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PRINCIPAL INVESTIGATOR: Jimmie D. Petty, Ph.D.

CONTRACTING ORGANIZATION: U.S. Geological Survey
Columbia, Missouri 65201

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Jimmie D. Petty, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Geological Survey
Columbia, Missouri 65201

E-MAIL:
jim_petty@usgs.gov

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13. ABSTRACT *(Maximum 200 Words)* U.S. military personnel may be exposed to a wide variety of potentially toxic airborne and waterborne chemicals. Such situations include troop deployment in third world countries with major pollution problems, prolonged exposure in enclosed areas such as submarines, training exercises involving chemicals, and in combat conditions. Unfortunately, existing chemical monitoring technology is limited or inadequate for determining the broad range of chemicals potentially present in areas of concern. Scientists at the USGS's Columbia Environmental Research Center have developed and patented a semipermeable membrane device (SPMD) for integrative monitoring of hydrophobic chemicals. The objective of this research project was to develop a prototype Area Monitor based on the concept of integrative sampling and to expand this approach to monitor more hydrophilic chemicals, toxic metals, and vapor phase neutral metals. Application of bioindicator tests designed to define exposure to complex chemical mixtures having various modes of action, provides a unique approach for determining not only the presence of a wide array of toxic chemicals, but also their potentail tpxicological significance. During the course of this research project, integrative samplers for airborne vapor phase metals, specifically mercury, waterborne ionic metals, i.e., Cd, Cu, Ni, Pb, and Zn, and waterborne hydrophilic organic chemicals were designed and developed. Also, the incorporation of bioindicator tests to define the toxicological relevance of exposure to complex mixtures of chemicals was validated. In combination with the SPMD trchnology, the research described in this report has resulted in the development and proof-of-concept validation of technology forming the basis of Area Monitors for use by the DOD in situations requiring exposure assessment. Further refinements of the technology, including miniaturization and remote sensing approaches are possible.

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ABSTRACT

U.S, military personnel may be exposed to a wide variety of potentially toxic airborne and waterborne chemicals. Such situations include troop deployment in third world countries with major pollution problems, prolonged exposure in closed-in spaces such as submarines, training exercises involving a variety of chemicals, and in combat conditions. Unfortunately, existing chemical monitoring technology is limited or inadequate for determining the broad range of contaminants potentially present in areas of concern. Scientists at the USGS's Columbia Environmental Research Center have developed and patented a semipermeable membrane device (SPMD) for integrative monitoring of hydrophobic chemicals. Research results indicate that the SPMD technology can be tailored to successfully mimic key aspects of human respiratory and waterborne chemical uptake. The purpose of this research project was to develop a prototype area monitor based on the concept of integrative sampling and to expand this approach to monitor more hydrophilic chemicals, toxic metals, and vapor phase neutral metals. Application of bioassay procedures designed to define exposure to complex chemical mixtures having various modes of action, provides a unique approach for determining not only the presence of a wide array of toxic compounds, but also their potential toxicological significance. During the course of this research project, integrative samplers for airborne vapor phase metals, specifically mercury, waterborne ionic metals, i.e., Cd, Cu, Ni, Pb, and Zn, and waterborne hydrophilic organic chemicals were designed and developed. Proof-of-concept field deployments were conducted to test these samplers under actual field deployment conditions. Also, the incorporation of bioindicator test to define the toxicological relevance of exposure to complex mixtures of chemicals was validated. The research described in this report has resulted in the development and proof-of-concept validation of technology forming the basis of Area Monitors for use by the DOD in situations requiring exposure assessment. Further refinement of the technology, including miniaturization and remote sensing approaches are possible.

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INTRODUCTION

In performing their duties, U.S. Military personnel may be exposed to a wide variety of potentially toxic airborne and waterborne chemicals. Such military environments include troop deployments in third world countries with major pollution problems, prolonged exposure in closed-in spaces such as submarines, training exercises involving a broad spectrum of chemicals, and combat conditions. Toxic chemicals encountered by military personnel include a wide array of organic compounds, organometallic species, and metals. Knowledge of the presence of potentially deleterious levels of contaminants in areas used by the military is critically needed to ensure the health and safety of personnel. Unfortunately, existing contaminant monitoring approaches are severely limited or inadequate in terms of the range of detectable toxic chemicals, sensitivity and portability of the monitoring systems, turnaround time of analytical results, and level of exposure and potential toxicity to personnel.

While progress has been made in improving active water and air sampling technology, such devices suffer the disadvantages of complexity and mechanical operation. Further, no active approach is designed to phenomenologically mimic the uptake of contaminants by living organisms. Moreover, the incorporation of in vitro bioassay methods to rapidly determine the potential for adverse effects due to contaminant exposure is conspicuously absent from current monitoring approaches.

Scientists at the USGS's Columbia Environmental Research Center (CERC) have developed a semipermeable membrane device (SPMD) for integrative sampling of airborne and waterborne contaminants. This technology is the subject of two U.S. Government patents. The device consists of layflat polyethylene tubing containing a thin film of a large molecular weight neutral lipid. The polymeric semipermeable membrane used in the SPMD functions by allowing bioavailable (i.e., vapors in the case of airborne chemicals and dissolved residues in the case of waterborne chemicals) hydrophobic molecules to pass through transient membrane cavities approaching 10 Å in cross sectional diameter (1). Transfer of chemicals through these transport corridors appears to be very similar to the transport of chemicals through biomembranes (2) and thus, the device only samples the readily bioavailable, toxicologically active portion of environmental residues. While the SPMD technology is applicable for monitoring hydrophobic organic chemicals, no such approach exists for integratively sampling polar, i.e., hydrophilic organic chemicals. Further, a similar sampling approach for toxic metals has not been demonstrated. Because the deleterious effects of polar organic chemicals and toxic metals have been well documented, their transport, concentration, and bioavailability are important aspects of any monitoring program. Consequently, new passive integrative approaches are needed for extending monitoring efforts to include polar organic chemicals and toxic metals.

