Chemical and Toxicologic Assessment of Organic Contaminants in Surface Water Using Passive Samplers

David A. Alvarez,* Walter L. Cranor, Stephanie D. Perkins, Randal C. Clark, and Steven B. Smith USGS

Passive sampling methodologies were used to conduct a chemical and toxicologic assessment of organic contaminants in the surface waters of three geographically distinct agricultural watersheds. A selection of current-use agrochemicals and persistent organic pollutants, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and organochlorine pesticides, were targeted using the polar organic chemical integrative sampler (POCIS) and the semipermeable membrane device passive samplers. In addition to the chemical analysis, the Microtox assay for acute toxicity and the yeast estrogen screen (YES) were conducted as potential assessment tools in combination with the passive samplers. During the spring of 2004, the passive samplers were deployed for 29 to 65 d at Leary Weber Ditch, IN; Morgan Creek, MD; and DR2 Drain, WA. Chemical analysis of the sampler extracts identified the agrochemicals predominantly used in those areas, including atrazine, simazine, acetochlor, and metolachlor. Other chemicals identified included deethylatrazine and deisopropylatrazine, trifluralin, fluoranthene, pyrene, *cis*- and *trans*-nonachlor, and pentachloroanisole. Screening using Microtox resulted in no acutely toxic samples. POCIS samples screened by the YES assay failed to elicit a positive estrogenic response.

Anthropogenic pollution is recognized as a global problem contributing to the degradation of ecosystem quality, the loss of numerous plant and animal species, and adverse affects on human health. A multitude of point and nonpoint pollution sources exist that contain a broad spectrum of agricultural, industrial, and petroleum-related chemicals. Increasingly, environmental scientists are recognizing that in addition to contaminants of historic origin and concern, emerging contaminants are playing an ever-increasing role as potential environmental stressors and actual sources of adverse environmental effects. The intensive nature of modern animal husbandry practices, limited or no-till crop production, and increasing urbanization of vast portions of the nation contribute to the pollution of a wide variety of aquatic systems. Further, rapid urbanization is occurring in many areas with limited water resources that must be used for multiple purposes. Consequently, treated wastewater is often used to recharge water supplies for recreation, consumption, and the maintenance of viable ecosystems (Daughton and Ternes, 1999; Kolpin et al., 2002).

Exacerbating the current situation is the uncertainty of the potential adverse effects resulting from organism exposure to the complex mixture of contaminants present in the nation's aquatic resources. Most traditional methods of collecting a sample, such as liquid–liquid extraction or solid-phase extraction (SPE), are limited by the volume of water that can be transported to the laboratory, which can result in a lack of detection of low concentrations of many chemicals (Barceló and Hennion, 1997). This means of sample collection, whether a grab or composite sample, only provides data on the presence of chemicals at the moment the sample was taken. On the other hand, the use of on-site automated sampling systems can be costly and difficult to maintain. Episodic events, such as a spill- or storm-related runoff, are often missed due to the logistic and financial hurdles of collecting repetitive samples. An approach for providing a time-weighted average (TWA) assessment is critical for an improved

Copyright © 2008 by the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Published in J. Environ. Qual. 37:1024–1033 (2008).
doi:10.2134/jeq2006.0463
Received 25 Oct. 2006.
*Corresponding author (dalvarez@usgs.gov).
© ASA, CSSA, SSSA
677 S. Segoe Rd., Madison, WI 53711 USA

D.A. Alvarez, W.L. Cranor, S.D. Perkins, and R.C. Clark, U.S. Geological Survey Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201; S.B. Smith, U.S. Geological Survey, MS 433, National Center, 12201 Sunrise Valley Dr., Reston, VA 20192.

Abbreviations: CPRG, chlorophenol red-β-D-galactopyranoside; DMSO, dimethylsulfoxide; E2, 17β-estradiol; EAF, exposure adjustment factor; EC_{50}, effective concentration required to elicit a 50% response in the exposed organisms; EEQ, estradiol equivalent factor; GC, gas chromatograph; IIS, instrumental internal standard; MQL, method quantitation limit; OCP, organochlorine pesticide; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PES, polyethersulfone; POCIS, polar organic chemical integrative sampler; PRC, performance reference compound; QC, quality control; SEC, size exclusion chromatography; SPMD, semipermeable membrane device; TWA, time-weighted average; YES, yeast estrogen screen.

understanding of the consequences of prolonged exposure to environmental contaminant mixtures.

Passive, integrative samplers provide a means of measuring the TWA concentrations of dissolved organic chemicals while meeting many of the detection limit requirements of common instrumental techniques by sampling large volumes of water over prolonged exposure periods (Huckins et al., 2006; Alvarez et al., 2007). Numerous passive, integrative sampling technologies exist, including the semipermeable membrane device (SPMD), polar organic chemical integrative sampler (POCIS), passive in situ concentration and extraction sampler, Chemcatcher, and polyethylene strips (Huckins et al., 2006). The SPMD has gained worldwide acceptance as a tool for monitoring for lipophilic organic contaminants in water (Booij et al., 2003; Petty et al., 2004; Huckins et al., 2006). The POCIS was developed to provide data for a wide array of bioavailable waterborne polar-organic contaminants (Alvarez et al., 2004, 2005, 2007; Jones-Lepp et al., 2004; Petty et al., 2004). The combined use of the POCIS and the SPMD provides a means to sample environmentally relevant mixtures of contaminants for chemical and biological testing. The complex mixtures of chemicals obtained from the samplers can also be used to potentially link organic contaminant occurrence to biotic effects through the use of a series of bioindicator tests, including the Microtox acute toxicity screen and the yeast estrogen screen (YES) assay, for estrogenic activity (Johnson et al., 2000; Petty et al., 2004; Rastall et al., 2004; Gilli et al., 2005; Vermeirssen et al., 2005; Matthiessen et al., 2006).

Theory and Modeling

Derivation of Water Concentrations from Semipermeable Membrane Devices and Polar Organic Chemical Integrative Sampler Residues

Semipermeable membrane device and POCIS uptake kinetic data are required to accurately estimate aquatic concentrations of environmental contaminants. Using models previously developed (Alvarez et al., 2004, 2007; Huckins et al., 2002, 2006) and data from the analysis of the performance reference compounds (PRCs) concentrations and from calibration studies (when available), the bioavailable (i.e., via respiration from the dissolved phase) concentrations of analytes in POCIS and SPMDs deployed in the study sites can be estimated for select chemicals.

The effects of exposure conditions on SPMD and POCIS uptake and dissipation rates are largely a function of (i) exposure medium temperature; (ii) facial velocity-turbulence at the membrane surface, which in turn is affected by the design of the deployment apparatus (i.e., baffling of media flow-turbulence); and (iii) membrane biofouling. Performance reference compounds are analytically noninterfering organic compounds with moderate to high fugacity from SPMDs that are added to the lipid before membrane enclosure and field deployment (Huckins et al., 2006). By comparing the rate of PRC loss during field exposures to that of laboratory studies, an exposure adjustment factor (EAF) can be derived and used to adjust laboratory sampling rates to more accurately reflect actual in situ sampling rates. A mixture of PRCs is often used to ensure at least one will have the optimal 20 to 80% loss (Huckins et al., 2002). PRCs undergo increased loss as their log K_{ow} value decreases. The amount of loss is dependent on environmental factors such as exposure time, surficial flow/velocity, temperature, and biofouling. Due to the strong sorptive properties of the adsorbents used in the POCIS, attempts to incorporate PRCs into the POCIS have failed (Alvarez et al., 2007).

