INVESTIGATION OF AN INVERTEBRATE KILL IN THE RIVER WEY AT BORDON – METHOD DEVELOPMENT AND FIELD VALIDATION OF THE POCIS FOR THE INTEGRATIVE SAMPLING OF CROTAMITON

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EXECUTIVE SUMMARY

The work was conducted as part of a continuing collaborative effort between the U.S. Geological Survey's Columbia Environmental Research Center (CERC) and the Environment Agency (EA) of England and Wales to further develop the polar organic chemical integrative sampler (POCIS). The POCIS passively samples the bioavailable fraction of the more hydrophilic organic contaminants (i.e., pesticides, pharmaceuticals, natural hormones, etc.) in the water column. The POCIS were fabricated at CERC and deployed in the River Wey below the Bordon sewage treatment works in the United Kingdom by EA researchers. The POCIS were returned to CERC for processing and analysis of crotamiton, diuron, isoproturon, estriol, 17β-estradiol, 17α-ethynylestradiol, and estrone. Quantifiable levels of crotamiton and diuron were found in the POCIS. Concentrations of crotamiton averaged 72 ng/POCIS and diuron was detected in one sampler at 27 ng/POCIS. Isoproturon and the estrogens were not detected.

INTRODUCTION

Anthropogenic activities continue to adversely effect ecosystems by both point and non-point source pollution inputs. In addition to the contaminants of historic concern (e.g., organochlorine pesticides [OCs], polychlorinated biphenyls [PCBs], polycyclic aromatic hydrocarbons [PAHs], etc.), emerging contaminant issues are increasingly being recognized as sources of adverse effects on human health and ecosystem quality. These "new" contaminants include pharmaceutically related chemicals such as natural and synthetic hormones, certain industrial chemicals (e.g., surfactants and biocides), and current use pesticides. Assessment of the potential effects of exposure to the complex mixture of chemicals present in the environment is problematic and requires innovative approaches.

The Environment Agency of England and Wales is a leader in conducting research designed to define the presence and potential effects associated with pollution of aquatic systems in Great Britain. Historically, the majority of water sampling techniques used were based on the measurement of chemicals present in a limited volume of water (i.e., ≤ 5L) at a single point in time, and consequently do not provide an integrated assessment of exposure or sufficient contaminant mass for determining the presence of trace, yet toxicologically relevant, levels of waterborne contaminants.

As the basis for a holistic approach for assessing exposure to environmental contaminants, scientists at the USGS's Columbia Environmental Research Center

(CERC) have developed a variety of integrative samplers designed to determine the ecological relevance of organism exposure to complex mixtures of waterborne and airborne contaminants. Two of these integrative samplers, the semipermeable membrane device (SPMD) and the polar organic chemical integrative sampler (POCIS) are applicable to sampling hydrophobic and hydrophilic waterborne contaminants, respectively. SPMDs have been shown to be of great utility for monitoring the presence of trace to ultra-trace levels of bioavailable neutral organic chemicals (1, 2, 3, 4, 5). SPMDs sample only the dissolved (readily bioavailable) portion of waterborne chemicals on which water quality criteria are based. The SPMD samplers function as an abiotic physical model of the bioconcentration process by which aquatic organisms concentrate, via respiration, dissolved lipophilic chemicals in water.

The POCIS is a newly developed integrative sampler designed to sequester polar organic chemicals from aquatic systems (6, 7, 8). The POCIS is constructed by enclosing a sorbent in a hydrophilic membrane. Waterborne polar organic chemicals diffuse through the hydrophilic membrane and are subsequently retained by the sorbent. As with the SPMD, the POCIS samples only the readily bioavailable portion of the polar organic contaminants and not that portion associated with particulate matter. As a corollary to the SPMD, the POCIS represents the abiotic physical model of organism exposure process for more hydrophilic organic contaminants. The use of an integrative sampler such as POCIS is particularly important, because polar organic contaminants rapidly dissipate, increasing the probability that episodic events are undetected using conventional methods.

The Environment Agency has a particular interest in enhancing its ability to determine, in an integrated manner, the presence of polar organic contaminants. As an integral part of the development and field validation of the POCIS device, scientists at the CERC and the Environment Agency conducted an exploratory project incorporating the deployment of these samplers in the River Wey below the Bordon sewage treatment works (STW). This site was of immediate interest due to an unexplained invertebrate kill in the area. Analysis of water samples by EA laboratories identified the chemical, crotamiton, present in the water at approximately 2 ppb (9). Crotamiton (crotonyl-N-ethyl-o-toluidine) is commonly used as a scabicide, insecticide and an antipruritic agent (10). Review of the literature revealed methods of analysis for crotamiton in creams, ointments, plasma and urine (10, 11, 12). The lack of information on the environmental presence of crotamiton initiated the method development work as described in the appendix of this report. Presented herein are the results of the analysis of these field deployed POCIS and SPMD devices.

EXPERIMENTAL

Materials: Polyethersulfone membrane (pore size – 0.1 μm) was purchased from Pall Gelman Sciences, Inc. (Ann Arbor, MI). Isolute® ENV+ solid exchange resin was purchased from Jones Chromatography (Lakewood, CA). Ambersorb® 1500 was obtained from Rohm and Haas (Philadelphia, PA). S-X3 Bio-Beads (200-400 mesh)

were purchased from Bio-Rad Laboratories (Hercules, CA). The Oasis HLB sorbent was supplied by Waters Corp. (Milford, MA). The stainless steel materials used in construction of POCIS were purchased from McMaster-Carr (Elmhurst, IL). Aluminum washers comprising the POCIS housing were manufactured by Phoenix Speciality Mfg. Co. Inc. (Bamberg, SC). PVC (schedule 40) used in the construction of the deployment canisters were obtained from local hardware stores. Figure 1 depicts the deployment canister and sampler array.LDPE layflat tubing was purchased from Environmental Sampling Technologies, St. Joseph, MO. The tubing was a 2.54 cm wide, no. 940, untreated (pure PE; no slip additives, antioxidants, etc.) clear tubing. The wall thickness of this lot ranged from 84 to 89 μm. Triolein (1,2,3-tri-[cis-9-octadecenoyl]glycerol) was obtained from NuChek Prep, Inc., Elysain, MN and was ≥ 99% pure. All organic solvents were Optima grade from Fisher Scientific.

POCIS Preparation: Construction of the POCIS involves placing a cleaned membrane disk on one half of the support ring system and weighing 200 mg of the sorbent (either an admixture of 40 mg S-X3 dispersed A-1500 and 160 mg Isolute ENV+ or 200 mg of the Oasis HLB sorbent) onto the center of this membrane. The sorbent was spread evenly about the inside of the disk and subsequently covered with the second membrane disk. The remaining half of the support ring was positioned and fastened together with thumb screws and wing nuts. After assembly, POCIS are mounted to a support rod and secured inside the deployment canister. Each canister housed 3 POCIS with the Oasis HLB and 3 POCIS containing the sorbent admixture.