The current research project concerns the development of area monitors based on our experience in developing in situ passive integrative samplers for hydrophobic organic chemicals. The research is designed to expand the application of integrative sampling and to develop approaches for polar organic chemicals, toxic metals and vapor phase

neutral metal species. The ultimate goal of the research is to enhance the Department of Defense's (DOD) capabilities to permit sampling of a broad array of toxic chemicals and to provide proof-of-concept for generating rapid toxicological assessment of chemicals present in areas of concern to DOD. This involves the use of bioindicator tests for determining the toxicity of the chemicals sequestered by the area monitors. Further, the results of this research will establish a foundation for the development of a troop dosimeter, capable of providing timely information on toxic chemical exposure events and the potential toxicological consequences. Moreover, as envisioned these approaches may well be compatible with remote sensing technology. The current research is being conducted in parallel with the development of toxicological indicators of neurotoxin exposure and other bioassay assessment techniques (e.g., AHH induction, estrogenic effects, etc.), which can be readily applied to residues sampled by area monitors or troop dosimeters.

EXPERIMENTAL DESIGN

The research being conducted during the course of this project involves five areas; 1) continued development of the SPMD technique for integrative sampling of hydrophobic chemicals, 2) evaluation of a deployment apparatus for use with SPMD samplers, 3) continued development of an integrative sampling approach for toxic metals and initiation of the development of an integrative sampler for volatile metals, specifically mercury (Hg) vapor, 4) initiation of development of an integrative sampling approach for more hydrophilic chemicals, and 5) evaluation of extracts from standard SPMDs deployed in the field using a variety of bioindicator tests to determine the applicability of incorporating an integrative sampling approach into holistic exposure assessment paradigms. The last area of research is an integral part of the biological research being conducted by Drs. Edward Little and Susan Jones.

During the final reporting period, the focus of the research has been in areas 3, specifically the development of a neutral mercury sampling device, 4 development of an integrative sampling approach for hydrophilic chemicals, and 5, evaluation of SPMD extracts using a suite of bioassay procedures. A detailed summary of the research results involving the remaining areas was provided in the first annual report (3). Consequently, emphasis in the final report will be placed on reporting results relating to area 3, specifically development of an integrative sampler for neutral mercury species, area 4, and area 5. Each research area will be addressed individually. To ensure continuity, a brief summary of the previously reported research results from areas 1, 2, and portions of 3 will be presented.

RESEARCH DESCRIPTION AND PROGRESS

Each of the research areas is addressed below. The research performed during this reporting period is described and the results of the research are discussed and summarized.

Research Area 1-

Continued Development of the SPMD Technique for Integrative Sampling of Hydrophobic Chemicals-

As part of our ongoing research into the applicability of the SPMD approach for integrative sampling of the bioavailable portion of waterborne hydrophobic chemicals, we completed the research designed to define the sampling rate of standard SPMDs for organochlorine (OC) pesticides. This research was the subject of a DOD interagency agreement and the research is described in detail in the final report entitled, "Evaluation of the Semipermeable Membrane Device (SPMD) as a Passive In Situ Concentrator of Military Organic chemicals in Water." This report was approved for distribution on September 11, 1997.

Another CERC project concerned the determination of SPMD sampling rate data for the EPA priority pollutant polycyclic aromatic hydrocarbons (PAHs). This CERC research project was performed separately from the current research, however, the data are applicable to the development of the Area Monitors. The sampling rate data from this research was reported previously (3).

Research Area 2-

Evaluation of Deployment Apparatus-

During the first reporting period, we obtained and evaluated an apparatus for deployment of SPMD samplers in a wide variety of monitoring situations. The apparatus consists of a stainless steel canister capable of holding five individual SPMD samplers. This apparatus offers several advantages; 1) decontamination is easily accomplished using normal cleaning procedures which include acid treatment and solvent rinsing, 2) the design is very sturdy and is capable of being deployed in aquatic systems ranging from low energy lakes to highly energetic streams, 3) the apparatus is also applicable for use in air sampling deployments in indoor and outdoor scenarios, and 4) the apparatus is commercially available. During this reporting period of the project we evaluated a second deployment apparatus. This deployment device also consisted of a stainless steel canister, but is smaller and will accommodate four standard SPMDs. The second apparatus is capable of deployment in areas similar to the larger version, but can be sealed (fully loaded with SPMDs) in a metal can. While deploying loaded sampling devices results in less handling of the samplers under field conditions, loading this deployment device must be done under laboratory conditions. Both devices have been used under field conditions and have proven to be completely acceptable.

Research Area 3-

Development of an Integrative Sampler for Metal Residues-

The third research area involved continued development of an integrative sampler for toxic metals, specifically, lead, cadmium, copper, zinc, and nickel. The research results reported previously (3) were a continuation of efforts begun in 1995. As a result, Dr. Bill

Brumbaugh received his Doctor of Philosophy in analytical chemistry from the University of Missouri-Columbia in December 1997. The results of this research were reported previously (3).

Convenient sampling of mercury vapor in air or dissolved in water employing an SPMD containing a mixture of dilute HNO_3 and 1 ppm Au^{+3} was demonstrated and reported previously (3). Additional research was conducted during this reporting period and is summarized below.

Research Results for the Final Reporting Period of the Project in Area 3-

The rationale for the development of the passive integrative mercury sampler (PIMS) employing a mixture of nitric acid and gold chloride enclosed in a polymeric membrane is based on the following; 1) non-porous membranes such as low density polyethylene (LDPE) exclude the mass transfer of analytes associated with particles, 2) Hg vapor readily diffuses through LDPE, and 3) gold chloride is an effective oxidant for Hg in water. Consequently, the permeability characteristics of LDPE and the ready transformation of the neutral mercury species into a soluble ionic species by the acidified gold result in an ideal design for a passive integrative sampling approach.

