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# A holistic passive integrative sampling approach for assessing the presence and potential impacts of waterborne environmental contaminants

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**Abstract**

As an integral part of our continuing research in environmental quality assessment approaches, we have developed a variety of passive integrative sampling devices widely applicable for use in defining the presence and potential impacts of a broad array of contaminants. The semipermeable membrane device has gained widespread use for sampling hydrophobic chemicals from water and air, the polar organic chemical integrative sampler is applicable for sequestering waterborne hydrophilic organic chemicals, the stabilized liquid membrane device is used to integratively sample waterborne ionic metals, and the passive integrative mercury sampler is applicable for sampling vapor phase or dissolved neutral mercury species. This suite of integrative samplers forms the basis for a new passive sampling approach for assessing the presence and potential toxicological significance of a broad spectrum of environmental contaminants. In a proof-of-concept study, three of our four passive integrative samplers were used to assess the presence of a wide variety of contaminants in the waters of a constructed wetland, and to determine the effectiveness of the constructed wetland in removing contaminants. The wetland is used for final polishing of secondary-treatment municipal wastewater and the effluent is used as a source of water for a state wildlife area. Numerous contaminants, including organochlorine pesticides, polycyclic aromatic hydrocarbons, organophosphate pesticides, and pharmaceutical chemicals (e.g., ibuprofen, oxindole, etc.) were detected in the wastewater. Herein we summarize the results of the analysis of the field-deployed samplers and demonstrate the utility of this holistic approach.
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## 1. Introduction

Anthropogenic contaminants are present in aquatic systems worldwide and are nearly universally present as complex mixtures. These pollutants range from contaminants of historic concern such as organochlorine

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pesticides (OCs), polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans (PCDDs, PCDFs), and polycyclic aromatic hydrocarbons (PAHs) to emerging contaminants of concern such as pharmaceutical chemicals and personal care products (Lebo et al., 1995; Petty et al., 1995, 1998; Doughton and Jones-Lepp, 2001; Koplin et al., 2002). As the global population increases and drought conditions worsen in many areas, the demand for water continues to grow. Water supplies in many areas, particularly in those with limited water resources, must be reused to satisfy a multitude of needs. Increasingly, multiuse water, including treated municipal wastewater, functions as a source of water for the maintenance of ecosystems and ultimately as the source of drinking water. Consequently, the determination of the presence and ultimate effects on ecosystems of bioavailable mixtures of anthropogenic contaminants in environmental waters (i.e., exposure assessment) is of critical concern.

Nearly all currently employed analytical and biomonitoring based sampling techniques are inherently limited in their ability to provide a holistic exposure assessment because: (1) they lack the capability to integratively sample through time, i.e., sampling represents one or more points in time, (2) analytical sensitivity and selectivity are insufficient to detect and quantify ultra-trace to trace levels of complex mixtures of contaminants in water, (3) site conditions (e.g., water quality) impact the survival of sentinel organisms (e.g., caged fish or bivalves), and exposure information is lost due to species-specific metabolism and depuration of contaminants of interest, and (4) causal links between observed biological effects and environmental contaminant mixtures are seldom established.

A great deal of effort has gone into improving traditional analytical monitoring methods and these refinements may partly address these limitations. However, to adequately define organism exposure to waterborne complex contaminant mixtures and to reliably screen the potential biological consequences of such exposures require more holistic approaches. Scientists at the US Geological Survey's Columbia Environmental Research Center (CERC) have developed a suite of passive integrative samplers for ultra-trace to trace levels of complex mixtures of waterborne contaminants. These integrative samplers include: the semipermeable membrane device (SPMD) used for sequestering hydrophobic waterborne contaminants (Huckins et al., 1993; Petty et al., 1995, 1998, 2000a; Gale et al., 1997; Huckins et al., 1999), the polar organic chemical integrative sampler (POCIS) applicable for sequestering waterborne hydrophilic contaminants (Alvarez, 1999; Alvarez et al., 2000; Alvarez et al., in press), the stabilized liquid membrane device (SLMD) for sequestering waterborne toxic metal ions, specifically Cu, Cd, Pb, Zn, Ni (Brumbaugh et al., 2002), and the passive integrative mercury sampler (PIMS) applicable to monitoring neutral vapor and dissolved phase neutral mercury species (Brumbaugh et al., 2000). This suite of integrative samplers provides a means to sequester and concentrate a broad spectrum of waterborne contaminants present as complex mixtures in aquatic ecosystems and forms the basis for a holistic approach to the assessment of organism exposure and for screening the potential consequences of such exposures.

In addition to analyzing contaminant mixtures in enriched extracts from this suite of samplers deployed in the field, the extracts can also be employed to expose laboratory organisms followed by measurement of physiological endpoints or be incorporated into numerous bioindicator tests (Petty et al., 2000b). In the study described herein, we conducted instrumental analysis of sample extracts, and measured the estrogenic potential of the POCIS extracts using the yeast estrogen screen (YES) assay technique.

As a proof-of-concept field study, we deployed three of our in situ passive integrative samplers, i.e., SPMDs, POCIS, and SLMDs in a constructed municipal wetland and the adjacent Missouri Department of Conservation (MDC) Eagle Bluffs Conservation Area (a wetland complex), which receives water from the constructed wetlands. The PIMS sampler was not used in this study due to ongoing laboratory studies to optimize the deployment configuration. The municipal wetlands, three cells with a fourth cell completed shortly after this study, provide the final polishing of the treated wastewater from Columbia, MO. The water from these wetlands serves as the major source of water for the MDC Eagle Bluffs Conservation Area. An average daily flow of 12 million gallons of wastewater passed through the municipal treatment system during the deployment period. Additional water is supplied to the Eagle Bluffs Conservation Area via a distribution channel from the Missouri River. The suite of three integrative samplers was deployed at five locations within this system. Presented herein are the results of this initial application of our approach for the assessment of the exposure of fish, wildlife and ultimately humans, to waterborne hydrophobic and hydrophilic organic contaminants, and labile forms of toxic ionic metals.