Uptake of hydrophobic chemicals into SPMDs follows linear, curvilinear, and equilibrium phases of sampling. Integrative (or linear) sampling is the predominant phase for compounds with log K_{ow} values ≥5.0 and exposure periods of up to 1 mo. During the linear uptake phase, the ambient chemical concentration (C_w) is determined by

$$C_w = N/R_s t \quad [1]$$

where N is the amount of the chemical sampled by an SPMD (typically ng), R_s is the SPMD sampling rate (L/d), and t is the exposure time in days. Estimation of a chemical's site-specific R_s in an SPMD and its ambient C_w requires the derivation of the EAF as described by Huckins et al. (2002). A key feature of the EAF is that it is relatively constant for all chemicals that have the same rate-limiting barrier to uptake, allowing PRC data to be applied to a range of chemicals. Then the in situ or site-specific sampling rate (R_{si}) of an analyte is the EAF times its laboratory calibration R_s.

Uptake of hydrophilic organic chemicals by the POCIS is controlled by many of the same rate-limiting barriers, allowing the use of the same models to determine ambient water concentrations. Previous data indicate that pesticides of interest remain in the linear phase of sampling for at least 56 d (Alvarez et al., 2004, 2007); therefore, the use of a linear uptake model (Eq. [1]) for the calculation of ambient water concentrations is justified.

Determination of Estradiol Equivalent Factors from Yeast Estrogen Screen Data

Estradiol equivalent factors (EEQ) for the samples were calculated by measuring the effective concentration required to elicit a 50% response in the exposed organisms (EC_{50}) for the 17β-estradiol (E2)–positive control and determining the percent of sample required to give an equivalent response (same adjusted absorbance indicating the equivalent amount of conversion of CPRG). The adjusted absorbance, compensating for turbidity caused by the growing yeast, is calculated as

$$\mathrm{Abs}_{\mathrm{corrected}} = \mathrm{Abs}_{540} - (\mathrm{Abs}_{620} - \mathrm{Abs}_{\mathrm{MNC620}}) \quad [2]$$

where Abs_{540} and Abs_{620} are the measured absorbances at 540 and 620 nm, and Abs_{MNC620} is the mean negative control absorbance at 620 nm (Rastall et al., 2004). Because plate-to-plate variability can occur during the development, EEQs are calculated using the positive control from that plate instead of an average value (Rastall et al., 2004).

$$\begin{aligned} \mathrm{EEQ} &= \mathrm{EEQ}\ (\mathrm{ng\ E2/POCIS}) \\ &= \mathrm{EC}_{50}\mathrm{E2}\ (\mathrm{ng\ E2/mL})/\mathrm{EC}_{50}\mathrm{test}\ (\mathrm{POCIS/mL}) \end{aligned} \quad [3]$$

Table 1. Sequestered chemical residues and average estimated aqueous concentrations of agrochemicals in deployed polar organic chemical integrative sampler (POCIS) at each study site (n = 2).†

Analyte	Leary Weber Ditch (IN)		Morgan Creek (MD)		DR2 Drain (WA)	
	ng POCIS^{-1}	ng L^{-1}	ng POCIS^{-1}	ng L^{-1}	ng POCIS^{-1}	ng L^{-1}
Atrazine	3400	350	1200	170	33	2.1
Ametryn	<0.67‡	ND§	45	4.6	<0.67	ND
Atraton	150	7.2	<6.7	ND	<6.7	ND
Metribuzin	<3.3	ND	<3.3	ND	<3.3	ND
Prometon	<3.3	ND	<3.3	ND	<3.3	ND
Prometryn	<0.67	ND	<0.67	ND	<0.67	ND
Propazine	<0.67	ND	<0.67	ND	<0.67	ND
Simazine	<3.3	ND	44	6.9	55	3.8
Simetryn	<0.67	ND	35	3.2	<0.67	ND
Terbuthylazine	<0.67	ND	<0.67	ND	<0.67	ND
Terbutryne	<0.67	ND	<0.67	ND	<0.67	ND
Deethylatrazine	2000	190	27	3.6	<3.3	ND
Deisopropylatrazine	490	54	<33	ND	<33	ND
Acetochlor	1400	85	88	7.5	<0.67	ND
Alachlor	<0.67	ND	170	17	<0.67	ND
Metolachlor	440	24	2100	170	<0.67	ND
Chlorpyrifos	<0.67	ND	<0.67	ND	<0.67	ND
Diazinon	<0.67	ND	<0.67	ND	<0.67	ND
Fonofos	<0.67	ND	<0.67	ND	<0.67	ND
Malathion	<6.7	ND	<6.7	ND	<6.7	ND
Methyl parathion	<0.67	ND	<0.67	ND	<0.67	ND
Dacthal	<0.67	ND	<0.67	ND	<0.67	ND
EPTC	<0.67	ND	<0.67	ND	<0.67	ND
Fipronil	75	NA¶	<0.67	ND	<0.67	ND
Pendimethalin	<3.3	ND	<3.3	ND	<3.3	ND
Trifluralin	<0.67	ND	<0.67	ND	33	NA

† Water concentrations were estimated using preliminary sampling rate data for these chemicals. These estimates are provided for informational purposes only and should not be considered to be definitive values.

‡ "<" values indicate chemical was at a concentration less than the method detection limit (MDL = mean + 3 × SD).

§ ND, not determined. Water concentration was not estimated for values less than the method detection limit.

¶ NA values indicate chemicals for which sampling rate data do not exist.

Table 2. Sequestered chemical residues and average estimated aqueous concentrations of organochlorine pesticides and polychlorinated biphenyls (PCBs) in deployed semipermeable membrane devices (SPMDs) at each study site (n = 2).†

Analyte	Leary Weber Ditch (IN)		Morgan Creek (MD)		DR2 Drain (WA)	
	ng SPMD^{-1}	ng L^{-1}	ng SPMD^{-1}	ng L^{-1}	ng SPMD^{-1}	ng L^{-1}
Trifluralin	<6.3‡	ND§	<6.3	ND	<6.3	ND
Hexachlorobenzene	***4.0***¶	ND	<1.7	ND	<1.7	ND
Pentachloroanisole	26	290	8.5	48	<0.79	ND
α-BHC	<13	ND	<13	ND	<13	ND
Diazinon	14	2300	***12***	ND	<4.5	ND
Lindane	<5.4	ND	***5.9***	ND	<5.4	ND
β-BHC	<0.32	ND	***0.48***	ND	<0.32	ND
Heptachlor	<1.4	ND	<1.4	ND	<1.4	ND
δ-BHC	<3.9	ND	<3.9	ND	<3.9	ND
Dacthal	<0.36	ND	<0.36	ND	<0.36	ND
Chlorpyrifos	***26***	ND	<10	ND	<10	ND
Oxychlordane	<1.8	ND	<1.8	ND	<1.8	ND
Heptachlor Epoxide	7.6	41	***2.4***	ND	***2.4***	ND
trans-Chlordane	***4.2***	ND	***3.9***	ND	<2.4	ND
trans-Nonachlor	2.8	55	2.9	29	<0.12	ND
o,p′-DDE	<1.6	ND	<1.6	ND	<1.6	ND
cis-Chlordane	<8.2	ND	<8.2	ND	<8.2	ND
Endosulfan	<1.7	NA#	<1.7	N/A	<1.7	NA
p,p′-DDE	<12	ND	***17***	ND	720	4700
Dieldrin	31	420	***11***	ND	<8.1	ND
o,p′-DDD	<1.2	ND	***1.4***	ND	<1.2	ND
Endrin	<18	ND	<18	ND	<18	ND
cis-Nonachlor	1.3	24	2.6	24	<0.35	ND
o,p′-DDT	<4.2	ND	<4.2	ND	<4.2	ND
p,p′-DDD	<2.5	ND	***3.8***	ND	<2.5	ND
Endosulfan-II	***1.1***	NA	***1.2***	N/A	<0.39	NA
p,p′-DDT	<16	ND	<16	ND	<16	ND
Endosulfan sulfate	<1.1	NA	<1.1	N/A	<1.1	NA
Methoxychlor	<4.6	ND	<4.6	ND	<4.6	ND
Mirex	<0.12	ND	<0.12	ND	<0.12	ND
cis-Permethrin	<4.0	ND	<4.0	ND	<4.0	ND
trans-Permethrin	<0.42	ND	<0.42	ND	<0.42	ND
Total PCBs	<0.8	ND	<0.8	ND	***1.3***	ND