Sampler Preparation:
Segments of lay-flat LDPE tubing were cut to desired length with a scalpel. The standard PIMS sampler configuration employed consisted of a 15 cm length of tubing containing 10 to 20 mL of the sequestration media plus an additional 5 to 10 cm segment to allow for construction of tether loops or tags on each end for suspending or hanging the PIMS. During construction, one end of each PIMS was heat sealed (molecular weld), the exterior was rinsed with spectroscopy grade acetone, and the tubing was filled with the 10% HNO_3, 1 ppm Au^{+3} mixture and allowed to soak overnight (approximately 15 hours) to extract Hg from the tubing prior to use. After the oxidative extraction mixture was discarded, the interior of the PIMS was flushed with approximately 10 mL of the acid/gold reagent, and the PIMS was subsequently charged with 10 to 20 mL of the sequestration reagent and the open end was heat-sealed. Air pockets were carefully removed prior to sealing. After sealing, the exterior of each PIMS was rinsed sequentially with dilute nitric acid, ultra-pure deionized water, and spectral grade menthol. The PIMSs were placed in acid rinsed zip-seal type plastic bags and stored in tightly sealed acid-cleaned glass jars until use. Samplers to be used as method blanks were stored in the sealed jars prior to processing and analysis. At no time did the samplers come into contact with any metal. Once sealed and rinsed, each PIMS was handled only by the tag or loop end. For environmentally deployed PIMS, a "clean hands-dirty hands" technique was employed.

Transfer and Analysis:
Following deployment of the PIMS, the samplers were returned to the laboratory sealed in acid washed bottles. Each PIMS was rinsed with dilute nitric acid and methanol. A small 0.5 cm cut was made just below the heat seal on one end of the PIMS with an acid rinsed stainless steel scalpel. The PIMS was opened by pulling the tag or loop and

subsequently tearing the polyethylene along the cut. The bulk of the liquid contents were carefully transferred to an acid cleaned 25 mL glass test tube with was sealed with a polypropylene screw cap and finally a polypropylene cap cover. Analysis of the sequestration reagent was performed by using either flow-injection cold vapor atomic absorption spectrophotometry (4) or inductively coupled plasma mass spectrometry (ICP-MS) employing a Meinhard-type nebulizer for sample introduction (5). The ICP-MS method resulted in a lower detection limit (about 0.01 compared to 0.04 μg/L for the atomic absorption method) and was therefore used for the determination of all environmentally deployed samplers. Accuracy of instrument calibration standards were verified to be within 5% by daily analysis (at the beginning and end of an analytical run) of a separate solution prepared from a National Institute of Standards and Technology reference standard.

Temperature Effects on PIMS Sampling Rate:
Experiments were conducted employing 1 L glass jars as the exposure chamber. One mL of Hg (about 13 g) and one PIMS per jar were used to ensure maximum vapor phase saturation of Hg in the exposure chamber so that steady state gas-phase concentrations could be assumed. Samplers were prepared using 20 mL of sequestration reagent and each jar was allowed to equilibrate for 24 hr at the exposure temperature prior to adding the PIMS. Four temperatures, specifically –11, 4, 21, and 40 °C and two exposure periods, 1 d and 7 d, were employed.

Environmental Air Sampling:
A set of PIMSs containing 10 mL of sequestration reagent were deployed on the roof of the Federal building in downtown Columbia, MO. The site is about 0.5 km north of a coal-fired power plant, a potential source of vapor phase neutral mercury species. Five sets of triplicate samplers were deployed: three sequential 4-week exposures; one 8-week exposure, and one 12-week exposure. Samplers to be analyzed as method blanks were also maintained in triplicate for each of the five PIMS sets. The PIMSs were constructed with a 1 cm loop on each end for horizontal deployment between two steel rods affixed to a steel base. The base/rod assembly was initially deployed with nine samplers to be collected at the 4, 8, and 12 week sampling periods. The three PIMSs which were removed after the first 4-week interval were immediately replaced with a new set of PIMSs to monitor the second 4-week interval, and likewise for the third 4-week interval.

Results and Discussion for Area 3-

An important consideration for the sequestration reagent for concentrating neutral Hg species is that it be compatible with analysis of Hg residues will minimal processing. As configured, the PIMS sequestration reagent is directly analyzable by commonly employed analytical methods. Maximizing the retention of Hg by the sampler would obviously improve the detection limit for a monitor designed to assess the presence of vapor phase neutral Hg species. As reported previously (3), the combination of 10 % HNO_3 and 1ppm Au^{+3} was found to be optimum for the sequestration of Hg by the PIMS with only minimal improvement being observed for higher reagent concentrations (up to 50 % HNO_3 and 10 ppm Au^{+3}).

Presumably, the maximum sequestration rate for the PIMS is obtained when the oxidation rate of Hg inside the sampler is rapid relative to the permeation rate of Hg through the membrane and associated boundary layers (i.e., the probability that a Hg atom inside the sampler is oxidized is considerably greater than the probability that the atom will diffuse back into the membrane). Once oxidized to the aqueous ion, the loss of Hg by transfer across the hydrophobic LDPE membrane is negligible. We hypothesize that each reagent component functions to retain Hg in the PIMS in the following manner: 1) HNO_3 and gold chloride oxidize the Hg atoms to ions, and 2) the gold chloride stabilizes the Hg ions and prevents the reduction of ionic Hg to the neutral state, for instance photo chemically, with subsequent loss of Hg residues by diffusion out of the PIMS. In reality, the exact role of the Au^{+3} is unclear. Higher concentrations of gold chloride alone may have oxidized the Hg° just as effectively, but this option was not pursued because of the potential analytical problems associated with gold concentrations above 1 ppm. It appears from our investigations that the reagent mixture functions in a synergistic manner, since the sequestration rate and concentration factor was about 3 times greater for the mixture than for the sum of the individual reagents.