## 2. Experimental

### 2.1. Integrative samplers

The preparation of the SPMDs, POCIS, and SLMDs has been previously described in detail (Huckins et al., 1993; Petty et al., 1995, 2000a,b; Alvarez, 1999; Alvarez et al., in press; Brumbaugh et al., 2002). Briefly, SPMDs (US Patents, 5,098,573, Huckins et al., 1992; 5,395,426, Huckins et al., 1995) are constructed of a thin film of the

neutral lipid, triolein, sealed inside a layflat, thin-walled tube of nonporous, low-density polyethylene. The diameters of the transient thermally mediated cavities range up to about 10 Å, effectively precluding sampling of any contaminant molecules associated with dissolved organic matter or particulates. This cavity size limitation has an important consequence: in general, only dissolved chemicals with molecular masses less than about 600 Daltons are sampled by the SPMD (Ellis et al., 1995), and this molecular mass limitation is very similar to that imposed by the pores of biomembranes (Opperhuizen et al., 1985). SPMDs used were of the "standard design" as defined earlier (Petty et al., 2000a) and were 2.54 cm wide by 91.4 cm long. The POCIS device is prepared from an admixture of solid phase sequestering media (which can be tailored to specific applications) encased in a microporous polyethersulfone membrane (Alvarez et al., 2000). The POCIS device (US Patent 6,478,961, Petty et al., 2002) integratively samples dissolved polar organic contaminants in aquatic environments. A detailed description of this technology will be presented elsewhere (Alvarez et al., in press). The SLMD (US Patent 6,296,760, Petty et al., 2001) consists of low-density polyethylene layflat tubing containing an equal mixture (v/v) of oleic acid (*cis*-9-octadecanoic acid) and 7-[4-ethyl-1-methyloctyl]-8-quinolinol. The basis for sampling by the SLMD is the controlled release of the water-insoluble liquid complexing reagent mixture and the formation of stabilized metal complexes on the outer membrane surface. Continual formation of highly insoluble metal-oleates (primarily calcium and magnesium) on the surface of the hydrophobic membrane results in a wax-like hydrophobic medium in which the alkylated quinolinol is mobile. Because of the process-enhanced stability of the complexing media, the SLMD is applicable for long-term, low-level sequestration of labile i.e., readily complexed by a chelating agent (Florence, 1982), forms of toxic metals (e.g., Pb, Cd, Cu, Ni, Zn, etc.).

### 2.2. Materials and reagents

Florisil (60–100 mesh) was obtained from Fisher Scientific, Pittsburgh, PA, and was heated at 475 °C for 8 h and stored at 130 °C prior to use. All organic solvents were Optima grade from Fisher Scientific, except methyl-*tert*-butyl ether (Chromatographic grade, Baxter Health Care, McGraw Park, IL). Silica gel (SG-60, 70–230 mesh) was purchased from Thomas Scientific, Swedesboro, NJ. The silica gel was washed with 40:60 (v:v) methyl *tert*-butyl ether:hexane followed by 100% hexane, and subsequently heated at 130 °C for 72 h prior to use. Potassium silicate (KS) was prepared by treating silica gel, SG-60 (300 g) with methanolic potassium hydroxide solution (168 g potassium hydroxide in 570 ml of methanol). The air dried KS was placed in a glass column and subsequently washed with 100 ml of methanol followed by 170 ml of methyl *tert*-butyl ether and heated for a minimum of 12 h at 130 °C prior to use. Phosphoric acid/silica gel was prepared by reacting silica gel washed as described above, with organic free phosphoric acid (500 g SG-60; 330 g ACS Reagent grade phosphoric acid, Fisher Scientific). The mixture was shaken until free flowing and stored in a desiccator until use. All organic solvents were Optima grade from Fisher Scientific, except methyl *tert*-butyl ether (Chromatographic grade), which was obtained from Baxter Healthcare. Isolute ENV + resin was obtained from Jones Chromatography, Lakewood, CO. S-X3 Bio-Beads (200–400 mesh) were from Bio-Rad Laboratories, Hercules, CA, and Ambersorb 1500 was obtained from Rohm and Hass, Philadelphia, PA. Oleic acid was from Baker, Phillipsburg, NJ and 7-[4-ethyl-1-methyloctyl]-8-quinolinol was obtained from Rhodia Chemicals, Paris, France. High purity nitric acid was from Baker.

### 2.3. Sample extract enrichment and analysis

#### 2.3.1. SPMDs

The sample processing and residue enrichment procedures have been described in detail previously (Petty et al., 1995, 1998, 2000a; Huckins et al., 1999). In summary, processing of the SPMDs generally involves the following steps: (1) removal of exterior surfical periphyton and debris; (2) organic solvent dialysis; (3) size-exclusion chromatography (SEC); and (4) chemical fractionation, e.g., Florisil, silica gel and/or alumina sorption chromatography. The extracts from two individual SPMDs were combined to prepare a composite sample.

Gas chromatographic (GC) analyses were conducted using either an AgilentTechnologies 5890 series GC equipped with an Agilent 7673A autosampler and an electron capture detector or a HNU photoionization detector (PID) with a 9.5 eV lamp operating at 270 °C (HNU Inc., Newton, MA). Typical chromatographic conditions have been described in detail previously (Petty et al., 1995, 1998, 2000a; Huckins et al., 1999).

#### 2.3.2. POCIS

To maximize retention of the broadest possible spectrum of polar organic chemicals, the POCIS sampler extracts were subjected to minimal processing and fractionation procedures. Briefly, each POCIS sampler was rinsed under warm running water to remove any material adhering to the membrane surfaces. Each POCIS device was carefully opened and the sorbent mix was transferred into individual 1 cm (i.d.) glass chromatography columns for subsequent solvent elution. The sorbent mix was eluted with 50 ml of a 10 + 10 + 80 (v:v:v) mixture of methanol:toluene:dichloromethane. The eluate was reduced in volume to about 1 ml using

rotary evaporation. Each extract was subsequently dried using a mini-column of anhydrous sodium sulfate (2 cm bed volume) contained in a disposable Pasteur pipet. The mini-columns were rinsed with isopropyl alcohol (three times). The extract and rinses were collected in glass culture tubes and subsequently reduced in volume to about 1 ml using nitrogen blow down. The final extracts were combined into two and four POCIS equivalent composites for each deployment site and analyzed for sequestered contaminants.

Gas chromatography–mass spectrometry (GC/MS) analysis was conducted using a Finnigan MAT 95 GC–MS-system (Finnigan MAT, Inc., Bremen, Germany) equipped with a 0.25 mm × 0.25 μm × 60 m DB-5 capillary GC column (J&W Scientific, Palo Alto, CA). The following temperature program was used; inject at 50 °C, hold for 3 min, then ramped at 20 °C/min to 150 °C, followed by a 2.5 °C ramp to 250 °C, then a 20 °C ramp to 300 °C and hold for 10 min. Detection was by a magnetic sector-MS with electron impact ionization.

