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Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary

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ABSTRACT

Novel methods utilizing liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry were validated for low-level detection of 104 pharmaceuticals and personal care products ingredients (PPCPs) and four alkylphenols (APs) in environmental samples. The methods were applied to surface water, sediment, and mussel tissue samples collected from San Francisco Bay, CA, USA, an urban estuary that receives direct discharge from over forty municipal and industrial wastewater outfalls. Among the target PPCPs, 35% were detected in at least one sample, with 31, 10, and 17 compounds detected in water, sediment, and mussels, respectively. Maximum concentrations were 92 ng/L in water (valsartan), 33 ng/g dry weight (dw) in sediments (triclocarban), and 14 ng/g wet weight (ww) in mussels (N,N-diethyl-m-toluamide). Nonylphenol was detected in water (<2–73 ng/L), sediments (22–86 ng/g dw), and mussels (<0.04–95 ng/g ww), and nonylphenol mono- and diethoxylates were detected in sediments (<1–40 ng/g dw) and mussels (<5–192 ng/g ww). The concentrations of PPCPs and APs detected in the San Francisco Bay samples were generally at least an order of magnitude below concentrations expected to elicit toxic effects in aquatic organisms. This study represents the first reconnaissance of PPCPs in mussels living in an urban estuary and provides the first field-derived bioaccumulation factors (BAFs) for select compounds in aquatic organisms.

1. Introduction

The continuous introduction of pharmaceuticals and personal care product ingredients (PPCPs) to surface waters worldwide via the discharge of treated and untreated wastewater has lead to a number of efforts to assess their occurrence and potential impacts on nontarget organisms in aquatic environments (Berninger and Brooks, 2010; Bruce et al., 2010; Daughton and Ternes, 1999; Fent et al., 2006; Khetan and Collins, 2007; Kolpin et al., 2002). For pharmaceuticals, the majority of occurrence studies have focused on effluents and surface waters (e.g., Glassmeyer et al., 2005; Hummel et al., 2006; Kim and Carlson, 2007; Kolpin et al., 2002; Loffler et al., 2005; Ternes, 1998; Waiser et al., 2011), including drinking water sources (Benotti et al., 2009; Focazio et al., 2008), with a much smaller number of studies conducted on sediments (Burkhardt et al., 2005; Jelic et al., 2009; Kim and Carlson, 2007; Loffler et al., 2005; Martin et al., 2010; Stein et al., 2008). Studies of occurrence in aquatic life are few in number and have primarily focused on accumulation of select pharmaceuticals in wild-caught fish (reviewed in Ramirez et al., 2009), with a recent study also observing accumulation in caged mussels (Bringolf et al., 2010). Most of these efforts have been conducted in freshwater rivers and streams heavily impacted by wastewater effluent, where concentrations are anticipated to represent worst-case scenario conditions with regard to aquatic life exposure. However, because urban estuarine and marine environments typically receive inputs of complex mixtures of chemical contaminants from a variety of sources, including numerous municipal and industrial wastewater outfalls, characterization of PPCP concentrations in these environments is also important. Data on the occurrence in marine or estuarine systems for pharmaceuticals in particular are currently limited to a small number of surface water studies (Benotti and Brownawell, 2007; Langford and Thomas, 2011; Thomas and Hilton, 2004; Togola and Budzinski, 2008; Weigel et al., 2002; Wille et al., 2010; Yang et al., 2011), two of which also analyzed surface sediments (Langford and Thomas, 2011; Yang et al., 2011). To our knowledge, occurrence of pharmaceuticals in wildlife living in estuarine or marine environments has not yet been reported. Until recently, a lack of analytical methods for reliable, low level quantitation of PPCPs has limited the generation of occurrence data for PPCPs in systems with a high degree of wastewater dilution, particularly in sediments and tissues.

In contrast, it is well established that alkylphenols (APs) are common contaminants of surface waters and aquatic sediments worldwide, and can accumulate in wildlife tissues (David et al., 2009; Soares et al., 2008). APs are nonionic surfactants in widespread use in many industrial applications. Despite their frequent detection in environmental matrices, analytical methods for APs have historically

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been limited by high laboratory blank levels and high detection limits caused by matrix interference in complex matrices including sediments and tissues.

In this study we report the validation of analytical methods to significantly improve selectivity and sensitivity in the measurement of PPCPs in ambient estuarine waters, sediments, and tissues, and APs in tissues. For PPCPs, United States Environmental Protection Agency (USEPA) Method 1694 (US EPA, 2007) was modified to include additional compounds and extended to the analysis of tissue matrices. Multiple labeled internal standards and liquid chromatography–tandem mass spectrometry (LC/MS/MS) analysis with multiple reaction monitoring (MRM) were used to produce recovery corrected concentrations for all compounds. For APs, recovery correction using labeled APs, derivatization by acetylation, and gas chromatography–mass spectrometry (GC/MS) analysis with multiple ion detection (GC/MS MID) were employed for water and sediment analysis, while recovery correction using labeled APs combined with steam extraction and subsequent analysis by LC MS/MS/MRM were employed for tissue matrices. The AP method allows for determination of low-levels in tissue (2–50 ng/g) while eliminating the typically encountered tissue lipid interferences. We have applied these methods to characterize the occurrence of PPCPs and APs in surface water, sediments, and mussels collected from a representative urban estuary, San Francisco Bay, CA, USA. Further, application of these low-level detection methods provided an opportunity to calculate the first field-derived bioaccumulation factors (BAFs) for select compounds in marine invertebrates. To our knowledge, this study provides results from the first reconnaissance of a broad suite of PPCPs in marine mussels and advances our understanding of the bioaccumulation potential of these compounds and APs in the marine environment.