† Pyrene-d_{10} (k_{eprc} = 0.0051 d–1) was used for the sampling rate adjustments.

‡ "<" values indicate chemical was at a concentration less than the method detection limit.

§ ND, not determined. Water concentration was not estimated for values less than the method detection limit (MDL = mean + 3 × SD).

¶ Italicized values are below the method quantitation limit (MQL = mean + 10 × SD).

NA values indicate chemicals for which sampling rate data do not exist.

Materials and Methods

Chemicals

Analytical standards of all targeted chemicals (Tables 1–3) were obtained from AccuStandard Inc. (New Haven, CT), ChemService Inc. (West Chester, PA), Crescent Chemical (Islandia, NY), or Sigma Aldrich (St. Louis, MO). All laboratory chemicals were American Chemical Society reagent grade, and organic solvents were Optima grade from Fisher Scientific Co. (Pittsburgh, PA). Materials for the construction of the POCIS (Alvarez et al., 2004, 2005) and SPMDs (Huckins et al., 2002, 2006) and for the preparation of the chromatographic sorbents (Petty et al., 2000) have been described in detail elsewhere.

Site Selection and Field Work

Three sites potentially affected by diverse pesticide applications were selected for study (Vogel et al., 2008). Morgan Creek in Maryland (PODL) and Leary Weber Ditch in Indiana (WHMI) predominantly receive drainage from corn and soybean farming. DR2 Drain in Washington (CCYK) is in an area of mixed crops, including grapes, pumpkins, apples, corn, and hops. Two canisters of POCIS and one canister of SPMDs were deployed at each site for up to 2 mo beginning in May 2004. Each POCIS canister contained six individual POCIS devices, and the SPMD canister contained five SPMDs (Fig. 1). Site conditions are listed in Table 4.

Passive Sampler Preparation, Processing, and Analysis

The POCIS used in this study were of the "generic" configuration, containing the triphasic admixture of (80:20 W:W) Isolute ENV+ and S-X3 dispersed Ambersorb 1500 enclosed between two polyethersulfone (PES) membranes. Each POCIS unit had an effective sampling surface area of 41 cm^2 and a membrane surface area to sorbent mass ratio of ≈180 cm^2/g. This ratio conforms to the definition of a standard POCIS as defined by Alvarez et al. (2004). Each POCIS field deployment canister contained six individual POCIS units. The POCIS from each canister were combined into two 3-POCIS composite samples resulting in replicates for subsequent test-

Table 3. Sequestered chemical residues and average estimated aqueous concentrations of polycyclic aromatic hydrocarbons in deployed semipermeable membrane devices (SPMDs) at each study site (n = 2).†

Analyte	Leary Weber Ditch (IN)		Morgan Creek (MD)		DR2 Drain (WA)	
	ng SPMD^{-1}	ng L^{-1}	ng SPMD^{-1}	ng L^{-1}	ng SPMD^{-1}	ng L^{-1}
Naphthalene	<2.0‡	ND§	<2.0	ND	<2.0	ND
Acenaphthylene	<2.0	ND	<2.0	ND	<2.0	ND
Acenaphthene	<2.0	ND	<2.0	ND	<2.0	ND
Fluorene	<2.0	ND	10	79	10	270
Phenanthrene	***50***¶	ND	***60***	ND	***80***	ND
Anthracene	<2.0	ND	<2.0	ND	<2.0	ND
Fluoranthene	***50***	ND	90	540	130	1300
Pyrene	40	420	60	320	80	700
Benz[*a*]anthracene	<2.0	ND	<2.0	ND	<2.0	ND
Chrysene	<44	ND	<44	ND	<44	ND
Benzo[*b*]fluoranthene	<2.0	ND	<2.0	ND	<2.0	ND
Benzo[*k*]fluoranthene	<2.0	ND	<2.0	ND	<2.0	ND
Benzo[*a*]pyrene	<2.0	ND	<2.0	ND	<2.0	ND
Indeno[1,2,3-*c,d*]pyrene	<2.0	ND	<2.0	ND	<2.0	ND
Dibenz[*a,h*]anthracene	<2.0	ND	<2.0	ND	<2.0	ND
Benzo[*g,h,i*]perylene	<2.0	ND	<2.0	ND	<2.0	ND
Benzo[*b*]thiophene	<2.0	NA#	<2.0	NA	<2.0	NA
2-methylnaphthalene	<2.0	NA	<2.0	NA	<2.0	NA
1-methylnaphthalene	<2.0	NA	<2.0	NA	<2.0	NA
Biphenyl	<28	NA	<28	NA	<28	NA
1-ethylnaphthalene	<2.0	NA	<2.0	NA	<2.0	NA
1,2-dimethylnaphthalene	<2.0	NA	<2.0	NA	<2.0	NA
4-methylbiphenyl	<2.0	NA	<2.0	NA	<2.0	NA
2,3,5-trimethylnaphthalene	<2.0	NA	<2.0	NA	<2.0	NA
1-methylfluorene	<110	NA	<110	NA	<110	NA
Dibenzothiophene	<2.0	NA	<2.0	NA	<2.0	NA
2-methylphenanthrene	<2.0	NA	<2.0	NA	20	NA
9-methylanthracene	<2.0	NA	<2.0	NA	<2.0	NA
3,6-dimethylphenanthrene	<2.0	NA	<2.0	NA	<2.0	NA
2-methylfluoranthene	<2.0	NA	<2.0	NA	<2.0	NA
Benzo[*b*]naphtho[2,1-*d*] thiophene	<2.0	NA	<2.0	NA	<2.0	NA
Benzo[*e*]pyrene	<2.0	NA	<2.0	NA	<2.0	NA
Perylene	<2.0	NA	120	NA	10	NA
3-methylcholanthrene	<2.0	NA	<2.0	NA	<2.0	NA

† Pyrene-d_{10} (k_{eprc} = 0.0051 d^{-1}) was used for the sampling rate adjustments.
‡ "<" values indicate chemical was at a concentration less than the method detection limit.
§ ND, not determined. Water concentration was not estimated for values less than the method detection limit.
¶ Italicized values are below the method quantitation limit (MQL = mean + 10 × SD).
# NA values indicate chemicals for which sampling rate data do not exist.