High performance liquid chromatography–mass spectrometry (LC/MS) analyses were conducted using an Agilent Series 1100 LC/MS (Agilent, Inc., Palo Alto, CA) with electrospray ionization in both positive and negative mode. A $C_{18}$ reversed phase HPLC column, 15 cm × 4.6 mm i.d., 5 μm particles (180 Å), fully endcapped (Supelco, Inc., Bellefonte, PA) with a mobile phase consisting of 25:75 (v:v) water:methanol at a flow rate of 2.0 ml/min was used during the analysis. The quadrapole MS scanned from 100 to 1000 $m/z$ with the fragmentor at 70 V with positive or negative polarity. The source fragmentor voltage was decreased to 50, based on the results obtained with standards, to maximize formation of molecular ions and minimize adducts (positive mode) or fragmentation (negative mode). Additional analyses were conducted using a ThermoQuest Finningan LCQ configured with an electropsray ion source. Analyte separation was accomplished using a Restek Allure $C_{18}$ (15 cm × 3.2 mm i.d., 5 μm particles [60 Å], fully endcapped) column (Restek, Inc., Bellefonte, PA) and a gradient elution (98% water/1 nM ammonium acetate/0.1% acetic acid/1–99% methanol/1 nM ammonium acetate/0.1% acetic acid) over 20 min.

In addition to instrumental analysis, the POCIS extracts were examined using the yeast estrogen-screening assay (YES, Routledge and Sumpter, 1996; Rehmann et al., 1999; Alvarez et al., 2000). This estrogenic activity assay uses a genetically modified yeast strain, which expresses the human estrogen receptor (hER) in a form capable of binding to estrogen response elements (ERE) situated on an expression plasmid carrying a reporter gene (*lac*-Z). The binding of an appropriate ligand to a receptor results in the expression of the reporter gene and the secretion of β-galactosidase into the surrounding medium where it metabolizes a chromatogenic substrate. Estrogenic activity is subsequently determined by measuring absorbance at 540 and 620 nm.

The POCIS extracts were shipped to the University of Heidelberg sealed in amber glass ampoules. Upon receipt, the extracts, controls, etc., were transferred into test tubes and stored at 4 °C in the dark. Testing of these extracts was conducted according to slightly modified version of the YES protocols described previously (Routledge and Sumpter, 1996). Briefly, all samples tested (including a 17 β-estradiol positive control and an ethanol negative control) were added to 96 well microtiter plates. The maximum concentration of each test sample was added to the first well of each row and then serially diluted along the row. The negative control separated each test sample and all samples were tested twice. All wells were allowed to evaporate to dryness before addition of 200 μl assay medium containing about $4 \times 10^7$ recombinant yeast cells. The plates were then incubated for 72 h. The absorbance at 540 and 620 nm was determined following the incubation period.

#### 2.3.3. SLMDs

The sample processing procedures used for the SLMD sampler have been previously described in detail (Brumbaugh et al., 2002). Briefly, each exposed SLMD was rinsed with high-purity water and placed into LDPE bottles prior to extraction. Individual SLMD samplers were extracted twice in an ultrasonic bath for 15 min at 50 °C by addition of 50 ml of 20% (v:v) high purity nitric acid to the LDPE bottle. The two 50 ml extracts were combined and a 1 ml sub-sample was diluted with ultrapure water for subsequent analysis. Metal analyses were conducted using a Perkin–Elmer ELAN 6000 inductively coupled plasma–mass spectrometer (ICP–MS; Perkin–Elmer Inc., Norwalk, CN). Operating parameters were as recommended by the manufacturer (Brumbaugh et al., 2002).

### 2.4. Quality control

Field blank SPMDs, POCIS, and SLMDs accompanied the samplers during deployment, retrieval, and transportation to CERC. These field blanks were processed and analyzed exactly as deployed samples. All field blanks were found to contain no analyte residues at higher levels than those associated with the laboratory procedural blanks and were indicative of successful deployment and retrieval of all sampler types. Procedural blanks consisted of freshly prepared SPMDs, POCIS, and SLMDs taken through the entire processing and analysis sequence. Field deployed samples containing contaminant residues exceeding laboratory procedural blanks were considered positive and subsequently background corrected.

For SPMD samples, the analytes of interest were the priority pollutant PAHs, OCs, total PCBs, and selected

current use pesticides, specifically trifluralin, chlorpyrifos, diazinon, and *cis*- and *trans*-permethrin. The method detection limit (MDL) and method quantitation limit (MQL) were determined for each analyte by measuring the coincident instrumental response in SPMD blank samples taken through the entire processing and analysis procedure. The MDL was defined as the mean plus three standard deviations and the MQL was the mean plus 10 standard deviations of values so determined (Keith, 1991). For those analytes where the MQLs were below the level of the calibration curve employed in the GC analysis, the MQLs were set at the value of the lowest level of the calibration curve employed in quantifying the analyte levels. For contaminants present in the SPMD sample extracts, the MDL values determined as described above were used to express the ambient water MDLs employing equation 2 presented in Section 3. Recoveries of analytes from fortified blank SPMDs were incorporated as an overall assessment of the sample processing, residue enrichment and analysis. These SPMDs, $n = 3$, were prepared by adding 8 μg of each priority pollutant PAH, a mixture of 27 OCs at 40 ng each, and 8 μg of total PCBs (1:1:1:1 mixture of Aroclor 1242, 1248, 1254 and 1260). The mean recoveries were, PAHs = 66%, OCs = 71%, and PCBs = 169%. The source of the error associated with the PCB levels in the fortified SPMDs is unknown. However, no PCBs were found in field deployed SPMDs above the blank values (background = 86 ng total PCB/SPMD).

Because this field deployment of the POCIS devices represented the initial proof-of-concept application, the sample extracts were examined using GC/MS and LC/MS. In general, the instrumental analysis was designed to provide the maximum qualitative data possible, i.e., tentative identification of chemicals in the field deployed POCIS samplers but not present in POCIS blanks. Identification of analytes was based on comparison to either mass spectral library spectra or by comparison of the molecular weight and HPLC retention times with polar organic chemical target analytes on the USGS's National Water Quality Laboratory Schedule 9060 (National Water Quality Laboratory, Denver Federal Center, Denver, CO). For GC/MS analysis, analyte spectra that matched library spectra were ranked by their degree of fit to the library spectra and to the purity (i.e., lack of extraneous masses in the spectra). Values for the purity and fit parameters for matched spectra greater than 700 were considered to provide tentative identification. All other matched spectra with purity and fit values less than 700 were considered to be suggested identifications only.