SPMD Preparation: The SPMDs used in this project were constructed using 0.14 mL (0.13 g) aliquots of high purity triolein enclosed as a thin film in 13 cm lengths of LDPE tubing. Note that the design of the standard or commercially available SPMD (5) consists of a specified length (typically about 90 cm [distance between the inter molecular welds or the length of the triolein containing portion]) of 2.5 cm wide layflat LDPE tubing (additive free, wall thickness 70-95 μm), containing ≥ 99% purity triolein (1 mL used for the 91.4 cm length). After heat-sealing the triolein inside the LDPE tube, the resulting SA-V (membrane surface area to total SPMD volume) ratio is ≈ 90 cm^2/mL or ≈ 460 cm^2/mL of triolein, and the device consists of ≈ 20% triolein. For the 1 mL triolein configuration, the whole device typically weighs about 4.4 to 4.6 g. In our case, the area of the active exchanging or sampling surface of the finished device was approximately 65 cm^2. The SPMDs attached to each deployment device and in the Field Blanks were spiked with 8 μg of acenaphthylene-d_8, acenaphthene-d_{10}, fluorene-d_{10}, phenanthrere-d_{10}, and pyrene-d_{10} which are permeability/performance reference compounds (PRCs, see subsequent section on PRCs). Each SPMD was loaded into a small single SPMD deployment unit. The units were then placed into labeled, solvent rinsed, gas-tight cans. Afterwards, the cans were flushed with argon and sealed. These cans were placed in coolers and then shipped overnight along with the POCIS to the Environment Agency of England and Wales for deployment near the Bordon STW system.

Permeability/Performance Reference Compounds: PRCs are analytically non-interfering organic compounds with moderate to high fugacity from SPMDs that are added to the

lipid prior to membrane enclosure and field deployment (5). The functional basis of the PRC approach has been previously described in detail by Huckins et al. (13). In our study perdeuterated phenanthrene was used as the PRC. The rate constants for PRC dissipation from SPMDs at each sample site were determined and compared to PRC dissipation rate constants measured during laboratory calibration studies of target compounds. This method permitted the calculation of an exposure adjustment factor (EAF). Using the EAF ratios, calibration data (i.e., SPMD uptake rate constants for analytes of interest) were adjusted to more accurately reflect actual *in situ* sampling rates. As suggested earlier, the effects of exposure conditions on SPMD uptake and dissipation rates are largely a function of 1) exposure medium temperature, 2) facial velocity-turbulence at the membrane surface, 3) membrane biofouling, and 4) the design of the deployment apparatus (i.e., baffling of media flow-turbulence). Based on our PRC research (13), the use of EAFs should permit the estimation of analyte water concentrations within ± 75 % of the actual time weighted average values.

POCIS sampling rates are similarly affected by surrounding water conditions. The use of PRC-SPMDs allows the estimation of the effects of site conditions on relative sampling rates during deployment. This assessment is critical to the proper selection of sampling rate data for the estimation of ambient water concentrations.

Passive Samplers Deployment and Retrieval:

The samplers were deployed and retrieved by Jon Goddard and other scientists of the Environment Agency following CERC and EA protocols.

POCIS and SPMD Storage and Custody: Following receipt of the samplers at CERC and prior to processing, the SPMDs and POCIS were stored in a laboratory freezer at -15°C.

Sample Processing and Residue Enrichment: Sample processing was similar to procedures previously described (5, 8), with specific details noted in the following sections.

SPMD Membrane Cleaning: The exterior membrane surfaces of exposed SPMDs were cleaned prior to dialysis. This cleaning procedure was applied to all SPMDs received from the field as well as the quality assurance/quality control (QA/QC) SPMDs generated in conjunction with analysis sets. The steps associated with membrane cleaning were applied to each SPMD individually and sequentially, and were as follows. Sealed cans with SPMDs were opened and the SPMDs were removed and momentarily immersed (< 30 sec) in 100 mL of hexane. Afterwards, the hexane was discarded. Then the SPMDs were individually placed into a large flat stainless steel pan and washed thoroughly using running tap water and a clean brush to remove all remaining surface adhering material. Any SPMD tether loops outside the lipid containment seals were cut away and discarded. Next, the water was drained from the exterior of each SPMD. The SPMDs were then separately immersed in a glass tank containing 1 N HCl for a period of approximately 30 seconds. Subsequently, they were rinsed with tap water to remove the acid. Afterwards, all surface water was removed from individual SPMDs by using successive rinses of acetone followed by isopropanol.

SPMD Dialysis (i.e., Recovery of Analytes): Glass canning jars (one pint) with solvent-rinsed aluminum foil under the lid were used for the dialysis step. Each SPMD was submersed in 75 mL of hexane in separate jars and were dialyzed individually at 18 °C for 18 hours. The hexane was removed and transferred into an evaporation flask. A second volume of 75 mL of hexane was added to each sample jar and the SPMDs were dialyzed for an additional 4 hours at 18 °C. The second dialysate was transferred into the flask containing the first dialysate. At this point analyte recovery is complete and the SPMDs were discarded. The combined dialysates from each SPMD were reduced to a volume of 3 to 5 mL on a rotary evaporation system, and quantitatively transferred through a pre-rinsed glass fiber filter into appropriately labeled test tubes. The solvent volume was then reduced to about 1.0 mL, using high purity nitrogen.

SEC Cleanup: A Perkin-Elmer Series 410 HPLC (Perkin-Elmer, Inc., Norwalk, CN) was employed as the solvent delivery system for size exclusion chromatography (SEC) cleanup of samples. This HPLC unit was equipped with a Perkin-Elmer ISS-200 auto sampler. The SEC column was a 300-mm x 21.2-mm I.D. (10-µm particle size, 100 Å pore size) Phenogel column (Phenomenex, Inc., Torrance, CA), equipped with a 50-mm x 7.5-mm i.d. Phenogel guard column. The isocratic mobile phase was 98:2 (V:V) dichloromethane:methanol (DCM:MeOH) and the flow rate was set at 4.0 mL per minute. The SEC system was equipped with an ISCO Foxy® 200 (ISCO, Inc., Lincoln, NE) fraction collector connected to the output end of the SEC column.

The SEC system was calibrated on a daily basis by the injection of a solution of compounds representative of the analytes of interest and potentially interfering materials. The substances contained in this calibration solution, in sequence of elution, are diethylhexylphthalate (DEHP; a common plasticizer with lipid-like chromatographic behavior), biphenyl and naphthalene (small aromatic analytes), coronene (a large PAH later eluting than any anticipated analyte), and elemental sulfur (a problematic interference frequently encountered in environmental samples). Elution of these components from the SEC column was monitored by a UV detector (254 nm) and a strip chart recorder.

SEC cleanup was accomplished using a collect fraction (i.e., window in which target analytes elute) determined by the calibration of the system on the day of operation. The collect fraction was initiated at the point 70% of the time between the apex of the DEHP chromatographic peak and the apex of the biphenyl chromatographic peak and terminated at 70% of the time between the apex of the coronene chromatographic peak and the apex of the sulfur chromatographic peak. The fractions collected were amended with approximately 2 mL of isooctane, reduced to a volume of about 1 mL on a rotary evaporation system, and quantitatively transferred with hexane into appropriately labeled test tubes. Measurement of the PAHs and the PRCs in the SPMDs required concentration of the diluted samples to 1 mL to satisfy detection and quantitation limitations of the instrument.