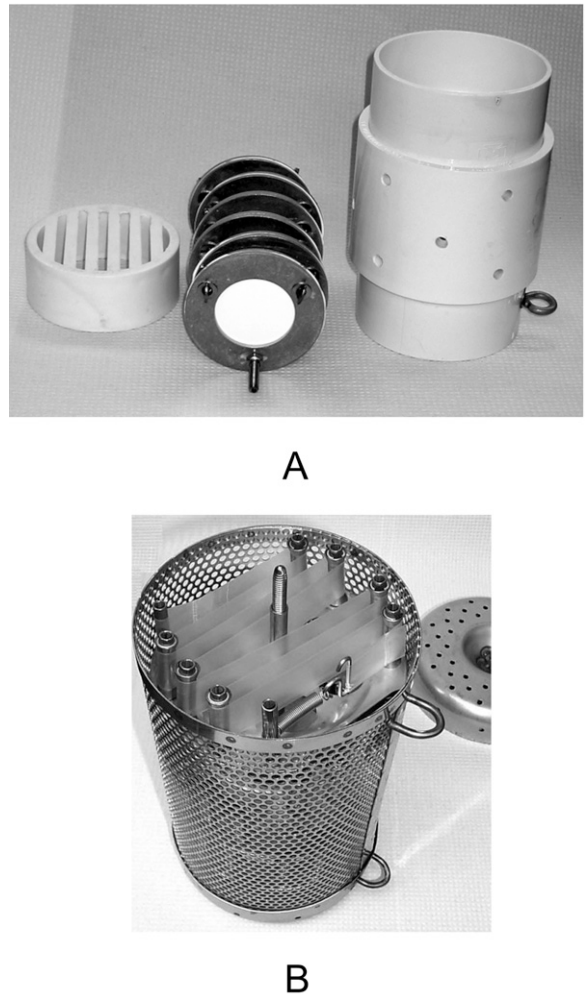

Fig. 1. Field deployment canisters. (A) Polar organic chemical integrative sampler (POCIS) deployment canister with six POCIS mounted on a support rod that secures the samplers inside the canister. (B) Semipermeable membrane device (SPMD) deployment canister containing five SPMDs (cover removed to show SPMDs secured on racks).

ing. One deployment canister was designated for chemical analysis, and the other was designated for testing by the Microtox and YES assays. Chemical analyses on the POCIS extracts targeted select agrochemicals, including triazine herbicides, amide herbicides, and organophosphate insecticides.

The SPMDs used in this project consisted of 97-cm-long (86 cm between the lipid-containment seals) by 2.5-cm-wide layflat LDPE tubing containing 1.0 mL of purified triolein (Huckins et al., 2006; Lebo et al., 2004). The membrane surface area to total SPMD volume ratio of SPMDs used in this study was ≈86 cm^2/mL, and triolein represented ≈20% of the mass of the SPMDs conforming to a "standard SPMD" as defined by Huckins et al. (2006). Five SPMDs were placed in each deployment canister. Two of the five SPMDs deployed at each site were fortified with 4 µg of each of the five perdeuterated polycyclic aromatic hydrocarbons (PAHs) selected as PRCs (acenaphthylene-d_{10}, acenaphthene-d_{10}, fluorene-d_{10}, phenanthrene-d_{10}, and pyrene-d_{10}). One of the two field-blank SPMDs was similarly spiked with PRCs, and the remaining SPMD was reserved for use as a biomarker blank. Two of the non-PRC SPMDs were used for the Microtox and YES assays, and the remaining non-PRC SPMD was archived. Chemicals analyzed for in the SPMD dialysates included PAHs, organochlorine pesticides (OCPs), and total polychlorinated biphenyls (PCBs).

A rigorous quality control (QC) plan was used to ensure the reliability of the data obtained. The QC samples for the POCIS and SPMDs consisted of fabrication (samplers prepared concurrently with the field samplers and stored in air-tight containers at −20°C to measure potential background contamination during sampler construction) and field blanks (samplers exposed to the air at each study site during the deployment and retrieval operations to measure sequestered chemicals not due to the water exposure). Laboratory controls, such as reagent blanks, matrix blanks, surrogate recovery, and fortified matrix

recovery checks, were included in the construction, deployment, and processing of the study samples. Detailed discussions on the benefits of each type of control sample have been reported (Alvarez et al., 2007; Huckins et al., 2006).

Polar Organic Chemical Integrative Sampler

The procedures used for preparing samples for analysis in this study are similar to published approaches (Alvarez et al., 2004; Jones-Lepp et al., 2004; Alvarez et al., 2005; Alvarez et al., 2007). The general sample processing and enrichment scheme is shown in Fig. 2. Briefly, the POCIS were gently cleaned, and the sorbents from each POCIS were transferred into glass gravity-flow chromatography columns (1 cm inner diameter) for extraction. Chemical residues were recovered from the POCIS sorbent using 50 mL of 1:1:8 (V:V:V) methanol:toluene:dichloromethane followed by 20 mL of ethyl acetate. The extracts were reduced in volume by rotary evaporation and under a gentle stream of nitrogen, filtered, and composited into 3-POCIS equivalent samples. The bioassay samples were transferred via solvent exchange into dimethylsulfoxide (DMSO) for the Microtox and into ethanol for the YES assays.

A modification of a size exclusion chromatographic (SEC) system fitted with a fraction collector was used to fractionate and enrich the POCIS samples designated for chemical analysis (Petty et al., 2000). Based on the elution of the target compounds and a SEC calibration standard, a collect window was initiated at the point of 5% of the time between the apexes of the diethylhexylphthalate (DEHP) and the biphenyl chromatographic reference peaks and terminated at 70% of the time between the apexes of the coronene and the sulfur chromatographic reference peaks. The post-SEC POCIS fractions were applied to Florisil for additional cleanup and removal of potential interferences. The samples were added to 5 g of Florisil in glass gravity-flow columns (1 cm inner diameter), and the targeted chemicals were eluted using 60 mL of 75:25 (V:V) methyl-*tert*-butyl ether (MTBE):hexane followed by 70 mL of acetone.

Gas chromatographic (GC) analyses for selected pesticides in the POCIS were performed using an Agilent 6890 GC (Agilent Technologies, Inc., Wilmington, DE) coupled to a 5973N mass selective detector (MSD) (Agilent Technologies, Inc., Palo Alto, CA). The separation was performed on a DB-XLB column (30 m by 0.25 mm inner diameter by 0.25 μm film thickness) (Agilent Technologies, Inc., Wilmington, DE) with the temperature program of injection at 90°C, held for 1 min, and ramped at 10°C/min to 180°C followed by a 5°C/min ramp to 230°C and a 10°C/min ramp to 320°C and held at 320°C for 2 min. Analytes were detected in the selected ion mode. Detector zone temperatures were set at 300°C for the MSD transfer line, 150°C at the quadrupole, and 230°C at the source. Quantitation was achieved using a calibration curve ranging from 10 to 4000 ng/mL with *p*-

Table 4. Field data during passive sampler deployment for each of the study sites.