For the SLMDs, blank values were measured in triplicate, which resulted in MDLs below values typically reported for conventional water sample analysis by ICP–MS (e.g., Cu = 0.02 μg/l, Ni = 0.006 μg/l, Co = 0.04 μg/l, etc.; Brumbaugh et al., 2002).

### 2.5. Deployment of integrative samplers

The sampling devices, i.e., SPMDs, POCIS, SLMDs, were deployed at five sites throughout the constructed wetlands complex and in the MDC's Eagle Bluffs Conservation Area (Fig. 1). The wastewater treatment plant has a flow ranging from 12 to 18 million gallons of water daily, which represents between 60% and 100% of the water supply for the Eagle Bluffs Conservation Area. The samplers were deployed at two sites in Eagle Bluffs and at three sites in the municipal wetlands during September 1999. Site 1 was in the Missouri River supply channel, which provides make-up water for Eagle Bluffs, principally during waterfowl hunting season. Site 2 was in pool 9 of the Eagle Bluffs Conservation Area, essentially the exit from the Conservation Area into the Missouri River. Sites 3–5 were part of the Columbia wastewater treatment wetland cells. Site 3 was in the water leaving the waste treatment plant into the first constructed wetland cell. Site 4 was situated at the exit of the first wetland cell and Site 5 was in the water leaving the final wetland cell prior to being pumped into the Eagle Bluffs Conservation Area wetland system. All the samplers were exposed for 28 days. The samplers were contained in stainless steel canisters (SPMDs, five per canister/two canisters per site; and POCIS, four per canister/two canisters per site) and plastic minnow buckets (SLMDs, six per bucket/one bucket per site), secured in place via screw ground anchors and woven metal airplane cable fitted with cable locks. Following retrieval, the SPMD and POCIS samplers were stored frozen at $\leqslant -15$ °C in vapor tight metal cans until processing was initiated. The SLMDs were frozen ($\leqslant -15$ °C) individually in pre-cleaned LDPE bottles sealed with LDPE lids until initiation of processing.

## 3. Results and discussion

### 3.1. SPMDs

The analysis of the field deployed SPMDs resulted in the detection of only a few priority pollutant PAHs. The lower molecular weight PAHs such as acenaphthylene, phenanthrene, anthracene, fluoranthene and pyrene were found at measurable levels up to 1.5 μg/sample at Sites 3 (exit of City of Columbia wastewater treatment plant) and 4 (exit of first constructed wetland cell). Due to the relatively low levels of the priority pollutant PAHs present in most of the SPMD extracts, the entire PAH response envelope from the GC analysis was quantified by comparing the total area to the response of a standard of the widely occurring PAH, pyrene. The levels of the total PAHs as pyrene ranged from 6.3 μg/sample at Site 1 (Missouri River water distribution channel) to 190 μg/sample at Site 3 (exit of wastewater treatment plant).

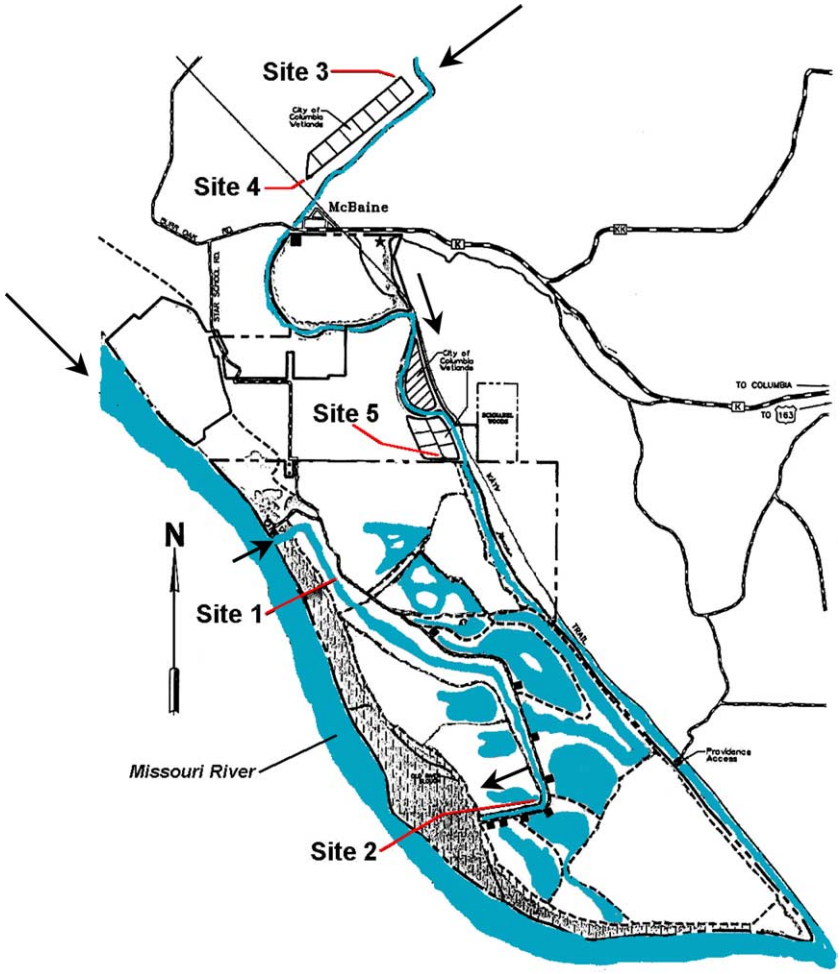


Fig. 1. Location of deployment sites throughout the city of Columbia, MO constructed wetland complex and the MDC's Eagle Bluffs Conservation Area. Arrows represent direction of water flow into and through area, e.g., Site 3 inflow, Site 2 outflow.