Gas Chromatographic Analysis of PRC-PAH Fractions: Analysis of PAHs and PRCs were conducted on an Agilent 6890 GC equipped with an Agilent 7683 autosampler (Agilent Technologies, Inc., Wilmington, DE). In all analyses, 1.0 µL of sample extract was injected using the "cool-on-column" technique with helium as the carrier gas. An HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) capillary column (Agilent Technologies, Inc., Wilmington, DE) was used with the following temperature program: injection at 50 °C, held for 2 min, then ramped at 25 °C/min to 130 °C, held for 1 min, followed by 6 °C/min ramp to 310 °C and held at 310 °C for 5 min. Detection was performed with a 5973 mass selective detector (Agilent Technologies, Inc., Palo Alto, CA) in the selected ion mode (SIM). (See following table for selected ions). Detector zone temperatures were set at 310 °C for the MSD transfer line, 150 °C at the quadrapole, and 230 °C at the source. Quantitation of the analytes was accomplished using a six-point curve with internal calibration. Calibration standard concentrations were 0.02, 0.05, 0.10, 0.50, 1.0, 2.0, and 4.0 µg/mL for each of the analytes with the internal standard, *p*-Terphenyl-d_{14}, maintained at 1 µg/mL. SIM parameters for identification are given in the Table I.

POCIS Cleaning and Extraction (i.e., Recovery of Analytes): Each POCIS was removed from its deployment canister and rinsed with water to remove any debris. The contents of the POCIS were then transferred with methanol into 1 cm (i.d.) glass chromatography columns fitted with a glass wool plug. Solvent extraction (elution) of sorbed analytes was achieved with the addition of 50 mL of 1:1:8 methanol:toluene:dichloromethane to the sorbent admixture POCIS and 40 mL of methanol for elution from the Oasis POCIS. The collected admixture eluates were evaporated by rotary evaporation to 2-3 mL, 20 mL

of methanol was added to the flask and evaporated again to approximately 1 mL. The additional methanol was necessary to form an azeotrope to facilitate the removal of the toluene. The Oasis eluates were similarly processed without the additional methanol azeotrope step. The samples were then quantitatively transferred through a pre-rinsed glass fiber filter into appropriately labeled test tubes with acetone and subsequently evaporated under N_2 to 0.5 mL. The samples were composited into 3 POCIS equivalent samples resulting in one Oasis and one sorbent admixture composites for the site. Following compositing, 1/3 of each sample type was removed and ampulated as an unadulterated sample. These ampulated samples were shipped to Jon Goddard for subsequent analysis for unknowns by EA scientists.

POCIS Sample Enrichment and Analysis: SEC fractionation was the first cleanup step employed for the POCIS samples. SEC operation was as previously described with the exception that the collect window was initiated at 30% of the way between the apex of the DEHP and biphenyl peaks. The earlier collect time is necessary to recover the more polar analytes of interest. Each sample was split into two injections to minimize the mass of coextracted compounds introduced on column to reduce the potential of overloading the SEC column. Following SEC, the samples were subjected to further cleanup by use of Oasis HLB SPE cartridges. Refer to the Method Validation section in the Appendix for a detailed description of the Oasis SPE technique. The samples were transferred into 10 mL of acetonitrile after the SPE cleanup. Half (5 mL) of each sample was removed, evaporated under N_2 and analyzed by HPLC. The remaining portions were evaporated under N_2 to dryness and redissolved in 0.5 mL dichloromethane. The portions in

dichloromethane were then fractionated on KS columns (see method validation section in Appendix for description). The KS-1 fraction contained crotamiton, diuron and isoproturon, and the KS-2 fraction contained the estrogens. Each fraction was evaporated to 1-2 mL by rotary evaporation then transferred with acetone into labeled HPLC sample vials, taken to dryness under N_2, and redissolved in 500 µL of 1:1 water:acetonitrile. The samples were then analyzed by HPLC-UV detection.

HPLC System and Conditions: The HPLC system consists of a Hewlett Packard Series II 1090 Liquid Chromatograph with a diode array detector (Hewlett Packard, Palo Alto, CA) with the ChemStation for LC software package revision A.08.03 (Agilent Technologies, Inc., Palo Alto, CA). A Phenomenex Luna C_{18} analytical column (150 x 4.6 mm, 5 µm d_p) and a Phenomenex Security Guard C_{18} cartridge were maintained at 30°C. An injection volume of 50 µL and a mobile phase of 55:45 water:ACN with a flow rate of 1 mL/min were used during analysis of the phenyl ureas and estrogens. Detection of the estrogens occurred at 281 nm, diuron and isoproturon were at 254 and 242 nm, respectively. Multi-point calibration curves (0.20, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 ng/µL of each estrogen; 0.20, 0.50, 1.0, 2.0, 3.0, and 4.0 ng/µL of diuron and isoproturon) were run with each sample set. Analysis of crotamiton required the use of a 55:45 0.01 M potassium phosphate monobasic (KH_2PO_4):acetonitrile mobile phase. The calibration curve of 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 ng/µL of crotamiton was used with detection at 220 nm. The analytical standard of crotamition consists of the trans and cis stereoisomers in the approximate ratio of 94:6 trans:cis. All determinations were based on the trans isomer due to its predominance.

RESULTS AND DISCUSSION

Quality Control: One field blank SPMD and one field blank POCIS accompanied the samplers during deployment, retrieval, and transportation to CERC. These field blanks were processed and analyzed exactly as the deployed samples. The field blank samples exhibited no coincident HPLC or GC-MSD peaks at levels significantly higher than those associated with the laboratory control blanks and were indicative of successful deployments and retrievals. The method detection limit (MDL) and method quantitation limit (MQL) for analysis of SPMD and POCIS samples were determined for each analyte by measuring the values of coincident GC-MSD or HPLC peaks for each compound in all blank samples processed with this study. The MDL was defined as the mean plus three standard deviations of values so determined (14). The MQL was defined as the mean plus 10 standard deviations of values so determined (14). For individual analytes having no coincident peak, an assumed value equal to the low sample reject for the analytical method was used to calculate the mean. In the cases where the MQLs were below the level of the calibration curve employed in the instrumental analysis, the MQLs were set at the value of the lowest level of the calibration curve employed in quantifying the analyte levels. The MDLs and MQLs for analysis of the study samples for each of the analytes are presented in Table II.

Observations and Findings: During the analysis of study sample fractions, conditions were optimized to give sufficient resolution for quantitation of the targeted analytes (Table III). The results of the analysis of the deployment samples are given for all

targeted analytes (Table IV). Figures II-V are representative chromatograms for standards, field blanks, and select deployed POCIS and SPMDs.

Derivation of Water Concentrations from SPMD Levels (Modeling): SPMD uptake kinetic data are required to accurately estimate aquatic concentrations of environmental contaminants. Using models previously developed (2, 5, 13), data from the analysis of the PRC levels, and from calibration studies, the bioavailable (i.e., dissolved phase) concentrations of analytes in SPMDs exposed to River Wey at Bordon waters can be estimated. For compounds with log K_{ow} values ≥ 5.0, sampling is integrative and reported water concentration values represent a time weighted average of residue concentration (i.e., residues were accumulated in an additive manner throughout the exposure with no significant losses of analytes) during the 30 day exposure period. For compounds with log K_{ow}s < 5.0, sampling is not integrative throughout the whole exposure period and reported water concentrations represent a portion of the 30 day exposure. Regardless of these considerations, water concentrations can be determined by using different assumptions and models (5).