2. Materials and methods

2.1. Sample collection

Co-located surface waters, sediments, and benthic mussels (Ribbed horsemussel, *Geukensia demissa*) were collected from five nearshore sites in San Francisco Bay, CA, USA in December of 2009 and January 2010 (Supporting information (SI) Table 1 and SI Fig. 1). Sample sites were spatially distributed throughout the major urbanized segments of the Bay and targeted areas historically influenced by a variety of potential contaminant sources (e.g. oil refineries, stormwater runoff, municipal and industrial wastewater). Whole water samples (unfiltered) were collected in high-density polyethylene (HDPE) bottles (3×1 L for PPCPs) or amber glass bottles (2×1 L for APs) and stored at 4 °C until analysis. Sediments were collected in HDPE and amber glass jars for PPCP and AP analyses, respectively. Mussels were collected from the sediment surface and placed in either re-sealable plastic bags or amber glass bottles for PPCP and AP analyses, respectively. Sediment and mussel samples were frozen until analysis. Mussel gut contents were not purged prior to freezing. Samples were extracted within one week of collection.

2.2. Target compounds

The 108 compounds analyzed in this study are listed in SI Table 2. All of the compounds were analyzed in surface waters and mussel tissues. A reduced list of 74 target analytes (those not indicated by an asterisk in SI Table 2) is reported for the sediment samples due to interference between the sediment matrix and some of the labeled standards used for quantification (research to resolve the issues for these compounds is ongoing). PPCPs selected for analysis were based on EPA method 1694 (US EPA, 2007) with 45 additional compounds targeted in Lists 3 and 4, and an additional run for List 5. This expanded EPA 1694 analyte list represents those PPCP compounds identified by the USEPA and other AXYS clients (AXYS Analytical Services, Sidney, BC, Canada) as priorities for assessment based on annual consumption, expected toxicity, and persistence. List 2 (14 tetracycline compounds) in the EPA 1694 method was not analyzed in the present study. The method was originally developed and validated to provide occurrence information for a broad spectrum of PPCPs in all relevant aqueous and solid matrices. Modification of EPA 1694 extraction processes and subsequent validation has allowed the application of the method to tissue matrices. The AP target compounds were 4-nonlyphenol (NP), 4-nonylphenol monoethoxylates (NP1EO), 4-nonylphenol diethoxylates (NP2EO), and octylphenol (OP), and were selected based on existing methods developed by AXYS.

2.3. Analytical methods

The analytical methods used in the present study are briefly summarized below. Method details, including quality assurance and quality control and method performance information, are provided in the Supporting information (SI).

2.3.1. PPCPs in water and sediment

The sediment samples (1 g dry weight) were first extracted with either an aqueous phosphate buffer (pH 2.0) for analysis of Lists 1, 3, and 5 compounds or with a pH 10 solution of NH_4OH for List 4 compounds. Each sample was further extracted with acetonitrile and the solvent evaporated to produce an aqueous solution. Sediment extracts and water samples were filtered (1.6 μm), adjusted to pH 2 by addition of HCl (for analysis of List 1, 3, and 5 compounds) or to pH 10 by addition of NH_4OH (for analysis of List 4 compounds). Na_4EDTA was added to each acidic extract prior to extraction on an Oasis HLB solid phase extraction (SPE) cartridge. The extracts were analyzed by LC/MS/MS operated in the ESI positive mode for List 1, 4 and 5 compounds and in the ESI negative mode for List 3 compounds.

2.3.2. PPCPs in tissues

Tissue samples (2.5 g wet weight for the acidic extraction and 1 g wet weight for the basic extraction) were extracted using the same procedure as for the sediment samples except that the extraction was with acetonitrile followed by the pH buffered aqueous solution. The acetonitrile and aqueous extracts from each extraction were combined and processed in the same manner as for the sediments.

2.3.3. APs in water and sediment

Sediment samples (5 g dry weight) were digested in methanolic KOH, extracted with hexane, and acetylated by treatment with acetic anhydride and pyridine. Water samples (1 L, unfiltered) were adjusted to pH 11–12, treated with acetic anhydride, and extracted with hexane at pH 6. Water and sediment extracts were cleaned up by silica column chromatography and analyzed by GC/MS operated in the multiple ion detection (MID) mode.

2.3.4. APs in tissue

Tissue samples (2 g wet weight) were mixed with water and then extracted by steam extraction into isooctane. The isooctane extract cleanup was performed using SPE on aminopropyl cartridges. Tissue extracts were analyzed using LC/MS/MS in the ESI negative mode for NP and OP and in ESI positive mode for NP1EO and NP2EO. For all matrices, NP, NP1EO, and NP2EO are reported as total concentrations, representing the sum of all the detected isomers in a specific target group.

2.4. Bioaccumulation factors (BAFs)

Field-derived BAFs were calculated for the target compounds detected in both mussel tissue and surface waters at a minimum of three sample sites. Biota-sediment accumulation factors (BSAFs) were not calculated because only one compound (triamterene) met this criterion (SI Table 8). Field-derived BAFs were calculated as the