The analysis of the SPMD sample extracts for OCs, PCBs, and the current use pesticides resulted in extremely complex GC–ECD chromatograms. However, identifiable and quantifiable levels of the individual OC pesticides were found at all sites. Examples of OCs present at appreciable levels included HCB, pentachloroanisole (a microbial metabolite of pentachlorophenol), lindane, *cis*- and *trans*-chlordane and the DDT complex. Residues of individual OCs ranged up to 200 ng/sample. Chlorpyrifos was the most commonly found current use pesticide and was present at levels up to 620 ng/sample. No quantifiable levels of PCBs were found in sample extracts from any deployment site. However, GC/MS examination of the SPMD sample extract resulted in the tentative identification of di- and tribromobenzenes and one chlorodibromobenzene.

The concentrations of contaminants present in the SPMD extracts can be used to estimate the ambient concentrations of these pollutants. Details of the model development and the estimation approach have been presented previously (Huckins et al., 1993; Petty et al., 1998; Huckins et al., 2002). Because the analytes present in the SPMD membrane as well as the triolein were recovered during the sample processing procedure, Eq. (1) was employed to estimate ambient contaminant concentrations:

$$C_W = C_{SPMD} M_{SPMD} / R_S t \quad (1)$$

where $C_{SPMD}$ is the concentration of the individual analyte in the SPMD and $M_{SPMD}$ is the mass of the SPMD (g), $R_S$ is the SPMD analyte sampling rate (l/d) and $t$ is deployment time in days. In the present case we used the uptake rate constant ($k_u$) defined as l/d g (liters per day per gram) of SPMD (membrane + lipid).

$$C_W = C_{SPMD} / k_u t \quad (2)$$

The SPMD sampling rates can change due to changes in temperature, flow velocity of the water, and build up of periphyton on the membrane surface. To correct for changes in these environmental variables relative to laboratory calibration studies, a permeability/performance reference compound (PRC) approach was developed and reported (Huckins et al., 2002). Briefly, PRCs are noninterfering (analytically) compounds, such as perdeuterated (PAHs) with moderate to fairly high fugacity, which are added to the SPMDs' triolein prior to deployment. Thus, measuring the PRC loss during the exposure period provides in situ loss rate constant ($k_e$) values, which when compared to the calibration $k_e$ values serves as an indicator of the differences in the exposure conditions. If large differences exist between the calibration and exposure $k_e$ values, adjustments are made to the laboratory calibration data to more accurately reflect the actual in situ sampling rates. The $k_{eprc}$ values for the field and laboratory are typically derived as follows:

$$C_{SPMD} = C_{SPMD_0} \exp(-k_{eprc} t) \quad (3)$$

and

$$k_{eprc} = \ln(C_{SPMD_0}/C_{SPMD})/t \quad (4)$$

where $C_{SPMD_0}$ is the initial concentration of the PRC and $C_{SPMD}$ is the concentration of PRC remaining in the SPMD following exposure. Comparison of the $k_{eprc}$ values derived from field-exposed SPMDs, to the $k_e$ values of the PRC measured in SPMD calibration exposures provides an exposure adjustment factor (i.e., $k_{eprc}/k_{ecal}$) $R_s$, providing an estimate of the relative effect of environmental variables on SPMD sampling. Thus, estimates of in situ sampling rates, $R_{sf}$, are given by

$$R_{sf} = (k_{eprc}/k_{ecal}) R_{sc} \quad (5)$$

Estimated water concentrations based on PRC-corrected $R_s$ values were calculated for selected analytes using laboratory calibration data at 18 °C and are presented in Table 1. The data in Table 1 demonstrate that Site 3 (exit of the wastewater treatment plant) contained the largest number of analytes and their concentrations were highest of all the sites, ranging up to 11 ng/l for anthracene. The majority of the OC pesticide residues likely originate from past usage of these chemicals, e.g., as treatments for termites or lawn chemicals. The PAH residues are typical of combustion related residues and likely originate from urban runoff. Ambient analyte concentrations fell as the wastewater flowed through the constructed wetland cells (Sites 4 and 5) and the levels were, with the exception of dieldrin, lowest in the water (Site 2) at the exit of Eagle Bluffs Conservation Area. The water originating from the Missouri River (Site 1) had the lowest ambient concentrations of all analytes and was similar to previous samples from the Missouri River (Petty et al., 1995).

These data provide evidence that the constructed wetlands and the natural wetlands of the Eagle Bluffs Conservation Area function as designed, to remove hydrophobic contaminants from the wastewater stream.

Table 1
SPMD derived ambient water concentrations (pg/l) of selected hydrophobic contaminants

| Analyte | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
|---|---|---|---|---|---|
| HCB | 12 | 26 | 140 | 59 | 32 |
| PCA | 71 | 51 | 430 | 55 | 60 |
| α-BHC | ND[a] | 88 | 100 | 19 | 18 |
| Lindane | ND | 120 | 3300 | 210 | 100 |
| Dacthal | 46 | 140 | 240 | 250 | 120 |
| Oxychlordane | <7.4[a] | 37 | 160 | 38 | 18 |
| Heptachlor epoxide | 39 | 69 | 350 | 140 | 54 |
| *trans*-Chlordane | 39 | 93 | 1400 | 810 | 310 |
| *trans*-Nonachlor | 21 | 55 | 510 | 270 | 120 |
| *p,p′*-DDE | 25 | 89 | 140 | 59 | 25 |
| *cis*-Chlordane | 44 | 200 | 1500 | 1400 | 590 |
| Dieldrin | 140 | 1400 | 630 | 720 | 290 |
| *cis*-Nonachlor | 8.5 | 25 | 170 | 77 | 31 |
| *p,p′*-DDD | 10 | 140 | 210 | 30 | 9 |
| *p,p′*-DDT | 5.2 | ND | 11 | 24 | 2.8 |
| Methoxychlor | 3.5 | 37 | 890 | 230 | 99 |
| Phenanthrene | <950 | <950 | 4200 | 5000 | <950 |
| Anthrancene | <500 | <500 | 11,000 | 2700 | <500 |
| Fluoranthene | <1200 | <1200 | 3900 | <1200 | <1200 |
| Pyrene | <820 | <820 | 9400 | 1200 | <820 |

[a] Less than values are MDLs expressed as ambient water concentrations calculated from contaminant residues in SPMD samples.

Further, the ambient concentrations of these typical bioconcentratable contaminants in the Eagle Bluffs Conservation Area (Sites 1 and 2) are present at pg/l or part-per-quadrillion levels and should be of minimal concern for potential ecological effects.