An example of the overall modeling procedure for compounds with log K_{ow}s ≥ 5.0 is as follows. Note that Huckins et al. (5) have also described modeling procedures for water concentration estimates of compounds with log K_{ow}s < 5.0. The analyte sampling rate (R_{sw}) is determined from laboratory exposures conducted under some of the same conditions (i.e., water temperature and exposure duration) as the current study. The

linear or integrative uptake of OCs by SPMDs has been described by Huckins, et al. (5) as follows:

$$C_{SPMD} = C_w k_o K_{mw} A t / V_{SPMD} \quad (1)$$

substituting R_{sw} for $k_o K_{mw} A$ in equation 1 gives

$$C_{SPMD} = C_w R_{sw} t / V_{SPMD} \quad (2)$$

where C_{SPMD} is the concentration of the analyte in the whole SPMD (i.e., the membrane + lipid), C_w is the concentration of the analyte in the water, t is the exposure time in days, and V_{SPMD} is the volume of the SPMD. Rearranging equation 2 results in

$$C_w = C_{SPMD} V_{SPMD} / R_{sw} t \quad (3)$$

In the present case we use the uptake rate constant (k_{uw}) defined as L/d g (liter/day·gram) of SPMD (membrane + lipid).

$$C_w = C_{SPMD} / (R_{sw} / M_{SPMD}) t = C_w = C_{SPMD} / k_{uw} t \quad (4)$$

where M_{SPMD} is the mass of the SPMD and is substituted for V_{SPMD}.

SPMD sampling rates can change due to changes in temperature, turbulence/facial velocity of water at the membrane surface, and buildup of periphyton on the membrane surface. To account for changes in these variables from the laboratory calibration studies, PRCs are used to allow estimation of actual *in situ* sampling rates. The PRC concept was described earlier. However, models to enable the use of a PRC were not. Measuring the loss of a PRC over a study exposure period provides in situ k_e values which when compared to the calibration k_e values can serve as an indicator of differences in the environmental conditions. If large differences exist between the k_e calibration and

exposure values, adjustments must be made to the laboratory calibration data (Table V). The k_{eprc} values are derived as follows

$$C_{SPMD} = C_{SPMDo} \exp(-k_{eprc} t) \quad (5)$$

$$k_{eprc} = \ln (C_{SPMDo}/C_{SPMD})/t \quad (6)$$

where C_{SPMDo} is the day 0 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 the field-exposed SPMDs (Equations 7 or 8), to the k_e values of the PRCs measured in SPMD calibration exposures (i.e., k_{eprc} / k_{ec}), provides an estimate of the relative effect of environmental variables on SPMD sampling. Laboratory k_{ec} values of PRCs are determined by direct measurement or by

$$k_{ec} = R_s/K_{SPMD} V_{SPMD} d_{SPMD} \quad (7)$$

where K_{SPMD} is the equilibrium SPMD-water partition coefficient and d_{SPMD} is the SPMD density (g/mL), which is 0.91. Estimates of in situ R_s values from the k_{ec}s of PRCs can be made with the following relationship

$$R_{sf} = (k_{eprc}/k_{ec}) R_{sc} \quad (8)$$

These models and assumptions as well as others have been incorporated into an Excel spreadsheet (5) for rapid estimation of water concentrations and the Excel calculator was used for water concentration estimates in this work (Table V). Note that an average temperature of 18 °C was assumed for POCIS and SPMD exposure at all sample sites.

SUMMARY

This exploratory investigation into the cause of an invertebrate kill in the River Wey below the Bordon STW was part of a continuing collaboration between the U.S. Geological Survey and the Environment Agency of England and Wales serving to further the development and field validation of the POCIS for the integrative sampling of hydrophilic organic chemicals. Crotamiton, which had previously been identified in water samples at levels of 2 ppb by EA laboratories, was present in the POCIS with an average concentration of 72 ng/POCIS (Table IV). Diuron was identified in one POCIS at a level of 27 ng/POCIS (Table IV). Isoproturon and the estrogens, 17β-Estradiol, 17α-Ethynylestradiol and Estrone were not found in any of the sampler extracts. A GC-MSD scan for various pesticides/herbicides along with a general unknown scan failed to identify any compounds of interest.

The SPMD, while primarily present as a PRC sampler, was screened for priority pollutant PAHs. Several of the PAHs, most notably fluorene, phenanthrene, fluoranthene, pyrene, and chrysene, were at quantifiable levels (Table IV). Because both fluoranthene and pyrene undergo photolysis to highly toxic degradation products, their estimated water concentrations (Table VI) are sufficiently high to be of concern.

While the estimated water concentrations are generally low, parts per trillion, or not possible due to the lack of calibration data, they are indicative of organism exposures to

complex mixtures of contaminants. Further, the long term effects of such exposure are not known, but deserve additional assessment.

Clearly, additional information is needed before the POCIS is ready for routine use. Calibration data, sorption kinetics, optimal configurations, etc. are just some of the factors undergoing intense scrutiny in the laboratory. Continued collaboration with our partners is vital to the evolution of this technology.

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Table I

SIM parameters used for identification of PAHs

Compound	Target Ion		Qualifier Ion 1		Qualifier Ion 2	
	Mass	Dwell (msec)	Mass	Dwell (msec)	Mass	Dwell (msec)
Naphthalene	128	75	127	75		
Acenaphthylene-d_8	160	50	158	50		
Acenaphthylene	152	50	151	50		
Acenaphthene-d_{10}	164	50	164	50		
Acenaphthene	154	50	153	50		
Fluorene-d_{10}	176	65	174	65		
Fluorene	166	65	165	65		
Phenanthrene-d_{10}	188	65	189	65		
Phenanthene	178	65	176	65		
Anthracene	178	65	176	65		
Fluoranthene	202	65	200	65		
Pyrene-d_{10}	212	65	211	65		
Pyrene	202	65	200	65		
p-Terphenyl-d_{14}	244	75	243	75		
Benz[a]anthracene	228	100	226	100		
Chrysene	228	100	226	100		
Benzo[b]fluoranthene	252	100	250	100	253	100
Benzo[k]fluoranthene	252	100	250	100	253	100
Benzo[a]pyrene	252	100	250	100	253	100
Indeno[1,2,3-cd]pyrene	276	100	277	100	278	100
Dibenz[a,h]anthracene	278	100	276	100	277	100
Benzo[g,h,i]perylene	276	100	277	100	278	100

Note: Agilent 6890 series gas chromatograph (GC) equipped with a HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) capillary column (Agilent Technologies, Inc., Wilmington, DE) with the following temperature program: injection at 50 °C, held for 2 min, then 25 °C/min to 130 °C, held for 1 min, followed by 6 °C/min to 310 °C and held at 310 °C for 5 min.

