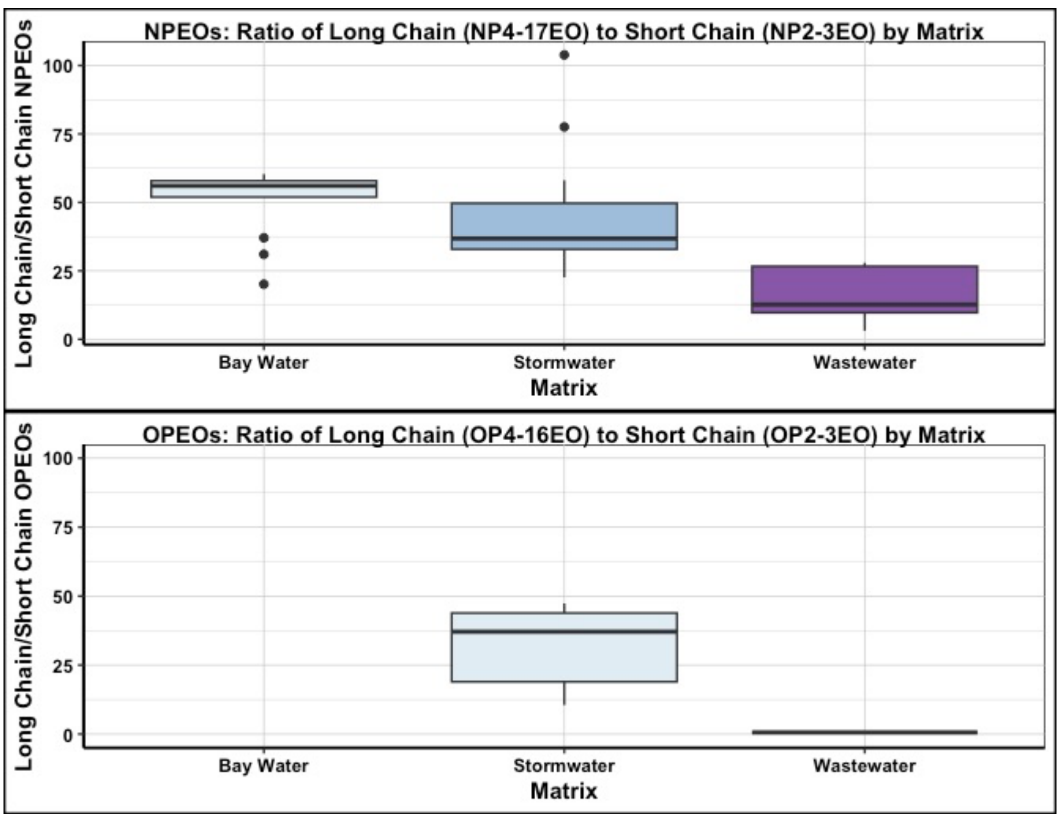


Figure 7. Ratios of Long to Short Chain APEOs by matrix

The main pathway of degradation of NPEO and OPEO is through loss of ethoxy groups, thus shortening of the EO chain to produce short chain NPEO and OPEO and eventually nonylphenol and octylphenol (Björklund et al., 2009). Ratios of long chain (EO units 4-17) to short chain (EO units 2-3) were determined for NPEO and OPEO. This ratio is a measure of degradation of these ethoxylates. The higher the ratio, the less degraded the mixture is. Figure 7 shows the distribution of these ratios in each matrix for NPEO and OPEO. There were no detectable ratios for OPEO in ambient Bay water due to concentrations of short chain below the limit of detection.

A one-way ANOVA and generalized linear models were used to compare the ratios of long chain to short chain for NPEO and OPEO to understand degradation extent in each matrix. The mean ratio of long chain to short chain NPEOs in Bay water is significantly higher than the mean ratio in wastewater (p-value < 0.001). This suggests that Bay water is less degraded than wastewater. Wastewater is biologically treated, and Bay water is not, therefore it is expected that wastewater would be more degraded. The mean ratio in stormwater is higher than the mean ratio in wastewater (p-value < 0.001) indicating more degradation in wastewater. There was no significant difference between the ratios in Bay water and stormwater. Suggesting that the profile of degradation of NPEOs in Bay water and stormwater are more similar than between other matrices. A higher ratio in Bay water could be due to removal of the short-chain NPEOs by sorption and sedimentation rather than less degradation because the more hydrophobic compounds (APs and short chain APs) have a stronger affinity to bind to sediments (Lara-Martín et al., 2014 and Klečka et al., 2010). There were no significant differences in ratio of long chain to short chain OPEOs between matrices.

3.3 Ratio of Linear to Branched AEO Isomers

As mentioned previously, alcohol ethoxylated surfactants can have linear and branched alkyl isomers. In this study the concentrations of linear and branched isomers were determined for each AEO. POTW sites are dominated by branched $C_{11}EO$, $C_{12}EO$, and $C_{13}EO$, with little proportions of linear AEOs. Stormwater sites contain mostly branched $C_{11}EO$, $C_{12}EO$, and $C_{13}EO$, with 10% linear $C_{11}EO$. The profile of S16 is almost half branched $C_{11}EO$ (44%) with branched $C_{12}EO$ and $C_{13}EO$ also abundant. S15 has a similar profile but a smaller proportion of branched $C_{11}EO$ and a higher proportion of branched $C_{12}EO$ than S16. S12 has relatively even proportions of branched $C_{11}EO$, $C_{12}EO$, and $C_{13}EO$, and linear $C_{11}EO$. Linear AEOs are the predominant form used in products as they are known to degrade easier, therefore the higher proportion of detected branched AEOs could be a result of biodegradation removing the linear AEOs (Talmage, 1994). Bay water is overall dominated by linear $C_{12}EO$ (37%) with smaller proportions of linear $C_{11}EO$, branched $C_{12}EO$, and branched $C_{13}EO$ (around 15%). The profiles of B2 and B9 match the overall profile with B2 having a higher proportion of linear $C_{12}EO$ (42%), $C_{13}EO$ (10%), and $C_{14}EO$ (10%). B9 is dominated by linear $C_{12}EO$ and branched $C_{13}EO$, with smaller proportions of the rest. The dominant presence of linear AEOs suggests that there is less biodegradation occurring in the Bay and that stormwater runoff and wastewater effluent are likely not sources to the Bay.