### 3.2. POCIS

The POCIS samples were processed in a minimally invasive manner to ensure that analytes of potential interest were not lost. Due to the unknown nature of the broad spectrum of polar organic contaminants present in the waters, portions of the POCIS extracts were examined by GC/MS (Site 5) and LC/MS (Sites 1–5) in both electrospray flow-injection analysis and HPLC electrospray analysis. Compounds presented in Table 2 fit the GC/MS selection criteria for spectral fit and purity but are considered to be tentative identifications. In addition to those compounds presented in Table 2, a variety of other chemicals (these did not meet the GC/MS selection criteria) are strongly suggested by their mass spectra. These compounds ranged from a variety of phthalates to various fragrance-related terpenes and essential oils.

The LC/MS analysis of the POCIS extracts resulted in very complex total ion current chromatograms. Analytes were identified by matching LC retention times with retention times of 53 current use pesticides, herbicides and their metabolites (USGS National Water Quality Laboratory Schedule 9060). Due to the complexity of the ion current chromatogram, only a limited number of chemicals on the 9060 Schedule list could be positively identified (Table 3). Other contaminants potentially present in the sample extracts as indicated by flow injection analysis/MS (FIA/MS) included oxytetracycline, sulfadiazine, sulfamerizine, metalochlor, acetochlor, alachlor, 17 α-ethynyestradiol, and surfactants.

Table 2
Tentatively identified compounds (GC/MS) in POCIS sample extracts

| Tentative identification | Use |
|---|---|
| Ibuprofen | Analgesic |
| Nonyl-phenol | Surfactant degradation product |
| Ephedrine acetate | Decongestant metabolite |
| Nicotinic acid | Vitamin |
| Oxindole | Treatment for viral infections |
| 4-Hydroxyindole | Fragrance |
| 5-Phenyhydantoin | Metabolite of phenytoin (anti-convulsant) |
| Phenelzine | Anti-depressant |
| Mephenytoin | Anti-convulsant |
| Ethotoin | Anti-convulsant |

Table 3
Compounds identified from LC/MS analysis of POCIS sample extracts

| Sample | Compound identity |
|---|---|
| Site 1, river distribution channel | Hydroxyatrazine<br>Atrazine |
| Site 2, pool 9 | Desethyl-desisopropyl atrazine<br>Hydroxyatrazine<br>Atrazine |
| Site 3, wastewater inflow | Desethyl-desisopropyl<br>Atrazine<br>Caffeine |
| Site 4, midpoint constructed wetlands | Desethyl-desisopropyl<br>Atrazine |
| Site 5, wastewater outflow constructed wetlands | Desethyl-desisopropyl<br>Atrazine<br>Caffeine<br>Propoxur |
| Field blank | No target analytes present |
| Laboratory process blank | No target analytes present |
| Reagent blank | No target analytes present |

Atrazine was quantified in the Site 1 (Missouri River water distribution channel) POCIS sample extract by LC/MS. This extract, a composite of three individual POCIS samplers, contained 19 μg of atrazine. Estimation of the ambient water concentration of atrazine was performed using the previously derived sampling rate (Alvarez, 1999) and Eq. (6):

$$C_W = C_{POCIS} M_{POCIS} / R_S t \quad (6)$$

where $C_{POCIS}$ is the analyte concentration in the POCIS sorbent, $M_{POCIS}$ is the mass of the POCIS sorbent, $R_S$ is sampling rate (in this case, the sampling rate/POCIS multiplied by three) and $t$ is the deployment time in days. Using this approach, the ambient water concentration of atrazine in the Missouri River distribution channel was determined to be 0.93 μg/l = 0.93 ppb. Atrazine concentrations in this region of the Missouri River are typically in the range of 1–3 μg/l (USGS, NASQAN Data). During agricultural use of this herbicide or surface run-off events, the level may reach 4–6 μg/l. The NASQAN database indicated an atrazine concentration of 1.16 ppb on 6 June 1999.

Also, bulk water samples (4 l) were obtained at Sites 2, 3, and 5. These water samples were immediately taken to the CERC, filtered through a 2.7 μm depth filter and subsequently extracted using 100 mg of the POCIS sorbent admixture contained in a 1 cm (i.d.) chromatography column. Following extraction using the POCIS solvent mixture, the water extracts (in methanol)

were analyzed using LC/MS. These grab water samples were found to contain the surfactants, polypropylene (PPG) and polyethylene (PEG) glycolates as witnessed by the presence of the homologous series of ions either 58 amu (PPG) or 44 amu (PEG) apart. While evidence of these surfactants in the POCIS sample extracts was present, identification was not possible due to the lack of a definitive fragmentation pattern. The results of the analysis of the grab samples for these widely occurring contaminants strongly supports their probable presence in the POCIS samples.

In addition to the analytical determination of the presence of a wide variety of polar organic chemicals, the POCIS approach provides ready access to environmentally relevant mixtures of waterborne contaminants for toxicological screening. This approach has been applied using the SPMD technology and provides valuable information relating to organism exposure and potential biological effects (Petty et al., 2000b). In this study, extracts from POCIS samplers were also examined using the YES assay (Alvarez et al., 2000).

The results from the YES assays depicted in Fig. 2 clearly indicate the presence of both estrogenic and toxic compounds in each sampler extract tested. The apparent lack of activity at high sample test volumes (i.e., $\geqslant 3.12$ μl of 1 ml extract) was the result of sample toxicity as indicated by the absence of yeast growth (Fig. 2). Sample test volumes $\leqslant 1.56$ μl produced a significant estrogenic response. Comparison of these data to a positive control suggest that sample test volumes ranging from 0.5 to 1.0 μl of each POCIS sample extract produced estrogenic activity approximately equivalent to 1.0 nM 17 β-estradiol. It should be noted that accompanying POCIS processing and field blanks produced no demonstrable estrogenic activity. A phenomenon termed the "alkylphenol effect", which is indicative of the presence of alkylphenols in a sample extract, was observed in each of the field deployed POCIS sample extracts. This effect, where both octyl- and nonyl-phenol will migrate and contaminate adjacent negative controls in a microtiter plate, has been described previously (Beresford et al., 2000). The presence of alkylphenols was confirmed by the GC/MS analyses (Table 2).