Table II

Background, MDL, & MQL Values for Targeted Analytes

Analyte	MDL ng injected*	MQL ng injected
Crotamiton	8.8	13
Diuron	2.0	10
Isoproturon	1.3	10
Estriol	5.0	25
17β-Estradiol	5.0	25
17α-Ethynylestradiol	5.0	25
Estrone	5.0	25

* ng of analyte injected on the HPLC column

Analyte	MDL µg/SPMD	MQL µg/SPMD
Naphthalene	0.01	0.02
Acenaphthylene-d_8	0.01	0.02
Acenaphthylene	0.01	0.02
Acenaphthene-d_{10}	0.01	0.02
Acenaphthene	0.01	0.02
Fluorene-d_{10}	0.01	0.02
Fluorene	0.01	0.02
Phenanthrene-d_{10}	0.01	0.02
Phenanthene	0.01	0.02
Anthracene	0.01	0.02
Fluoranthene	0.01	0.04
Pyrene-d_{10}	0.02	0.05
Pyrene	0.01	0.02
Benz[a]anthracene	0.12	0.30
Chrysene	0.02	0.04
Benzo[b]fluoranthene	0.01	0.02
Benzo[k]fluoranthene	0.01	0.02
Benzo[a]pyrene	0.07	0.21
Indeno[1,2,3-cd]pyrene	0.09	0.09
Dibenz[a,h]anthracene	0.10	0.10
Benzo[g,h,I]perylene	0.05	0.05

Table III

Elution Order of Targeted Analytes During Analysis*

POCIS Analytes (column C_{18})	Retention Time Min.	SPMD Analytes (column HP-5MS)	Retention Time Min.
Crotamiton[a]	8.51	Naphthalene	6.74
		Acenaphthylene-d_8	10.20
Isoproturon[b]	6.31	Acenaphthylene	10.23
Diuron[b]	6.64	Acenaphthene-d_{10}	10.70
Estriol[b]	2.25	Acenaphthene	10.78
17β-Estradiol[b]	6.32	Fluorene-d_{10}	12.28
17α-Ethynylestradiol[b]	8.49	Fluorene	12.36
Estrone[b]	9.57	Phenanthrene-d_{10}	15.55
		Phenanthene	15.63
		Anthracene	15.79
		Fluoranthene	20.08
		Pyrene-d_{10}	20.81
		Pyrene	20.87
		p-Terphenyl-d_{14}	21.92
		Benz[a]anthracene	25.60
		Chrysene	25.74
		Benzo[b]fluoranthene	29.54
		Benzo[k]fluoranthene	29.63
		Benzo[a]pyrene	30.57
		Indeno[1,2,3-cd]pyrene	34.02
		Dibenz[a,h]anthracene	34.16
		Benzo[g,h,i]perylene	34.69

[a] HPLC method using 55:45 0.01 M KH_2PO_4:acetonitrile as the mobile phase.
[b] HPLC method using 55:45 water:acetonitrile as the mobile phase.

* NOTE: Slight variations in retention times were noted on a run by run basis.

Table IV

Bordon STW POCIS

Analyte	Sorbent Admixture ng/POCIS	Oasis ng/POCIS
Crotamiton (GPC/Oasis)	85	81
Crotamiton (GPC/Oasis/KS)	68	76
Diuron	<MDL	27
Isoproturon	<MDL	<MDL
Estriol	<MDL	<MDL
17β-Estradiol	<MDL	<MDL
17α-Ethynylestradiol	<MDL	<MDL
Estrone	<MDL	<MDL

Analyte	µg/SPMD
Naphthalene	<MDL
Acenaphthylene	<MDL
Acenaphthene	0.06
Fluorene	0.05
Phenanthrene	0.10
Anthracene	<MDL
Fluoranthene	0.21
Pyrene	0.24
Benz[a]anthracene	0.02
Chrysene	0.14
Benzo[b]fluoranthene	0.03
Benzo[k]fluoranthene	0.03
Benzo[a]pyrene	<MDL
Indeno[1,2,3-cd]pyrene	<MDL
Dibenz[a,h]anthracene	<MDL
Benzo[g,h,i]perylene	<MDL

Table V

Permeability Reference Compound Recovery

QA/QC Sample	µg PRC
Acenaphthylene-d_8	4.49*
Acenaphthene-d_{10}	4.90*
Fluorene-d_{10}	5.03*
Phenanthrene-d_{10}	4.92*
Pyrene-d_{10}	3.71*

Exposure Site	µg PRC
Acenaphthylene-d_8	0.31**
Acenaphthene-d_{10}	0.56**
Fluorene-d_{10}	0.69**
Phenanthrene-d_{10}	1.46**
Pyrene-d_{10}	3.32**

Exposure Site	k_{eprc} (d^{-1})
Acenaphthylene-d_8	0.089
Acenaphthene-d_{10}	0.072
Fluorene-d_{10}	0.066
Phenanthrene-d_{10}	0.040
Pyrene-d_{10}	0.0037

$$k_{eprc} = \frac{\ln(C_{SPMDo} / C_{SPMD})}{t}$$

* C_{SPMDo}

** C_{SPMD}

Table VI

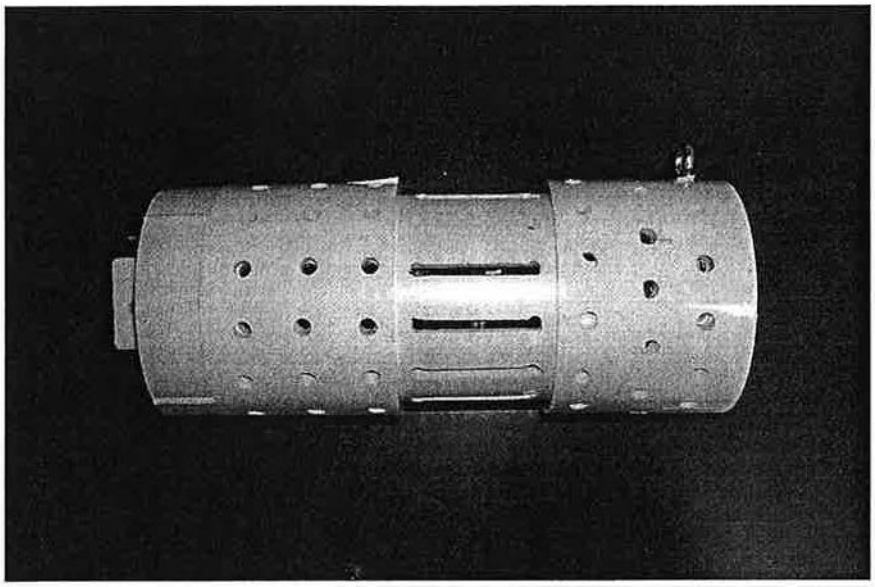
Estimated* Aqueous Concentrations of PAHs in Deployed SPMDs

Contaminant	ng/L
Naphthalene	ND
Acenaphthylene	ND
Acenaphthene	8.1
Fluorene	4.9
Phenanthrene	8.3
Anthracene	ND
Fluoranthene	4.6
Pyrene	4.5
Benz[a]anthracene	ND
Chrysene	1.7
Benzo[b]fluoranthene	0.2
Benzo[k]fluoranthene	0.1
Benzo[a]pyrene	ND
Indeno[1,2,3-cd]pyrene	ND
Dibenz[a,h]anthracene	ND
Benzo[g,h,i]perylene	ND

* Estimated to two significant figures using extrapolated value for PRC corrected R_s, where "ND" = Not Determined. ND values indicate concentrations of sequestered contaminants below quantification limits. The PRC phenanthrene-d_{10} was used for the R_s adjustments.