Field parameter	Leary Weber Ditch (IN)	Morgan Creek (MD)	DR2 Drain (WA)
Station ID	03361638	01493500	462023120075200
Deployment date	6 May 2004	4 May 2004	3 May 2004
Retrieval date	16 June 2004	2 June 2004	7 July 2004
Total deployment time, d	41	29	65
Average temperature, °C†	16.2	21.5	16.3
Streamflow, m^3 sec^{-1}†	0.41	0.045	0.19

† Average temperature and streamflow values were obtained from the online USGS National Water Information System (http://nwis.waterdata.usgs.gov/; verified 7 Mar. 2007).

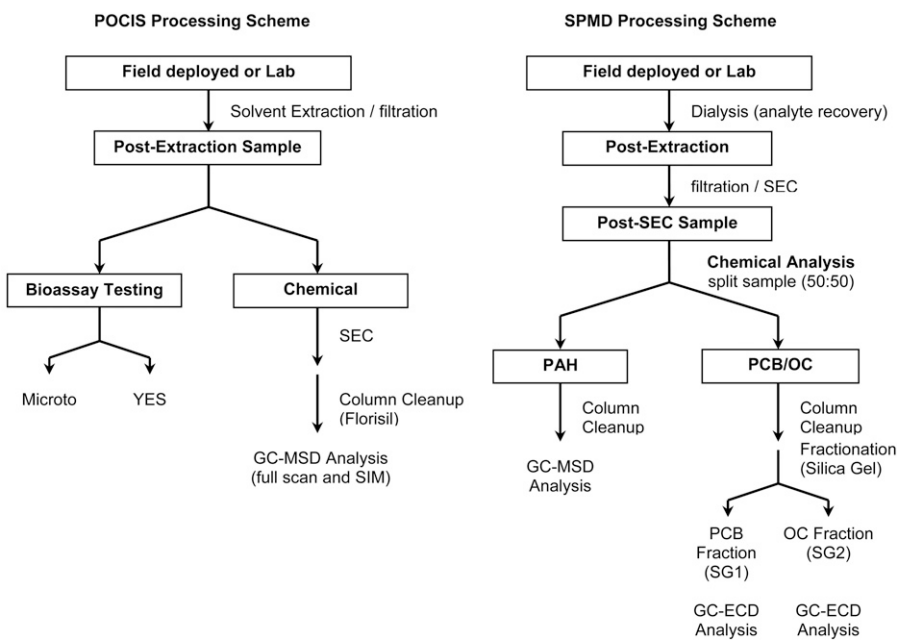

Fig. 2. Laboratory processing scheme for field-deployed and quality-control polar organic chemical integrative sampler (POCIS) and semipermeable membrane device (SPMDs). GC-ECD, gas chromatograph electron capture detector; GC-MSD, gas chromatograph-mass selective detector; OC, organochlorine; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; SEC, size exclusion chromatography; YES, yeast estrogen screen.

terphenyl-d_{14} at the instrumental internal standard (IIS). All samples were at a 1.0 mL volume in 3:1 (V:V) hexane:isopropanol.

Semipermeable Membrane Devices

The procedures used for preparing SPMD samples for analysis are similar to published approaches (Huckins et al., 2006; Petty et al., 2000). The general sample processing and enrichment scheme is shown in Fig. 2. Briefly, SPMDs were cleaned, and the target analytes were recovered by organic solvent dialysis. Samples were fractionated on SEC using the method described by Petty et al. (2000) with one modification (i.e., the collect window was initiated at 50% of the time between the apexes of the DEHP and the biphenyl chromatographic reference peaks). The post-SEC SPMD samples designated for Microtox were solvent exchanged into DMSO and were ready for testing without additional fractionation or enrichment. Because different enrichment techniques were required for the chemical analysis of SPMDs after SEC, each sample was split into two equal portions (equivalent to one SPMD) before further fractionation and enrichment. These were identified as the "PAH" fractions and the "OCP/PCB" fractions. The PAH fractions were processed using a tri-adsorbent column consisting of, from top to bottom; 3 g of phosphoric acid-silica gel, 3 g of potassium hydroxide-silica gel, and 3 g of silica gel (Petty et al., 2000), resulting in a solution suitable for the instrumental analysis of PAH residues. The OCP/PCB fractions were further enriched using a Florisil column followed by fractionation on silica gel (Petty et al., 2000). The first fraction (SG1; 46 mL of hexane) contains >95% of the total PCBs, hexachlorobenzene (HCB), heptachlor, mirex, and ≈40 to 80% of the *p,p′*-DDE when present in extracts. The second fraction (SG2; 75 mL of 40:60 [V:V] MTBE:hexane) contains the remaining 28 OCPs and ≤5% of the total PCBs (largely, mono- and dichlorobiphenyl congeners).

Gas chromatographic analyses for selected PAHs and PRCs were conducted using a gas chromatography–mass selective detector system described for the POCIS pesticides with the following changes. An HP-5MS (30 m by 0.25 mm inner diameter by 0.25 µm film thickness) capillary column (Agilent Technologies, Inc., Wilmington, DE) was used with the temperature program of injection at 50°C, held for 2 min, ramped at 25°C/min to 130°C, held for 1 min, then 6°C/min ramped to 310°C and held at 310°C for 5 min. Detector zone temperatures were set at 310°C for the MSD transfer line, 150°C at the quadrupole, and 230°C at the source. Quantitation of the analytes was accomplished using a six-point curve with internal calibration. Concentrations of calibration standards bracketed the range of 20 to 4000 ng/mL for each of the analytes with the 2-methylnaphthalene-d_{10} and benzo[*e*]pyrene-d_{12} maintained at 0.25 µg/mL as the IIS.

Analysis of the SPMD samples for PCBs and OCPs were conducted using a Hewlett-Packard 5890 series GC equipped with an electron capture detector (ECD) (Hewlett-Packard, Inc., Palo Alto, CA) that was maintained at 330°C. Analyses of SG1 and SG2 fractions for PCBs and OCPs were performed using a DB-35MS (30 m by 0.25 mm inner diameter by 0.25 µm film thickness) capillary column (J&W Scientific, Folsom, CA) with the temperature program of injection at 90°C, then ramped 15°C/min to 165°C, followed by a ramp of 2.5°C/min to 250°C, then 10°C/min to 320°C. Quantitation of OCPs and PCBs were accomplished using a six-point internal standard calibration curve with PCB congener I-30 as retention time reference compound and PCB congener I-207 as the IIS. The concentrations of the pesticide standards ranged from 1.0 to 80 ng/mL. The PCB calibration standards were composed of a 1:1:1:1 mixture of Aroclors 1242, 1248, 1254, and 1260 covering the range of 200 to 4000 ng/mL. The SG2 fractions were not included in the analysis for total PCBs. Carryover of mono- and dichlorobiphenyl congeners into the SG2 fraction represents, at most, an additional 5% of total PCBs. The more rigorous approach of analysis of the SG2 fraction for these few PCB congeners was beyond the scope of the project and was judged to be an excessive expenditure of time and effort for a minimal gain of information.

Microtox

The Microtox Basic (Strategic Diagnostics, Inc., Newark, DE) assay was used to assess the acute toxicity of sequestered waterborne contaminant residues. The assessment of the POCIS extracts and SPMD dialysates was conducted following the procedures outlined by Johnson et al. (2000) and Johnson and Long (1998). The analyzer, reagents, and freeze-dried bacteria were obtained from Strategic Diagnostics, Inc. (Newark, DE).