The sampling rate of a passive sampler is typically defined as the equivalent volume of air or water for which the analyte is completely removed per unit time. Air sampling rates were determined, as described previously (3), at four different temperatures with sampling intervals of 1 d and 7 d. These data are presented in Tables 1 and 1a.

Table 1. PIMS Hg° Sampling Rates at Selected Temperatures (one week exposure)

Temp (C°)	Vapor pressure (mm)	air conc (µg/L)	Sampled Hg (µg)		Sampling rate	
			Mean	S.D.	L-equiv/d	%RSD
			------	-----	----------	--------
-11	0.000054	0.59	2.84	0.12	0.68	4.1
4	0.000276	3.03	24.2	1.00	1.14	4.1
21	0.00131	14.4	211	4.96	2.10	2.3
42	0.00707	77.6	1140	57.9	2.09	5.1

Table 1a. PIMS Hg° Sampling Rates at Selected Temperatures (24 hr exposure)

Temp (°C)	Vapor pressure (mm)	air conc (μg/L)	Sampled Hg° (μg)	Sampling rate L-equiv/d
-11	0.000054	0.59	0.47	0.79
4	0.000276	3.03	4.1	1.35
21	0.00131	14.4	32.2	2.24
40	0.00608	66.7	200	3.00

The one week experiment yielded sampling rates approximately linear with temperature from –11 to 21 °C, but a plateau in uptake of Hg was observed at 42 °C. The extremely high Hg vapor concentrations produced for the 42 °C experiment may have eventually exceeded the capacity of the reaction medium, thus limiting the apparent sampling rate. This hypothesis is based on the observation that the Hg retained after one week (1140 μg, Table 1) was about 50 times the total quantity of Au^{+3} in the PIMS (20 μg). Repeating the experiment with only a 24 hour exposure (in order to reduce the total Hg load to the PIMS by a factor of 7) resulted in a linear relationship from –11 to 40 °C (Table 1a) suggesting that the sampler capacity did indeed become limiting during the one week exposure. This should not be a limitation for most environmental sampling conditions and is easily overcome by increasing the total sequestration reagent capacity.

Under the experimental conditions tested during the 24 hr exposure, the direct relationship of the sampling rate with temperature was highly linear (regression equation; $y = 0.0443x + 1.2476$, $R^2 = 0.9962$) with an increase in sampling rate of 0.44 L-equivalents/day for every 10 °C rise in temperature. Thus the sampling rate is only modestly affected by temperature (about a factor of 4 from –11 to 40 °C). Consequently, the precise determination of exposure temperature during field deployments is probably not critical for most applications. The lower temperature limit for the PIMS is the freezing point of the sequestration reagent (about –22 °C). The upper limit, while not known precisely, will be well above the 40 °C employed during this research.

There are at least three parameters that could contribute to the observed temperature dependence of the PIMS sampling rate; 1) the oxidation rate of Hg° inside the sampler, 2)

the air and liquid (sequestration phases) diffusion coefficients of the Hg species, and 3) the permeability coefficient of Hg in the LDPE membrane. If the oxidation reaction with the sequestration reagent is not rate limiting (which appears to be the case at room temperature ; otherwise higher sampling rates would have been observed for the 50% HNO_3/1 ppm Au^{+3} examined during the reagent optimization as reported previously [3]), the effects of temperature on the oxidation rate will be a minor factor in the temperature dependence observed for the sampling rate. Although the diffusion coefficient of a gas depends on temperature and cannot be ignored, this should be of minor importance over relatively small temperature ranges (e.g., 20 to 30 °C) observed for most environmental exposures. This results because between two temperatures, the diffusivity changes by the 3/2 power of the ratio of the absolute temperatures for the gas phase and by the first power of the temperature ratio times the appropriate viscosity for the liquid phase. For example, the gas diffusion coefficient and subsequently the sampling rate for a porous membrane sampler designed for gas phase control would be expected to increase by only 14% when the temperature is increased from 21 to 48 °C. However, when a nonporous membrane is used (as in the PIMS) the membrane permeability, i.e., parameter 3, may be significantly affected by temperature.

Most of the free volume in LDPE exists as transient cavities as opposed to porous membranes having a fixed pore distribution. At room temperature these cavities are thought to have cross-sectional diameters no greater than10 Å (1); whereas the cross-sectional diameter of a single Hg atom is about 3 Å (6). Because thermally mediated motions of the polymer chains increase with temperature, there is a corresponding increase in polymer free volume and in general the permeability (1, 7). Increases in these factors with temperature generally reduces resistance to mass transfer and enhances diffusion rates, which may account for the increase in sampling rate with temperature.

If the oxidation rate of Hg in the PIMS exceeds its maximum uptake flux, the permeation rate of the Hg through the membrane will limit the overall sampling rate (assuming resistance to mass transfer in the gas-membrane boundary is negligible). Consequently, the sampling rate might be increased by altering the membrane characteristics such as the polymer and the thickness of the membrane. However, significant decreases in membrane resistance could cause the sampling rate to be controlled by the boundary layer diffusion rate or the oxidation rate of Hg. Although it is possible that selection of a different membrane material could enhance the sampling rate, LDPE has several practical advantages; including low metal contamination, very good durability, and it is easily heat sealed. The thickness chosen for the membrane is a compromise between permeability and durability. We believe the 80 to 90 μm thickness is preferable because the PIMS is designed for extended deployments. Also, the thickness and type of membrane material used will yield not only different sampling rates, but a different temperature dependence as well. Therefore we recommend a single source of uniform and well characterized LDPE be employed in the construction of the PIMS.

Following the determination of the PIMS sampling rate for vapor phase Hg, we deployed samplers during a twelve week period on the roof (Figure 1 presents the deployment device) of the Federal building (three story building) in down town Columbia, MO.