Figure I

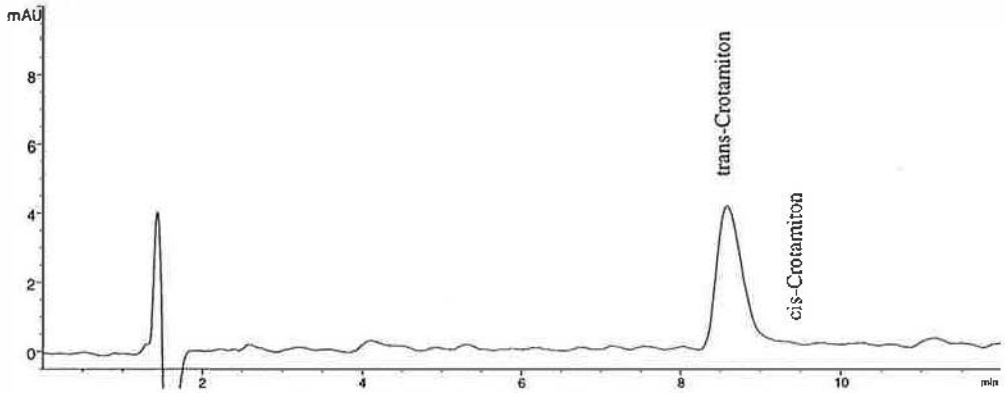
POCIS Deployment Configurations



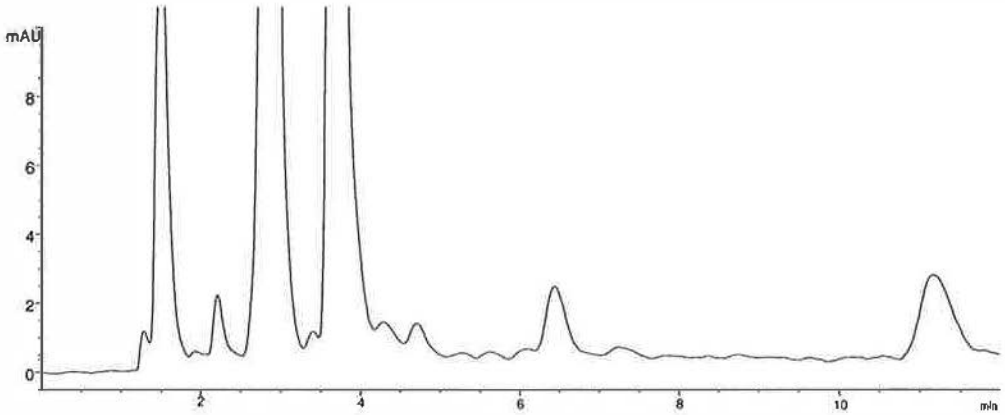
POCIS Protective Deployment Canister

POCIS on Support Rod Removed from Deployment Canister

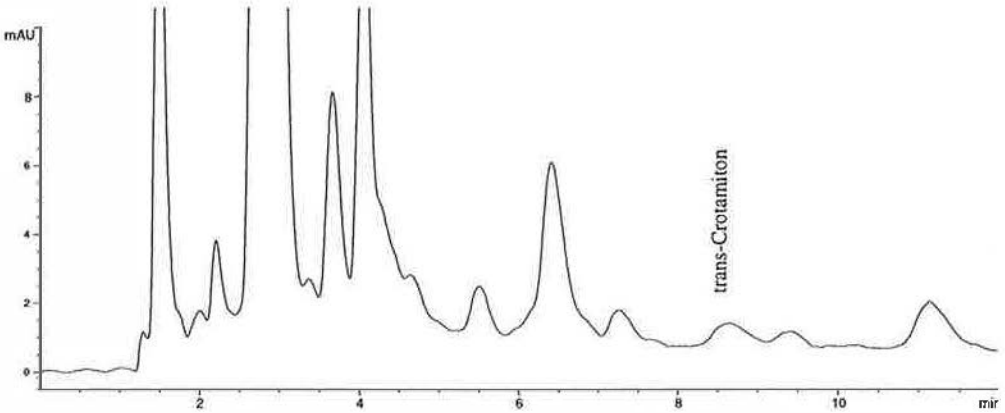
Figure II
HPLC Analysis for Crotamiton



2 ng/µL Crotamiton Standard

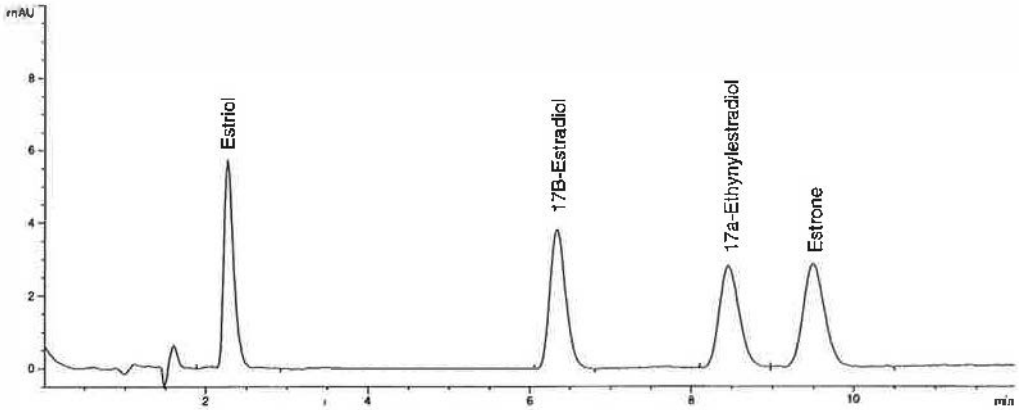


Bordon POCIS Field Blank (Sorbent Admixture)

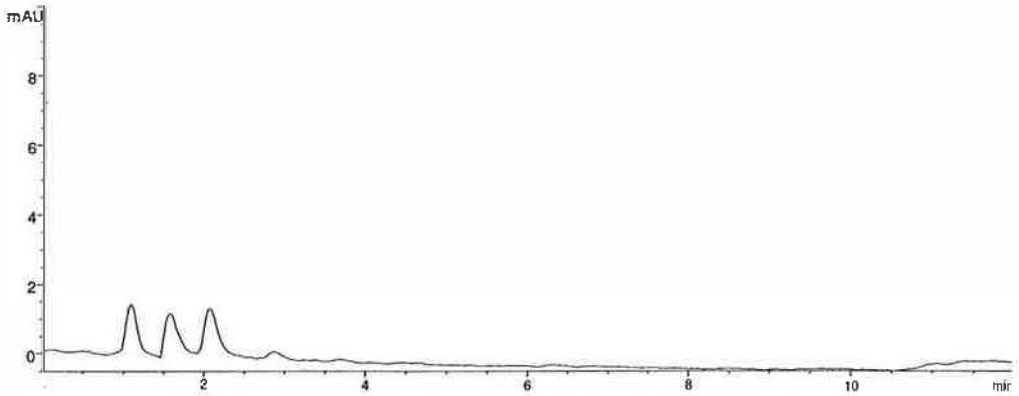


Bordon POCIS Deployed (Sorbent Admixture)