### 3.3. SLMDs

The third integrative sampler used in this assessment is the SLMD technology for waterborne, labile ionic metals (i.e., specifically Cu, Pb, Cd, Ni, Zn). Using sampling rates previously reported (Brumbaugh et al., 2002) and the following equation:

$$C_W = C_{SLMD}/R_{SLMD}t \quad (7)$$

where $C_W$ is the time weighted-averaged concentration of the specific metal in water, $C_{SLMD}$ is the concentration of the metal in the SLMD sampler, $R_{SLMD}$ is the SLMD sampling rate in units of liters per day (l/d) and $t$ is the deployment time in days. The 28-day average water concentrations (Brumbaugh et al., 2002) were calculated and are presented in Table 4. An examination of the data in Table 4 reveals that the 28-day average labile

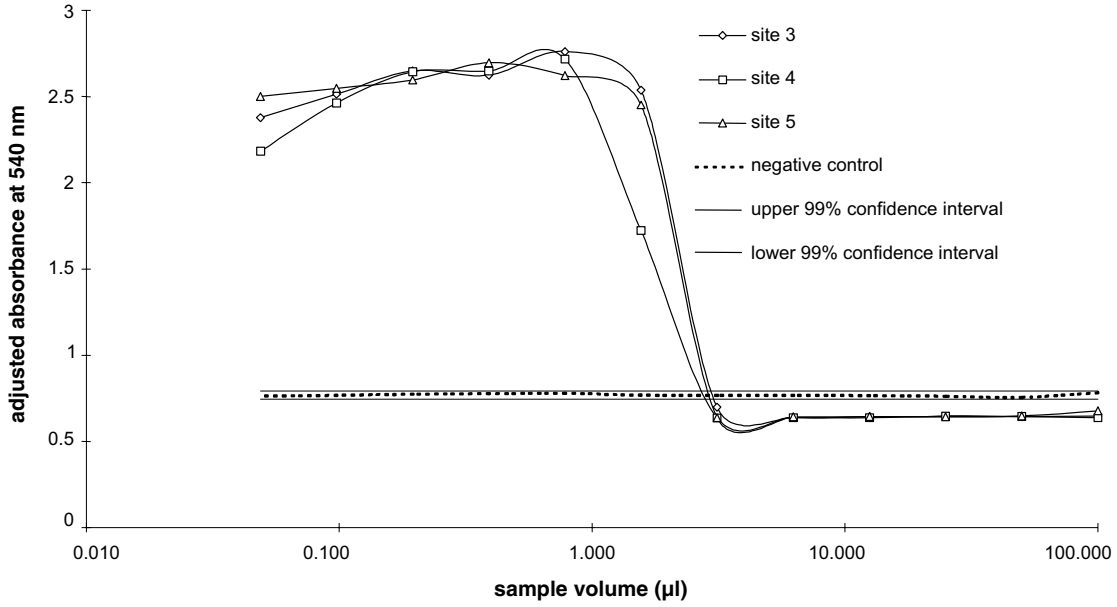


Fig. 2. Results of the YES assay performed on POCIS extracts.

Table 4
SLMD-derived 28 day average concentrations (μg/l) of labile Ni, Cu, Zn, Cd, and Pb

| Site description | Ni | Cu | Zn | Cd | Pb |
|---|---|---|---|---|---|
| Site 1, river distribution channel | <0.2 | 0.11 | 5 | 0.05 | 0.04 |
| Site 2, pool 9 | 0.24 | 0.18 | 5 | <0.02 | 0.13 |
| Site 3, wastewater inflow | <0.02 | 0.12 | 18 | <0.02 | 0.02 |
| Site 4, midpoint constructed wetlands | <0.02 | 0.12 | 4 | 0.04 | 0.05 |
| Site 5, wastewater outflow constructed wetland | 0.24 | 0.24 | 2 | <0.02 | 0.05 |
| Reporting limit | 0.20 | 0.02 | 1 | 0.02 | 0.02 |
| EPA chronic freshwater criteria[a] | 49 | 3.5 | 32 | 0.37 | 0.54 |

[a] USEPA. Guidance on the documentation and evaluation of trace metals data collected for clean water act compliance monitoring. July 1996, USEPA, Cincinnati, OH.

metal ion concentrations in the wetland complex were considerably below the EPA chronic freshwater criteria. Further, the concentrations of the metals of interest were similar at the various sampling sites. In essence, the wastewater treatment operation was not a source of significant waterborne ionic metal input into the wetland complex.

## 4. Summary

Passive integrative samplers designed for a broad array of environmental contaminants provide the basis of a holistic approach to the assessment of anthropogenic contaminant stressors in aquatic systems (Petty et al., 2000b). The use of three of our four integrative samplers to sequester and concentrate waterborne hydrophobic and hydrophilic organic contaminants, and ionic metals in the treated municipal wastewater source for a constructed wetland was demonstrated. Also, the data delineated the effectiveness of the wetlands as a final polishing for treated wastewater. A wide variety of waterborne contaminants were found to be present in the source water. These chemicals are typical of the types of contaminants expected to be present in all urban wastewater treatment plant effluents and, as such, are representative of the chemical stressors continually entering the Nation's aquatic ecosystems. Further, our exposure assessment approach (Petty et al., 2000b) has been expanded to include the sensitive YES procedure for screening for potential endocrine disrupting effects. The data from this study also demonstrate that the constructed wetlands are a successful adjunct to the secondary sewage treatment in terms of reducing levels of waterborne contaminants.

Our suite of integrative samplers is designed to provide organism exposure assessment and water quality information based on a time-weighted average basis. This approach minimizes cost, maximizes precision and breadth of application, and is suitable for systems with temporally variable concentrations of contaminants of concern. Future water quality assessments will incorporate these integrative samplers in combination with toxicity screening using an expanded suite of bioindicator tests. Refinement and extension of the analytical methods applied to the POCIS sample extracts is currently in progress. Thus, our holistic approach provides an enhanced assessment of the time integrated composition of the complex mixture of contaminants present in aquatic systems and concomitantly, toxicological-based screening data on the potential for adverse effects. Such an assessment could serve as a toxicity identification evaluation (TIE) procedure.

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Use of trade names or specific company names is for identification purposes only and does not constitute endorsement by the US Geological Survey or the US Environmental Protection Agency.

## References

Alvarez, D.A., 1999. Development of an integrative sampling device for hydrophilic organic contaminants in aquatic environments. Doctoral thesis, University of Missouri–Columbia, Columbia, MO.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Manahan, S.E., 2000. Development of an integrative sampler for polar organic chemicals in water. Presented at the 219th National Meeting of the American Chemical Society, Symposium on Issues in the Analysis of Environmental Endocrine Disruptors, San Francisco, CA, 26–31 March 2000.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Manahan, S.E. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Sci. Technol., in press.