The amount of Hg sequestered was found to decrease from 1.8 ng/PIMS during August to about 1.0 ng/PIMS for October. The decrease in Hg sequestered over this time period may have resulted from decreases in coal combustion at the nearby power plant and in part from lower sampling rates associated with seasonal decreases in average daily temperatures (about 27 °C for August to about 16 ° for October). Employing a 12 week average temperature of 21 °C and a sampling rate of 2.2 L-equivalents/day, the overall 12 week average air concentration of vapor phase neutral mercury was 25 ng/m^3. The summation of the results for the individual 4 week sampling periods was close to the results for the 8 week and 12 week values indicating a linear uptake rate over the 12 week deployment period.

The detection limit for a 4 week sampling (based on 3 times the standard deviation of the method blanks as determined by ICP-MS) was about 2 ng/m^3 which is near the typical background of 1 to 3 ng/m^3 (8). Method blanks were consistently about 0.25 ng Hg per sampler regardless of sampling intervals. We anticipate that the Hg background could be reduced significantly by the use of clean room facilities, however, for many applications, the Hg concentrations will be well above global background levels and the PIMS can be used by any competent analytical laboratory. For example, even with our less than ideal air handling system and the long deployment times, precision was excellent with RSDs of ≤5 % for triplicate samplers under laboratory conditions and 5 to 10 % for triplicate PIMS deployed under ambient outdoor conditions in a urban environment.

PIMS samplers were also utilized for the air monitoring of Hg at two indoor sites: 1) the CERC inorganic preparation laboratory where work with Hg metal was recently conducted and 2) the City/County (Howard) building in downtown, Columbia (the site of a recent Hg spill and intensive clean-up effort). At the Howard Building site, the PIMSs were placed in a health exam room where a Hg spill had occurred as well as in an adjacent office. In all, four sets of triplicate PIMS samplers were prepared for the 4-week indoor testing (3 locations and a blank set). Deployed samplers were thumb tacked to the ceiling in a corner of each room whereas the trip blank samplers were sealed in a glass jar over the 4-week interval. The results are summarized in Table 2.

Table 2. Mercury Results from 4-Week Indoor PIMS Deployment.

Sample ID	Description	PIMS Ng/mL	Total Hg (ng)	Mean (ng)	Std. Dev.	% RSD	Estimated Air Conc ($\mu g/m^3$)[a]
Howard-1-1	Exam room	11.4	114				
Howard-1-2	Exam room	10.5	105				
Howard-1-3	Exam room	13.0	130	116	13	11	1.7
Howard-2-1	Adjacent office	4.6	46				
Howard-2-2	Adjacent office	5.2	52				
Howard-2-3	Adjacent office	4.9	49	49	3	6	0.6
CERC lab-1	D-25 room 6	0.42	4.2				
CERC lab-2	D-25 room 6	1.1	11				
CERC lab-3	D-25 room 6	0.36	3.6	6.4	4.4	68	<0.25
Trip blank-1	Sealed in jar	1.18	11.8				
Trip blank-2	Sealed in jar	0.76	7.6				
Trip blank-3	Sealed in jar	0.89	8.9	9.4	2.2	23	------
Method detection limit[b]		1.6	16				0.25

[a]based on 2.2 L/d sampling rate and corrected for trip blanks.
[b]based on mean plus three time standard deviation of trip blanks.

Despite unusually high blank values associated with this batch of samplers, levels of airborne Hg clearly above the method blanks were detected by the PIMS samplers, both in the room where the spill occurred (1.7 $\mu g/m^3$) as well as in an adjacent office (0.6 $\mu g/m^3$) at the Howard Building. The results indicated the continued presence of elevated (compared to the general global background) Hg levels in the air despite a week-long clean-up effort by trained government personnel. Instrumental monitors used by the clean-up crews indicated no measurable Hg remained but the detection limit was 3 $\mu g/m^3$. No Hg was detectable (< 0.25 $\mu g/m^3$) for the PIMS samplers deployed at our laboratory.

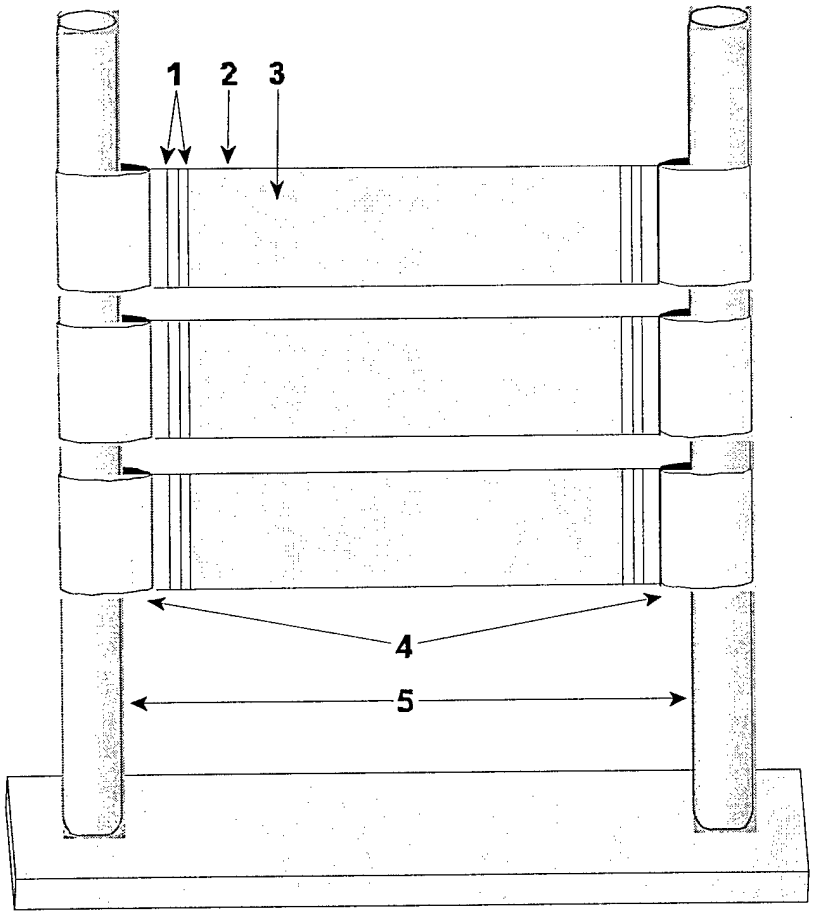


Figure 1. Configuration of PIMS for Environmental Air Sampling of Hg^0.
1 = heat seals, 2 = polyethylene tubing, 3 = sequestration reagent,
4 = tether loops, 5 = stainless steel rods on ringstand base