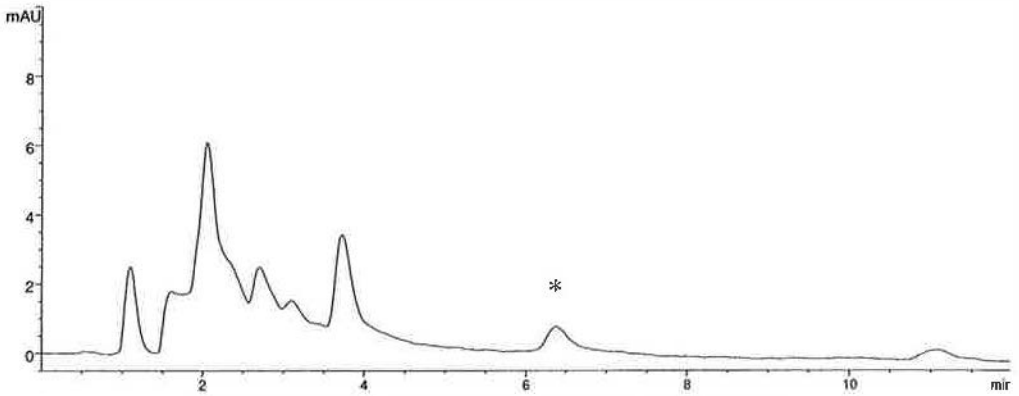
Figure III
HPLC Analysis of Estrogens



4 ng/µL Mixed Estrogen Standard

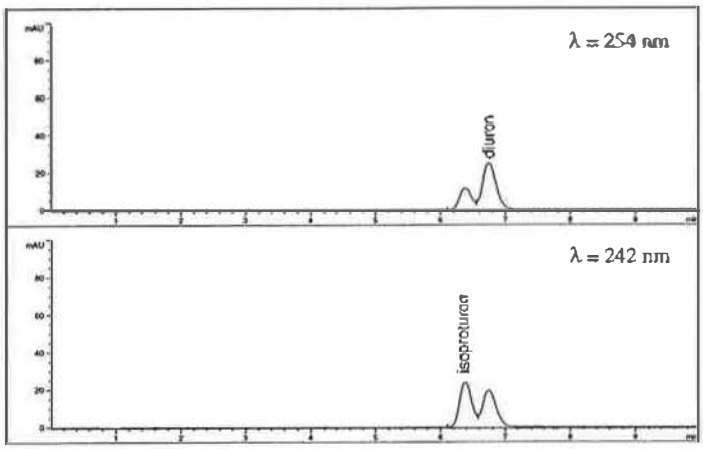


Bordon POCIS (Sorbent Admixture) Field Blank

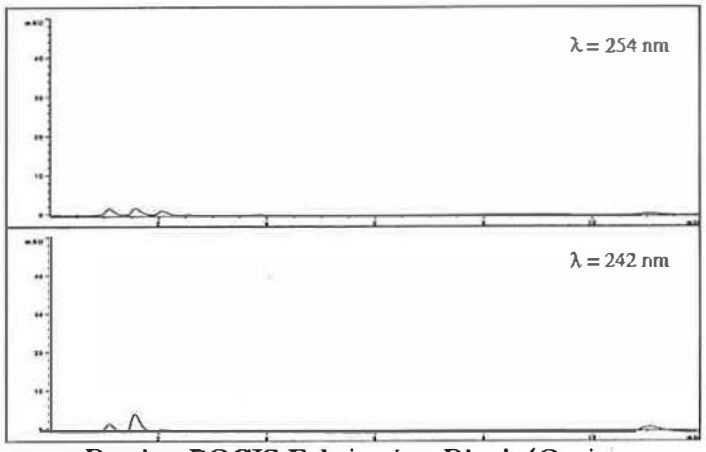


Bordon POCIS Deployed (Sorbent Admixture)
* peak does not match the uv spectral profile for 17β-estradiol

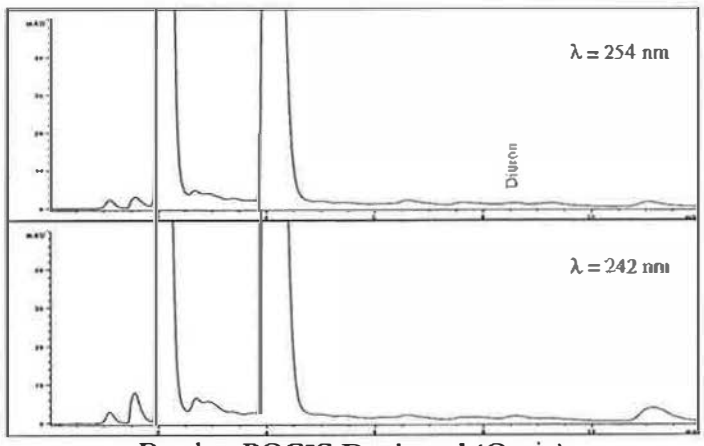
Figure IV
HPLC Analysis of Diuron and Isoproturon



2 ng/µL Diuron and Isoproturon standard

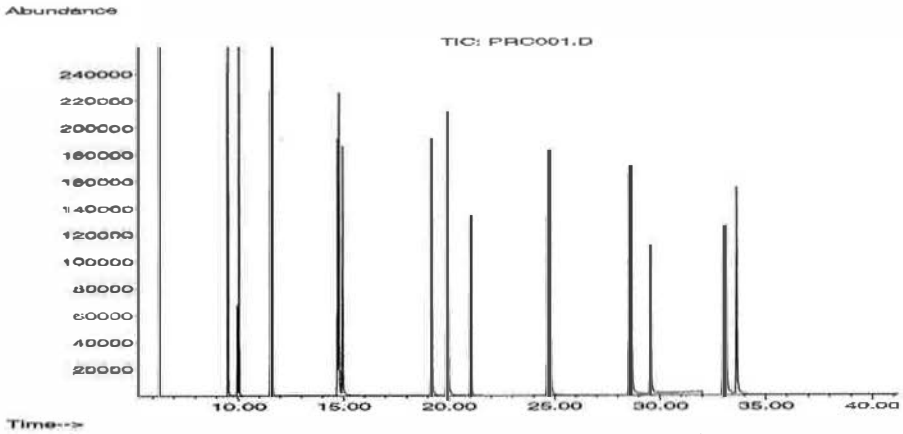


Bordon POCIS Fabrication Blank (Oasis)



Bordon POCIS Deployed (Oasis)

Figure V
GC-MSD Analysis of PRCs and PAHs



1.0 µg/mL PRC-PAH mixed standard

Bordon PRC-SPMD Field Blank at 1 mL volume (peaks represent PRC mixture)