Briefly, a suspension of luminescent marine bacteria, *Vibrio fischeri*, was added to an aliquot of the test sample and incubated at 15°C. Light readings of each test vial were taken before the addition of test samples and after a 5-min and 15-min incubation period. *V. fischeri* produces light as a byproduct of cellular respiration. When exposed to a toxicant, the rate of light production is reduced in proportion to the sample toxicity. To determine the dose–response and the concomitant toxicity, each sample solution was diluted into four test concentrations. Data were analyzed using the Microtox Omni software package (version 1.18; Strategic Diagnostics, Inc., Newark, DE). Phenol was used as the positive toxicity control for the Microtox assay. DMSO was used as the carrier vehicle for the samples and as the negative control. Results were reported in terms of an effective concentration, which was the estimated concentration of sample required to produce the desired level of metabolic inhibition (light loss). EC_{50} values (expressed as milligrams equivalent of sample) were reported as the means of three replicate determinations. Variability was expressed as SD.

Yeast Estrogen Screen Assay

The YES assay uses recombinant yeast cells transfected with the human estrogen receptor, which, through a cascade of events, releases β-galactosidase on binding with a suitable agonist (Routledge and Sumpter, 1996; Rastall et al., 2004). The β-galactosidase interacts with a chromogenic substrate (CPRG) in the media, producing a color change that can be measured spectrophotometrically. This color change is a measure of the estrogenic potential of chemicals in the sample. The YES procedure has been described in detail by Rastall et al. (2004). Briefly, test plates were prepared by adding a positive control (17β-estradiol) in the first row and alternating negative controls (200 µL ethanol) and test sample (100 µL extract diluted with 100 µL ethanol in triplicate) in the following rows. All samples and controls were serially diluted across the test plate. The

liquid in each well was allowed to evaporate before adding 200 μL of assay medium containing ≈4×10^7 recombinant yeast cells and CPRG. The plates were gently agitated, sealed, and incubated at 30°C for 48 h followed by an additional 24 h at room temperature. The plates were read using a Labsystems Multiskan MS type 352 with the Genesis II software (Labsystems, Finland) measuring the absorbance at 540 and 620 nm.

Results and Discussion

Chemical Analysis

A selection of persistent, bioaccumulative contaminants and herbicides of known or suspected use were targeted for analysis. Chemical residues sequestered by and analyzed for in the passive samplers are reported in Table 1 for the POCIS and in Tables 2 and 3 for the SPMDs. At each of the sites, the most prevalent chemicals identified in the passive samplers were herbicides associated with the agricultural practices in each drainage basin. Atrazine was measured at all three sites, with the highest concentrations occurring at Leary Weber Ditch (IN). Atrazine concentrations were approximately two orders of magnitude less at DR2 Drain (WA) than at the other two sites. Acetochlor and metolachlor were found at Leary Weber Ditch, and acetochlor, alachlor, and metolachlor were present at Morgan Creek (MD) (Table 1). PAHs identified in the SPMD samples included fluorene, phenanthrene, fluoranthene, and pyrene (Table 3). Few OCPs were found at concentrations greater than their method quantitation limits (MQLs). Heptachlor epoxide was identified at all sites, and pentachloroanisole (a degradation product of the wood preservative pentachlorophenol) was found at Leary Weber Ditch and Morgan Creek (Table 2). Other notable OCPs included the persistent and bioaccumulative *trans*-chlordane, *cis*- and *trans*-nonachlor, and dieldrin. The DDT complex was found in SPMDs at detectable concentrations but generally below the MQL (Table 2). PCBs were not found at levels above the method detection limit except in one SPMD from site DR2 Drain, where concentrations were at the MQL (Table 2). When possible, the chemical residues measured in the passive samplers were used in conjunction with the models described previously to estimate the ambient water concentrations (Tables 1–3). In this study, the chemical loss data for pyrene-d_{10} was used in the EAF correction of the sampling rates because >80% loss was observed for the other PRCs.

In samples from Leary Weber Ditch, atrazine, the atrazine metabolites deethylatrazine and deisopropylatrazine, acetochlor, and metolachlor were the major chemicals detected. These findings are consistent with historical chemical usage data for the watershed, which is used predominantly for corn and soybean row crops (USGS Fact Sheet 084-03, August 2003; U.S. Geological Survey, 2003c). Morgan Creek is also in a region commonly used for corn and soybean production primarily using the herbicides atrazine, simazine, and metolachlor (USGS Fact Sheet 080-03, August 2003; U.S. Geological Survey, 2003a). All three of these herbicides, as well as acetochlor, alachlor, and deethylatrazine, were found in the POCIS extracts from Morgan Creek. The DR2 Drain drainage basin is a multi-crop region where herbicides such as atrazine, simazine, and trifluralin are commonly used (USGS Fact Sheet 082-03, August 2003; U.S. Geological Survey, 2003b). At each of the study sites, herbicides known to be commonly applied in the area were identified in the POCIS extracts. Estimated water concentrations for agrochemicals identified in the POCIS extracts (Table 1) were determined using preliminary sampling rate data (Alvarez et al., 2007). This sampling rate data have not been fully validated. Therefore, any estimates of the water concentrations of these chemicals are provided for informational purposes only and should not be considered definitive values.

Chlorpyrifos, diazinon, and trifluralins were measured in SPMDs and POCIS. Dacthal was not identified in either sampler. Trifluralin was measured at DR2 Drain in the POCIS but not in the SPMDs. Chlorpyrifos (Leary Weber Ditch) and diazinon (Leary Weber Ditch and Morgan Creek) were not measured in the POCIS but were found at or below the MQL in the SPMD. Although there is an overlap in the types of chemicals sampled by each sampler, the POCIS, largely due to its smaller surface area, samples chemicals at a much lower rate than SPMDs (Alvarez et al., 2007). Differences in the sampling rates, volume of water sampled, and sampler-specific method detection limits and MQLs explain why a chemical may have been found in one type of sampler and not the other.

Numerous QC samples were used during the fabrication, field work, processing, and analysis of the SPMDs and POCIS in this study. Chemical analysis of the fabrication, field, and processing blanks did not reveal any significant bias due to contamination of the samplers at any step of the process. Any identified chemicals in these blanks were generally at or near the historic background levels previously observed (DeVita and Crunkilton, 1998; Huckins et al., 2006; Petty et al., 2000). Recovery of the ^{14}C phenanthrene SEC surrogate averaged 100% ($n = 4$), indicating proper operation of the instrument. The ^{14}C SPMD spike resulted in 89% recovery of the ^{14}C phenanthrene through dialysis and SEC. Recovery of the nonradiolabeled analyte list from SPMDs was generally within the expected range based on historical data, with the exception of a few chemicals (Table 5). Trifluralin, diazinon, chlorpyrifos, and *cis*- and *trans*-permethrin were components of a solution separate from the rest of the OC pesticides, which was used to spike the recovery SPMD. The recoveries (0–24%) of these five chemicals were significantly lower than commonly observed in our laboratory, and it was theorized that the wrong amount of this solution had been added to the SPMD. Because the recoveries of the rest of the test chemicals were within the expected range, it was determined that no processing errors had occurred. Recovery of test chemicals from the POCIS had not previously been reported and was deemed acceptable (Table 5).