Research Results for Final Reporting Period of the Project in Area 4-

Development of an Integrative Sampler for Hydrophilic Contaminants:
Concerns over the persistence of organic contaminants in the environment has lead to the development of more water soluble and environmentally friendly chemicals. Research indicates that the large quantities of these compounds (i.e. herbicides, insecticides, and pharmaceuticals) entering the water system may be responsible for not only acutely toxic effects, but also chronic abnormalities and endocrine disruption in aquatic organisms (9-13). At least 45 chemicals have been identified as potential endocrine disrupting contaminants for both terrestrial and aquatic organisms while many others are suspect (14). Numerous examples have been well-documented linking these chemicals abnormalities such as increased vitellogenin levels (an estrogen-controlled egg protein normally found only in females) in male carp from Las Vegas Wash, nonfunctional testes in male American alligators in Lake Apopka, Florida, reduced penis size in juvenile male

otters of the Columbia River, feminized behavior in male Western gulls of southern California, non-descended testicles in male Florida panthers, and masculinized female mosquito fish (15-17).

Atrazine, diazinon, and ethynylestradiol are representative of polar organic contaminants and were selected as model compounds to be used in the following research. Atrazine has been routinely ranked first in national herbicide usage and is commonly found in surface and subsurface waters. It is the most common of the triazine herbicides and is widely used as a pre- and post-emergence herbicide in corn production with 70-80 million pounds of active ingredient applied annually in the United States (18). Atrazine has recently been the focus of several newspaper articles reporting a possible EPA ban of the potentially carcinogenic chemical which has been found at high levels in the drinking water of many Midwestern rural communities (19-21). Diazinon, a typical organophosphate insecticide, has also gained the top national ranking among insecticides for home and garden use with 1.6 million pounds annually applied in the U.S. (22). Mammalian poisoning results in accumulation of acetylcholine at the neuromuscular junction producing rapid twitching of voluntary muscles, followed by paralysis and death due to respiratory failure. Ethynylestradiol is a synthetic hormone and the main component in oral contraceptive pills. Much of the ingested 17α-ethynylestradiol is excreted from the body as the Phase II glucuronide and sulfate metabolites which are deconjugated into the free biologically active form during sewage treatment processes (11, 23, 24). Ethynylestradiol has been found to produce an observed estrogenic response in male fish at concentrations as low as 0.1 ng/L, approximately 10-fold lower than natural estrogen in domestic sewage effluents (13).

The sampling of hydrophilic contaminants in aqueous media is problematic at best. Due to the high water solubility of these compounds, modifications to many standard sampling regimes are required. Techniques such as liquid-liquid extraction, involving the partitioning of contaminants into an immiscible organic solvent, may result in low recoveries due to a chemical's lower affinity for the solvent (25). The use of solid-phase extraction columns offer an attractive alternative for sampling, but must use specially modified resins in order to adequately sequester polar compounds (26). Large quantities of water, up to ten liters or more, are required by both methods in order to achieve the analyte mass necessary for instrumental detection. The handling and transport of such amounts of water to the laboratory is undesirable on a regular basis. These methods are further limited in that they only provide information on contaminant concentrations at the instant of sampling. A need exists for an integrative sampling method which can detect episodic or variable concentrations over extended time periods.

Using the SPMD as a template, an integrative sampling device capable of sequestering hydrophilic organic contaminants is a realistic goal. The development of such a sampler involves the following major points of emphasis addressed in this research.

- Selection of a suitable medium which will act as an infinite sink capable of sequestering hydrophilic compounds.

- Selection of a membrane material which will facilitate the uptake of these chemicals.
- Optimization of methods for the recovery of sequestered contaminants.
- Evaluation of the equilibrium and kinetic aspects of contaminant uptake.
- Applicability of design for extended field deployment.
- Proof-of-concept deployment at an environmentally relevant site.

To facilitate analysis of samples from each study, carbon-14 and tritium radiolabeled compounds were used throughout the research project. Liquid scintillation counting was used for all analytical measurements of the ^{14}C atrazine, ^{14}C diazinon, and ^{3}H ethynylestradiol. Hereinafter the use of atrazine, diazinon, and ethynylestradiol refers to the radiolabeled analogs unless specifically designated.

Methods and Instrumentation:
Liquid Scintillation Counting was performed on a Beckman LS 6500 Liquid Scintillation Counter (Beckman Instruments, Inc. Irvine, CA). Analysis of samples involved a count time of 10 min with automatic quench correction. Polypropylene LSC vials containing up to 10 mL of sample and 10 mL of scintillation cocktail were used. Scintillation cocktail used was Ecolume (ICN, Costa Mesa, CA) for general counting and Ready Organic (Beckman, Fullerton, CA) for the analysis of membranes. Water samples required the addition of 1-2 mL of Triton X-100 (Mallinckrodt Baker, Inc., Paris, KY) to prevent the formation of two phases in the vial. All exposures involving radiolabeled chemicals were at 100,000 DPM/L.