Bordon Deployment PRC-SPMD at 1 mL volume

APPENDIX

Method Validation Procedure for Crotamiton in POCIS

Analyte Recovery from POCIS: In order to evaluate the ability to recover crotamiton from the POCIS, 200 mg portions of the sorbent admixture (80%(w/w) Isolute ENV+ and 20%(w/w) S-X3 dispersed Ambersorb 1500) and Oasis HLB were placed separately in glass chromatography columns (1 cm i.d.). Prior to fortification, the sorbent was cleaned by a rinse of approximately 25 mL methanol. The columns were fortified in triplicate by the addition of 100 mL of deionized (DI) water spiked with 1000 ng of crotamiton (a water concentration of 10 ng/mL or 10 ppb). The spiked water samples were transferred into the columns with three successive 2 mL DI water rinses to assure analyte transfer. The water was allowed to drain from the columns and was discarded. The crotamiton was eluted from the sorbent admixture by the addition of 50 mL of 1:1:8 methanol/toluene/dichloromethane and from the Oasis by 40 mL of methanol. The collected eluate from each column was evaporated to 3-4 mL by rotary evaporation. The sorbent admixture samples required an additional 10-15 mL of methanol was added to the flask. The additional methanol is necessary to facilitate the removal of toluene from the sample. Rotary evaporation was continued until the volume was approximately 2-3 mL. The samples were then transferred into test tubes with 3 successive rinses of acetone. The samples in the tubes were evaporated to approximately 0.5 mL under N_2 and were transferred into amber sample vials with acetonitrile. The samples in vials were evaporated to near dryness under N_2 and were reconstituted to 1.0 mL with 0.01M KH_2PO_4:acetonitrile. This step results in a sample solvent composition similar to the

HPLC mobile phase conditions, which can be critical for optimized analytical conditions. Results are listed in Table 1.

HPLC Conditions:

The HPLC system consists of a Hewlett Packard Series II 1090 Liquid Chromatograph with a diode array detector (Hewlett Packard, Palo Alto, CA) with the ChemStation for LC software package revision A.08.03 (Agilent Technologies, Inc., Palo Alto, CA). A Phenomenex Luna C_{18} analytical column (150 x 4.6 mm, 5 μm d_p) and a Phenomenex Security Guard C_{18} cartridge were maintained at 30°C. An injection volume of 50 μL and a mobile phase of 55:45 KH_2PO_4:ACN with a flow rate of 1 mL/min were used during analysis. Detection of crotamiton occurred at 220 nm with a multi-point calibration curve (0.25, 0.50, 1.0, 2.0, 4.0. 6.0, and 8.0 ng/μL) ran on a daily basis.

Alternative Cleanup Procedures:

Techniques used to isolate the crotamiton from potential interferences present in the sample matrix include: solid-phase extraction (SPE), size exclusion chromatography (SEC), and the chromatographic sorbent potassium silicate (KS). Each of these techniques were evaluated individually by analyzing standard mixtures of the analytes without the influence of the sample matrices. A standard solution containing 1.0 μg of crotamiton was used for all spiking and fortification steps in this validation. Results for the recovery of crotamiton from each step are listed in Table 2. A description of the procedural steps for each technique is as follows:

SPE: Oasis HLB SPE cartridges (Waters Corp., Milford, MA) were used as the initial cleanup step for the raw sediment extracts. These cartridges (6 mL, 200 mg sorbent) contain both hydrophilic and lipophilic moieties within a macroporous copolymeric structure which enhances the extraction efficiencies of both polar and nonpolar compounds (1). The spiked sample, in acetonitrile (ACN), was transferred into a 50 mL conical tube and diluted with deionized (DI) water to a composition of 5% ACN-water at a total volume of 20 mL. The diluted sample was applied to the Oasis SPE cartridge. Prior to extract application, the SPE cartridge was cleaned and conditioned with successive 3 mL washes of methyl *tert*-butyl ether (MTBE), methanol (MeOH), and DI water. a vacuum manifold controlled solution flow and was the primary means of sample throughput. Following conditioning and sample loading, the system was washed with 5 mL portions of 40% MeOH/water, DI water, 10:2:88 (V:V:V) MeOH:NH_4OH:DI water, and DI water. Following the final wash, the analytes were eluted from the cartridge with 10 mL of 10% MeOH/MTBE.

SEC: Samples were evaporated to near dryness in conical SEC vials and redissolved in 98:2 (V:V) dichloromethane:methanol (DCM:MeOH) at a volume of ~ 950 μL for injection on the SEC. The SEC system consists of a Perkin-Elmer Series 410 HPLC pump (Perkin-Elmer, Inc., Norwalk, CN), a Perkin-Elmer ISS-200 autosampler, and an ISCO Foxy 200 (ISCO, Inc., Lincoln, NE) fraction collector connected to the output end of the SEC column. The SEC column was a 250 mm X 22.5 mm i.d. (10 μm d_p, 100 Å pore size) Phenogel column (Phenomenex, Inc., Torrance, CA), equipped with a 50 mm X 7.5 mm i.d. Phenogel guard column. An isocratic mobile phase of 98:2 (V:V)

DCM:MeOH was maintained at a flow rate of 4.0 mL per minute. The SEC system was calibrated on a daily basis by the injection of a solution of compounds representative of the analytes and potentially interfering materials. The substances contained in this calibration solution, in sequence of elution, were diethylhexylphthalate (DEHP; a model compound with lipid-like chromatographic behavior), biphenyl and naphthalene (small aromatic analytes), coronene (a large PAH later eluting than any anticipated analyte), and elemental sulfur (S_8, a problematic interfering substance encountered frequently in environmental samples). Elution of these components was monitored by UV detection at a wavelength of 254 nm. SEC cleanup was accomplished using a "Collect" fraction defined by the calibration of the system on the day of operation. The "Collect" fraction was initiated at the point 30% of the time between the apex of the DEHP chromatographic peak and the onset of the biphenyl chromatographic peak. The "Collect" fraction was terminated at 70% of the time between the apex of the coronene chromatographic peak and the onset of the sulfur chromatographic peak. This collected fraction was then ready for additional processing.

KS: Alkali metal silicates, such as KS, have the ability to retain acidic organic compounds with a pKa <12 from moderately polar solvents (2). This property makes KS a potentially suitable means of cleanup for estrogens containing a phenolic hydroxide group. Glass chromatography columns (1 cm i.d.) containing 3 g of KS were rinsed with 20 mL MeOH followed by 20 mL 75% DCM/Hexane prior to sample application. The sample was applied in ~ 0.5 mL DCM to the KS with 3 rinses of 75% DCM/Hexane. A total of 25 mL of 75% DCM/Hexane was used to wash the column following sample

application. Analyte elution follows with 20 mL of 2:49:49 MeOH:DCM:Hexane. This analyte fraction was ready for further cleanup and fractionation or HPLC analysis.

LITERATURE CITED

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Replicate	Sorbent Admixture (% recovery)	Oasis (% recovery)
A	113%	112%
B	103%	105%
C	106%	94.4%
average	**107%**	**104%**

Table 1 – Analyte recovery from each sorbent type used in the POCIS.

	Crotamiton % recovery
Oasis SPE	101%
SEC – 30% Initial Collect	84.4%
KS – fraction 1	76.5%
fraction 2	0%

Table 2 – Procedural technique recoveries of the crotamiton. Results are reported as percent recovery.