Degradation of alcohol ethoxylates varies by whether the alkyl chain is branched or linear. AEOs that are predominately linear have a higher degree of biodegradability than AEOs that have more alkyl chain branching (Talmage, 1994). The higher concentration of linear AEOs present in a sample would indicate less degradation because they are known to degrade more easily (Itrich and Federele, 2004). The ratio of linear to branched can also be used as an indicator of degradation (Figure 8). A higher linear to branched ratio indicates less degradation, however this indicator is confounded by source of ethoxylated surfactant (i.e., the product that AEO is used in). The following sites have the highest ratios of linear to branched isomers S16 ($C_{14}EO$), B2 ($C_{16}EO$), S10 ($C_{16}EO$), B2 ($C_{11}EO$), B6 ($C_{16}EO$), W7 ($C_{11}EO$), S16 ($C_{13}EO$).

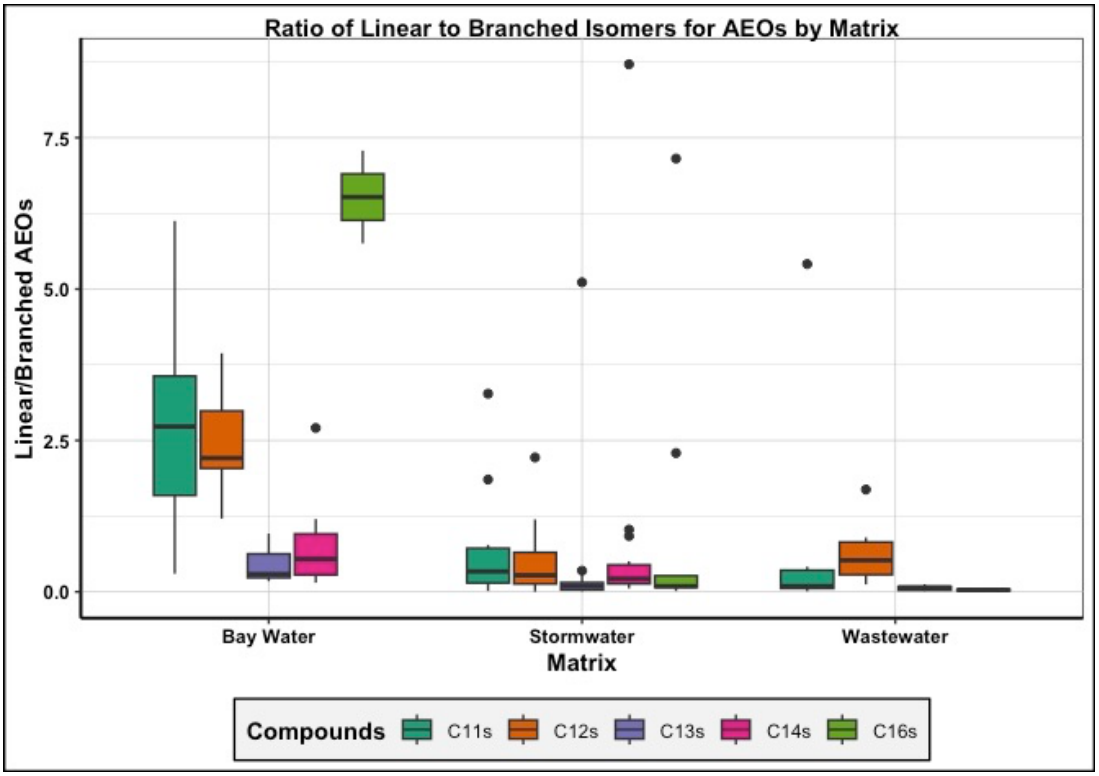


Figure 8. Ratio of Linear to Branched AEO Isomers by AEO and matrix.

ANOVAs and GLMs were used to compare ratios for each AEO across matrices. C_{11}EO and C_{12}EO have significantly higher linear to branched ratios in ambient Bay water than both stormwater and wastewater (at least p-value < 0.001), suggesting less degradation in Bay water. There were no differences in ratios between wastewater and stormwater for C_{11}EO and C_{12}EO. Ratios of linear to branched isomers for C_{13}EO and C_{14}EO were not significantly different between matrices. C_{16}EO does not have detectable ratios in wastewater due to concentrations below LOD. Linear to branched ratios of C_{16}EO are significantly higher in ambient Bay water than stormwater (p-value < 0.001). Higher ratios indicate less degradation in Bay water compared to both stormwater and wastewater. These results match NPEO long chain to short chain ratio results, suggesting that the least degradation is occurring in Bay water and the highest in wastewater.

4. Conclusion

This study has provided an assessment of contamination of polyethoxylated surfactants and their degradation products in an urban estuary, San Francisco Bay, CA. The presence of these surfactants suggests the need for further monitoring and testing to assess the profiles of these contaminants in the Bay and other urban estuaries. Comparison of long chain to short chain APEOs and linear to branched AEOs is a novel way to understand potential degradation extent between various matrices of concern.

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