Gas chromatographic analyses were conducted using a Hewlett Packard 5890 series gas chromatograph (GC) equipped with a Hewlett Packard 7673A autosampler (Hewlett Packard, Inc., Palo Alto, CA). In all analyses, 1.0 µL of sample extract was injected using the "cool-on-column" technique with hydrogen as the carrier gas. The chromatography column used was a DB-5 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) from J&W Scientific (Folsom, CA). The temperature program used was injection at 60°C, then 15°C/min to 165°C, followed by 2.5°C/min to 250°C, then 10°C/min to 320°C and held at 320°C for 1 min. Detectors used included a photoionization detector (PID) with a 9.5 eV lamp operating at 270°C (HNU, Inc., Newton, MA), an electron capture detector (ECD) operating at 330°C (Hewlett Packard, Inc., Palo Alto, CA), and a flame ionization detector (FID) also from Hewlett Packard.

Most of the membranes used in this project were not amenable to available sealing methods (i.e. heat) therefore a device had to be designed which would physically compress the membrane layers together. This compression seal was adequate as none of the material held between the membrane layers was lost in any exposure. This device consisted of 2 heavy-duty washers (1-5/16" ID, 2-3/4" OD, 0.125" thick) held together by 3 thumb screws (10-32 size, 3/4" long) and wing nuts (10-32 size). See Figure 2 for a schematic of the ring holder. This size of ring holder allows for the use of commercially available 47 mm membrane disks. The inner diameter of the washers is such to provide maximum surface area exposure while still providing sufficient overlap to ensure a good

seal. Thumb screws and wing nuts were selected to allow construction and disassembly of the device by hand. All parts were out of 18-8 stainless steel to prevent corrosion.

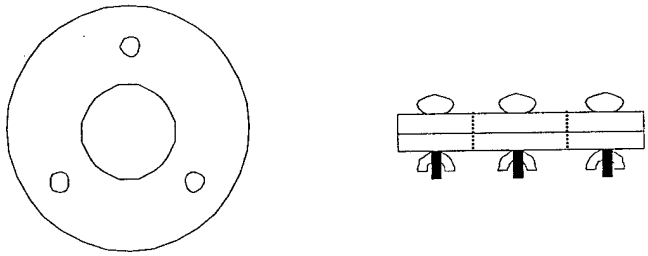


Figure 2 – Stainless Steel Ring Holder Schematic.

Devices were constructed immediately before exposure. Membrane Only devices were assembled by placing two membrane disks in the center of the washer and then fastening on the second washer with the screws. Construction of the resin-containing devices included an additional step of weighing 100 mg of the resin mix (20 mg S-X3 dispersed A-1500 and 80 mg Isolute ENV+) onto the center of the first membrane. The resin was spread evenly about the inside of the disk and subsequently covered with the second membrane disk. In some experiments, the resin mix was wet with DI water or methanol prior to covering with the second membrane disk. In cases involving wetting of the resin, the wetting solvent was added dropwise to the resin mix starting around the parameter working towards the center. This prevented unwanted spreading of the resin. Before the Empore containing devices were constructed, the Empore disk was conditioned with solvents in the order; 25 mL acetone, 10 mL isopropanol, 10 mL methanol, and 25 mL water as described in the product literature provided by the supplier. The conditioned disk was then placed in between two membrane disks and secured in the ring holders.

Methods of analysis varied depending on the type of device used. For devices consisting of membrane disks only, a dialysis was performed. The membrane disks were removed from the device and placed in an aluminum foil capped jar containing 50 mL of 10% isopropanol in hexane. The jars were placed in an incubator overnight at 18°C. The following morning, the solvent was transferred into an evaporating flask and an additional 50 mL of solvent was added to the jar which was placed back in the incubator for another 6 hours. The second portion of solvent was then combined with the first portion in the evaporating flask and reduced in volume by rotary evaporation. The resulting solvent was then transferred to a LSC vial for counting. Devices containing membrane and resin have to be carefully disassembled in order to prevent loss of any resin. The resin was transferred into a chromatography column fitted with a glass wool plug and eluted with 50 mL of 10/10/80 methanol/toluene/dichloromethane. The eluate was then reduced in volume by rotary evaporation ensuring the complete removal of dichloromethane which will quench LSC. The remaining membrane disks were placed in

a LSC vial with a ~5 mL hexane and counted by LSC. Empore disk containing devices were disassembled with the membrane disks being placed in a LSC vial and counted as previously described. The Empore disk was placed on a vacuum extraction apparatus and eluted with 50 mL of solvent mix. The eluate was evaporated and counted as previously described.

Results and Discussion for Research Area 4-

The Polar Organic Chemical Integrative Sampler (POCIS) was designed consisting of a sequestering media contained within a membrane enclosure. As there are no moving or mechanical parts, the POCIS is completely self-sufficient and does not require any attention during the exposure period. The intention is for the sampler to mimic the exposure, via respiration, of an organism without the inherent problems of metabolism, introduction of chemical from feeding, clearance of chemical, avoidance of a contaminated area, and possibly death that are present when working with live organisms. The POCIS device, made from synthetic materials, is superior to biomonitoring organisms in its reproducibility and simplified analytical cleanup. It is anticipated that standard analytical procedures can be applied to the device's extracts with minimal modifications.

Analyte uptake by the device needs to be essentially constant throughout the exposure period, typically up to 28 days. Constant uptake allows for the development of kinetic models which can be used to estimate actual environmental water concentrations. Assuming the kinetics will mimic that of the SPMD, uptake will occur linearly at first, changing to curvilinear then finally reach equilibrium after extended periods of time [26]. It is preferable to sample only during the linear phase which follows first-order kinetics. By incorporating a sequestering medium with a large enough capacity to act essentially as an infinite sink, the uptake should remain in the linear phase well beyond a typical 28 day exposure period.