Microtox

The Microtox technique for determining the acute toxicity of chemicals has been used worldwide for over 20 yr. The US Environmental Protection Agency has also suggested the use of the Microtox system as a standard method for monitoring whole effluent toxicity (40 CFR Part 122; April 6, 2004). This technique was used to screen for the acute toxicity of sequestered chemical(s) by the SPMDs and POCIS. The EC_{50} and toxic units were determined for each sample (Strategic Diagnostics,

Table 5. Recovery of targeted analytes in fortified polar organic chemical integrative sampler (POCIS) and semipermeable membrane devices (SPMDs).

Analyte	Recovery (n = 5)	Recovery (n =1)
	% (%RSD)	%
POCIS		
Atrazine	96 (1.7)	
Ametryn	37 (23)	
Atraton	55 (29)	
Metribuzin	77 (15)	
Prometon	54 (23)	
Prometryn	40 (21)	
Propazine	92 (1.5)	
Simazine	100 (2.1)	
Simetryn	37 (25)	
Terbuthylazine	99 (1.5)	
Terbutryne	43 (21)	
Deethylatrazine	100 (2.6)	
Deisopropylatrazine	110 (13)	
Acetochlor	110 (2.4)	
Alachlor	110 (1.6)	
Metolachlor	110 (1.6)	
Chlorpyrifos	100 (1.9)	
Diazinon	92 (0.5)	
Fonofos	83 (2.1)	
Malathion†	110 (16)	
Methyl parathion	100 (5.8)	
Dacthal	90 (1.8)	
EPTC	58 (8.0)	
Fipronil	180 (5.7)	
Pendimethalin	100 (2.7)	
Trifluralin	83 (2.1)	
SPMD		
Trifluralin‡		24
Hexachlorobenzene		85
Pentachloroanisole		100
α-BHC		63
Diazinon‡		0
Lindane		87
β-BHC		75
Heptachlor		91
δ-BHC		76
Dacthal		75
Chlorpyrifos‡		18
Oxychlordane		93
Heptachlor Epoxide		100
trans-Chlordane		91
trans-Nonachlor		76
o,p′-DDE		100
cis-Chlordane		88
Endosulfan		91
p,p′-DDE		150
Dieldrin		110
o,p′-DDD		120
Endrin		41
cis-Nonachlor		77
o,p′-DDT		110
p,p′-DDD		110
Endosulfan-II		100
p,p′-DDT		130
Endosulfan sulfate		84
Methoxychlor		110
Mirex		140
cis-Permethrin‡		7
trans-Permethrin‡		4

(cont'd)

Table 5. Continued.

Analyte	Recovery (n = 5)	Recovery (n =1)
Naphthalene		33
Acenaphthylene		43
Acenaphthene		51
Fluorene		60
Phenanthrene		74
Anthracene		75
Fluoranthene		88
Pyrene		85
Benz[*a*]anthracene		100
Chrysene		95
Benzo[*b*]fluoranthene		100
Benzo[*k*]fluoranthene		94
Benzo[***a***]pyrene		100
Indeno[1,2,3-*c,d*]pyrene		110
Dibenz[*a,h*]anthracene		110
Benzo[*g,h,i*]perylene		94
Total PCBs		75

† n = 3; an interference prevented determination in two of the five fortified matrix samples.

‡ These chemicals were part of a separate spiking solution in which the wrong amount was apparently added.

Inc., 2003; Gilli et al., 2005). Overall, no toxicity was observed in any of the extracts from the field deployed and blank SPMDs or POCIS. A known quantity of phenol, a toxicity surrogate, was tested independently and was added to the Leary Weber Ditch Replicate #2 SPMD sample and to the SPMD Reagent Blank. Equivalent EC_{50} values (14.4 for phenol, 14.7 for phenol + SPMD Leary Weber Ditch Replicate #2, and 14.1 for phenol + SPMD reagent blank) were obtained in all three cases, indicating that the test was performing within acceptable limits and that any apparent toxicity, if present, would have been measured. Of the chemicals identified in the SPMD and POCIS extracts, few have been reported to give a significant toxic response in the Microtox assay (Kaiser and Palabrica, 1991). PAHs, which are commonly more toxic than the herbicides used in the study areas, were found at very low concentrations in the SPMDs and did not produce a measurable toxic response in this assay.

Yeast Estrogen Screen

The potential for sequestered chemicals to act as an estrogenic mimic was measured by the YES assay. Each sample exhibited a maximal (Type 1) sigmodial dose-response curve, typical of receptor-mediated responses, allowing for the determination of EC_{50} concentrations. The Leary Weber Ditch Field Blank apparently had some unknown contamination that resulted in an EEQ 10 times greater than the other controls. The EEQ values averaged 5.7 ng per sampler, excluding the high Leary Weber Ditch Field Blank (Table 6). Serial dilutions of the each POCIS sample were run in triplicate, with variations ranging from 0.19 to 9.5%; however, most replicates exhibited <2% variation.

In general, EEQ values in the deployed samplers were not significantly greater than POCIS matrix controls. The POCIS extracts were added to the test plates at a concentration four times greater than typical in an attempt to detect any subtle estrogenic response that may have added to the elevated

background response. The positive responses observed in the POCIS matrix blanks are suspected to be the result of residual chemicals from the PES membrane remaining from the PES polymerization process during manufacture. Definitive identification of the causative chemical(s) present in the POCIS matrix has not been achieved.

The lack of a positive estrogenic effect from field-deployed POCIS might have been predicted by the presence of atrazine in extracts from each site. Atrazine and other chloro-*s*-triazine herbicides and metabolites have been shown to possess some antiestrogenic activity (Tran et al., 1996; Oh et al., 2003; Rastall, 2004). Rastall (2004) showed that atrazine was a potent antiestrogen capable of up to 66% inhibition of some estrogen mimics. When antiestrogens are present at sufficiently high concentrations, it is possible that any estrogenic activity due to other chemicals in the mixture may be inhibited to the extent where a positive YES response may not be observed.

Table 6. Screen for estrogenic activity of chemicals sampled by the polar organic chemical integrative sampler (POCIS) using the yeast estrogen screen (YES) assay.

Parameter	EC_{50} E2†	EC_{50} test‡	EEQ§
	M E2	mg POCIS	ng E2/POCIS
Fabrication blank	3.8×10^{-11}	0.40	10
Leary Weber (IN) field blank	5.2×10^{-11}	0.04	130
Morgan Creek (MD) field blank	3.7×10^{-11}	1.2	3.4
DR2 Drain (WA) field blank	4.2×10^{-11}	1.1	4.3
Leary Weber (IN) replicate #1	5.3×10^{-11}	1.2	5.0
Leary Weber (IN) replicate #2	5.0×10^{-11}	1.0	5.3
Morgan Creek (MD) replicate #1	3.2×10^{-11}	0.96	3.6
Morgan Creek (MD) replicate #2	4.9×10^{-11}	0.48	11
DR2 Drain (WA) replicate #1	4.0×10^{-11}	2.6	1.7
DR2 Drain (WA) replicate #2	4.5×10^{-11}	0.72	6.8

† EC_{50} E2: effective median concentration of 17β-estradiol producing 50% of the maximum response for the expression of *lac-Z* reporter gene of the yeast assay.

‡ EC50 test, concentration of sample needed to give a response equivalent to the EC50 E2.

§ EEQ, estradiol equivalent factor. EEQ (ng E2/POCIS) = EC50 E2 (ng E2/mL)/EC50 test (POCIS/mL) where POCIS/mL = % POCIS × 1-mL sample.