Beresford, N., Routledge, E.J., Harris, C.A., Sumpter, J.P., 2000. Issues arising when interpreting results from an in vitro assay for estrogenic activity. Tox. Appl. Pharmac. 162, 22–33.

Brumbaugh, W.G., Petty, J.D., May, T.W., Huckins, J.N., 2000. A passive integrative sampler for mercury vapor in air and neutral mercury species in water. Chem.: Global Change Sci. 2, 1–9.

Brumbaugh, W.G., Petty, J.D., Huckins, J.N., Manahan, S.E., 2002. Stabilized liquid membrane device (SLMD) for the passive, integrative sampling of labile metals in water. Water, Air, Soil Pollut. 133, 109–119.

Doughton, C.G., Jones-Lepp, T.L., 2001. Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues. ACS/Oxford University Press, Washington DC.

Ellis, G.S., Huckins, J.N., Rostad, C.E., Schmitt, C.J., Petty, J.D., MacCarthy, P., 1995. Evaluation of lipid-containing semipermeable membrane devices (SPMDs) and gas chromatography-negative chemical ionization–mass spectrometry for monitoring organochlorine contaminants in the Upper Mississippi River. Environ. Toxicol. Chem. 14, 1875–1884.

Florence, T.M., 1982. Development of physio-chemical procedures to investigate the toxicity of copper, lead, cadmium, and zinc towards aquatic biota. Anal. Chim. Acta 141, 73–94.

Gale, R.W., Huckins, J.N., Petty, J.D., Peterman, P.H., Williams, L.L., Morse, D., Schwartz, T.R., Tillitt, D.E., 1997. Comparison of the uptake of dioxin-like compounds by caged catfish and semipermeable membrane devices in the Saginaw River, Michigan. Environ. Sci. Technol. 31, 178–187.

Huckins, J.N., Lebo, J.A., Tubergen, M.W., Manuweera, G.K., Gibson, V.L., Petty, J.D., 1992. Binary concentration and recovery process, U.S. Patent, 5,098,573, March 24, 1992.

Huckins, J.N., Manuweera, G.K., Petty, J.D., MacKay, D., Lebo, J.A., 1993. Lipid-containing semipermeable membrane devices for monitoring organic contaminants in water. Environ. Sci. Technol. 27, 2489–2496.

Huckins, J.N., Petty, J.D., Zajicek, J.A., Gibson, V.L., 1995. Device for the removal and concentration of organic compounds from the atmosphere, U.S. Patent, 5,395,426, March 7, 1995.

Huckins, J.N., Petty, J.D., Orazio, C.E., Lebo, J.A., Clark, R.C., Gibson, V.L., Echols, K.R., 1999. Determination of uptake kinetics (sampling rates) by lipid containing semipermeable membrane devices (SPMDs) for polycyclic aromatic hydrocarbons (PAHs) in water. Environ. Sci. Technol. 33, 3918–3923.

Huckins, J.N., Petty, J.D., Lebo, J.A., Almedia, F.V., Booij, K., Alvarez, D.A., Cranor, W.L., Clark, R.C., Mogensen, B.B., 2002. Development of the permeability/performance reference compound approach for in situ calibration of semipermeable membrane devices. Environ. Sci. Technol. 36, 85–91.

Keith, L.H., 1991. Environmental Sampling and Analysis: A Practical Guide. CRC Press Inc, Boca Raton, FL. pp. 101–113.

Koplin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance. Environ. Sci. Technol. 36, 1202–1211.

Lebo, J.A., Gale, R.W., Petty, J.D., Tillitt, D.E., Huckins, J.N., Meadows, J.C., Orazio, C.E., Schroder, D.J., Inmon, L.E., 1995. Use of the semipermeable membrane device as an in situ sampler of waterborne bioavailable PCDD and PCDF residues at sub-part-per-quadrillion concentrations. Environ. Sci. Technol. 29, 2886–2892.

Opperhuizen, A., Velde, E.W.v.d., Gobas, F.A.P.C., Liem, D.A.K., Steen, J.M.D.v.d., 1985. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere 14, 1871–1896.

Petty, J.D., Huckins, J.N., Orazio, C.E., Lebo, J.A., Poulton, B.C., Gale, R.W., Charbonneau, C.S., Kaiser, E.M., 1995. Determination of waterborne bioavailable organochlorine pesticide residues in the Lower Missouri River. Environ. Sci. Technol. 29, 2561–2566.

Petty, J.D., Poulton, B.C., Charbonneau, C.S., Huckins, J.N., Jones, S.B., Cameron, J.T., Prest, H.F., 1998. Determination of bioavailable contaminants in the Lower Missouri River following the flood of 1993. Environ. Sci. Technol. 32, 837–842.

Petty, J.D., Orazio, C.E., Huckins, J.D., Gale, R.W., Lebo, J.A., Meadows, J.C., Echols, K.R., Cranor, W.L., 2000a. Considerations involved with the use of semipermeable membrane devices for monitoring environmental contaminants. J. Chromatogr. A 879, 83–95.

Petty, J.D., Jones, S.B., Huckins, J.N., Cranor, W.L., Parris, J.T., McTague, T.B., Boyle, T.P., 2000b. An approach for assessment of water quality using semipermeable membrane devices (SPMDs) and bioindicator tests. Chemosphere 41, 311–321.

Petty, J.D., Brumbaugh, W.G., Huckins, J.N., May, T.W., Wiedmeyer, R., 2001. Device for the removal and concentration of ionic metal species from water, U.S. Patent, 6,296,760, October 2, 2001.

Petty, J.D., Huckins, J.N., Alvarez, D.A., 2002. Device for sequestration and concentration of polar organic chemicals from water, U.S. Patent, 6,478,961, November 12, 2002.

Rehmann, K., Schramm, K.W., Kettrup, A.A., 1999. Applicability of a yeast oestrogen screen for the detection of oestrogen-like activities in environmental samples. Chemosphere 38, 3303–3312.

Routledge, E.J., Sumpter, J.P., 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem. 15, 241–248.

US Geological Survey, NASQAN Data–Mississippi Basin 1999–Missouri River at Hermann, MO. Available from <water.usgs.gov/nasqan/data/provdata/hermann.html>.