Conclusions

This study demonstrates the utility of passive samplers, such as SPMDs and POCIS, to identify and provide the TWA concentration of anthropogenic organic contaminants. This approach provides samples to be used in toxicity assessments representative of the bioavailable fraction of chemicals in watersheds. Using both samplers together allows determinations to be made on the water-soluble and sparingly soluble organic contaminants. Atrazine was the most prevalent of the chemicals identified and was present at each site. Acetochlor and metolachlor were quantified at two of the three sites. Few organochlorine pesticides and PAHs were detected in any of the samples. None of the samples elicited acute toxicity or estrogenic responses.

Acknowledgments

We gratefully acknowledge the funding by the USGS National Water Quality Assessment Program's Agricultural Chemical Team. Special recognition goes to Rajesh Shrestha, a senior chemistry student from Westminster College, Fulton, MO, whose help was invaluable in the processing of the SPMD samples. Also, the help of personnel involved in the deployment, retrieval, shipment, and delivery of these samples to the US Geological Survey's Columbia Environmental Research Center for processing and analysis is greatly appreciated.

References

Alvarez, D.A., J.N. Huckins, J.D. Petty, T.L. Jones-Lepp, F. Stuer-Lauridsen, D.T. Getting, J.P. Goddard, and A. Gravell. 2007. Tool for monitoring hydrophilic contaminants in water: Polar organic chemical integrative sampler (POCIS). p. 171–198. *In* R. Greenwood et al (ed.) Passive sampling techniques. Comprehensive analytical chemistry. Vol. 48. Elsevier, Amsterdam.

Alvarez, D.A., J.D. Petty, J.N. Huckins, T.L. Jones-Lepp, D.T. Getting, J.P. Goddard, and S.E. Manahan. 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23:1640–1648.

Alvarez, D.A., P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg, and M.T. Meyer. 2005. Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. Chemosphere 61:610–622.

Barceló, D., and M. Hennion. 1997. Sampling of polar pesticides from water matrices. Anal. Chim. Acta 338:3–18.

Booij, K., H.E. Hofmans, C.V. Fischer, and E.M. van Weerlee. 2003. Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. Environ. Sci. Technol. 37:361–366.

Daughton, C., and T. Ternes. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environ. Health Perspect. 107:907–938.

DeVita, W.M., and R.L. Crunkilton. 1998. Quality control associated with use of semipermeable membrane devices. p. 237–245. *In* E.E. Little et al. (ed.) Environmental toxicology and risk assessment. Vol. 7. ASTM STP 1333. American Society for Testing and Materials, West Conshocken, PA.

Gilli, G., T. Schiliro, C. Pignata, D. Traversi, E. Carraro, C. Baiocchi, R. Aigotti, D. Giacosa, and E. Fea. 2005. Application of semipermeable membrane device for assessing toxicity in drinking water. Chemosphere 61:1691–1699.

Huckins, J.N., J.D. Petty, and K. Booij. 2006. Monitors of organic chemicals in the environment: Semipermeable membrane devices. Springer, New York.

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

Johnson, B.T., and E.R. Long. 1998. Rapid toxicity assessment of sediments from large estuarine ecosystems: A new tandem in vitro testing approach. Environ. Toxicol. Chem. 17:1099–1106.

Johnson, B.T., J.N. Huckins, J.D. Petty, and R.C. Clark. 2000. Collection and detection of lipophilic chemical contaminants in water, sediment, soil, and air–SPMD-TOX. Environ. Toxicol. 15:248–252.

Jones-Lepp, T.L., D.A. Alvarez, J.D. Petty, and J.N. Huckins. 2004. Polar organic chemical integrative sampling (POCIS) and LC-ES/ITMS for assessing selected prescription and illicit drugs in treated sewage effluent. Arch. Environ. Contam. Toxicol. 47:427–439.

Kaiser, K.L.E., and V.S. Palabrica. 1991. *Photobacterium phosphoreum* toxicity data index. Water Pollut. Res. J. Can. 26:361–431.

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

Lebo, J.A., F.V. Almeida, W.L. Cranor, J.D. Petty, J.N. Huckins, A.C. Rastall,

D.A. Alvarez, B.B. Mogensen, and B.T. Johnson. 2004. Purification of triolein for use in semipermeable membrane devices (SPMDs). Chemosphere 54:1217–1224.

Matthiessen, P., D. Arnold, A.C. Johnson, T.J. Pepper, T.G. Pottinger, and K.G.T. Pulman. 2006. Contamination of headwater streams in the United Kingdom by oestrogenic hormones from livestock farms. Sci. Total Environ. 367:616–630.

Oh, S.M., S.H. Shim, and K.H. Chung. 2003. Antiestrogenic action of atrazine and its major metabolites in vitro. J. Health Sci. 49:65–71.

Petty, J.D., J.N. Huckins, D.A. Alvarez, W.G. Brumbaugh, W.L. Cranor, R.W. Gale, A.C. Rastall, T.L. Jones-Lepp, T.J. Leiker, C.E. Rostad, and E.T. Furlong. 2004. A holistic passive integrative sampling approach for assessing the presence and potential impacts of waterborne environmental contaminants. Chemosphere 54:695–705.

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

Rastall, A.C. 2004. The development of a biomimetic approach to the detection and identification of anthropogenic estrogen receptor agonists in surface waters. Ph.D. diss. The Open Univ., United Kingdom.

Rastall, A.C., A. Neziri, Z. Vukonvic, C. Jung, S. Mijovic, H. Hollert, S. Nikcevic, and L. Erdinger. 2004. The identification of readily bioavailable pollutants in Lake Shkodra/Skadar using semipermeable membrane devices (SPMDs), bioassays and chemical analysis. Environ. Sci. Pollut. Res. 11:240–253.

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

Strategic Diagnostics, Inc. 2003. Method for measuring the acute toxicity of wastewater and receiving water with the *Vibrio fischeri* (NRRL B-11177) Microtox test system (Strategic Diagnostics Microtox 1010). EPA document no.: EPA-HQ-OW-2003-0070-0225. Strategic Diagnostics, Inc., Newark, DE.

Tran, D.Q., K.Y. Kow, J.A. McLachlan, and S.F. Arnold. 1996. The inhibition of estrogen receptor mediated responses by chloro-*s*-triazine-derived compounds is dependent on estradiol concentration in yeast. Biochem. Biophys. Res. Commun. 227:140–146.

U.S. Geological Survey. 2003a. Morgan Creek Watershed Selected for a National Water-Quality Study. Fact Sheet 080-03. August. USGS, Washington, DC.

U.S. Geological Survey. 2003b. Granger Drain Subbasin Selected for a National Water-Quality Study. Fact Sheet 082-03. August. USGS, Washington, DC.

U.S. Geological Survey. 2003c. Sugar Creek Watershed Selected for a National Water-Quality Study. Fact Sheet 084-03. August. USGS, Washington, DC.

Vermeirssen, E.L., O. Körner, R. Schönenberger, M. Suter, and P. Burkhardt-Holm. 2005. Characterization of environmental estrogens in river water using a three pronged approach: Active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish. Environ. Sci. Technol. 39:8191–8198.

Vogel, J.R., M.S. Majewski, and P.D. Capel. 2008. Pesticides in four agricultural watersheds in the United States. J. Environ. Qual. 37:1101–1115.