Resin Characterization:
Recent advances in the area of sampling polar organic contaminants from water largely revolve around solid-phase extraction using specially modified polymeric resins in either a cartridge or embedded in an inert membrane disk (25, 26, 27, 28, 29-33). Resins commonly used in solid-phase extractions of polar compounds are the polystyrene-divinylbenzene copolymers along with various carbonaceous sorbents. These types of resins have also been incorporated into teflon membrane disks as an alternative to the classic column or cartridge configuration of extraction devices. Membrane extraction disks are attractive due to their high sample flow rates and extraction efficiencies compared to traditional packed-bed solid-phase extraction columns. A third variation in sampling is the use of resins embedded into diffusive polymeric gels. Although originally designed for the sampling of metal species, they can potentially be modified to sequester polar organics from water. Results from the thin gel evaluation have been previously reported (3).

Empore Extraction Disk Evaluation:

A recent innovation in solid-phase extraction technology by the 3M Corporation involved the development of the Empore™ extraction disk (29-33). The Empore extraction disk is a membrane composed of a 90%(w/w) mixture of solid-phase extraction resin particles intertwined in inert teflon fibers. The teflon comprises less than 1% of the total surface area and the open pore volume of the disk is approximately 60% (29). The membranes are constructed using smaller sized particles with a high surface area to obtain a uniform particle distribution with an extraction efficiency comparable to that of conventional packed extraction columns. Decreased channeling and improved mass transfer of the analytes during analysis are other advantages of resin-loaded membranes. Disks containing sulfonated polystyrene-divinylbenzene, referred to subsequently as Empore Sulfonated, were chosen for study. Sulfonated disks are designed for sampling drugs and metabolites, drugs of abuse, polar organic compounds, pesticides and metabolites, explosives, and amine-containing analytes. The sulfonation of the resin matrix allows water to come into intimate contact with the resin surface facilitating the transfer of analyte from the aqueous sample to the more hydrophobic interior resin surface (31). Retention of analytes on sulfonated resins can occur by either reversed phase interactions with the polystyrene-divinylbenzene polymer or by weak cation exchange with the sulfonic acid groups (30-31).

In order to determine if the Empore Sulfonated disks could actively extract polar analytes from water samples, 1-liter portions of DI water spiked individually with ethynylestradiol, atrazine, and diazinon were extracted using a glass vacuum filtration apparatus containing one Empore disk. The extracted water was collected with two 5 mL aliquots measured by means of Liquid Scintillation Counting (LSC). The Empore Sulfonated disk was eluted with 20 mL of 50/50 methanol/toluene followed by 30 mL of 10/10/80 methanol/toluene/dichloromethane. The eluates were reduced in volume and counted by LSC. Extraction data for ethynylestradiol, atrazine, and diazinon are listed in Table 3.

Analyte	Concentration	% in Water Effluent	% in Empore Disk[a]	Mass Balance
Ethynylestradiol	1.00 µg/L	0.89 ± 0.86	98.1 ± 3.56	99.0 ± 3.66
Atrazine	1.33 µg/L	10.0 ± 2.06	78.9 ± 1.07	88.9 ± 2.32
Diazinon	1.49 µg/L	0.87 ± 0.41	90.6 ± 1.76	91.5 ± 1.81

Table 3 – Extraction of 1 L water samples fortified with Ethynylestradiol, Atrazine, and Diazinon by Empore Sulfonated disks. Reported values are percent recovered ± standard deviation (n=3). [a] Empore recoveries based on % of analyte in Empore eluate and % of analyte remaining in the disk following solvent extraction.

Comparison of the overall mass balances for each analyte demonstrated the ability of the Empore Sulfonated disks to adequately sequester a range of polar contaminants. The nearly quantitative extraction of ethynylestradiol and diazinon along with the excellent recovery of each analyte demonstrated the potential usefulness of these disks as

environmental water samplers. Atrazine, probably due to its higher water solubility, proved more difficult to extract as evidenced by 10% remaining in the water following passage through the disk. The lower recovery of atrazine from the Empore disk may be due to secondary adsorption between the amine functionalities on atrazine and the sulfonic acid groups on the resin via an ion-dipole interaction. It has been reported that amine-containing analytes undergo selective cation exchange interactions with the sulfonic acid groups present from the disk sulfonation (30, 31).

The ability of Empore Sulfonated disks to passively sequester analytes was studied by securing a disk in a stainless steel housing and exposing it to ethynylestradiol in 1-liter of DI water with stirring for 1 week. Following exposure, the disks were removed, eluted with 50 mL of 10/10/80 methanol/toluene/dichloromethane and counted by LSC. Aliquots of the exposure water were also counted to determine the amount of ethynylestradiol remaining. Analysis of the data indicated 4.81% of analyte remained in the water and 97.4% was recovered from the Empore Sulfonated disk with a mass balance of 102.2%. This study demonstrated the suitability of this disk for sequestering polar compounds in a passive sampling manner. The disk alone was too fragile when wet to survive arduous environmental conditions, such as particulate filled flowing streams. Experiments which followed using the Empore disks involved placing the disk between two membranes in order to maintain the disk's integrity.

Polymeric Resin Evaluation:
The use of polymeric resins for the active sampling of environmental contaminants from water has been the focus of significant research efforts (25, 26, 28, 34-41). Specially modified polystyrene-divinylbenzene resins and carbonaceous sorbents have the ability to actively sequester polar organic contaminants. The resins used in the following research included Isolute ENV+ and a series of the Ambersorb® carbonaceous adsorbents. All of these resins are characterized as having fast mass transfer, high capacity, and excellent mechanical strengths.

Isolute ENV+ is a third generation hyper-crosslinked polystyrene-divinylbenzene resin which has been optimized for the retention of very polar water soluble analytes (27). Proprietary modifications to the resin has resulted in an inherent hydrophilicity which allows wetting of the resin to occur without the use of organic intermediaries. Analyte-resin sorption occurs by electron-donor interactions between the aromatic rings of the resin matrix and the aromatic and/or π bonds of the analyte (25). The irregularly shaped resin particles have a surface area of 980 m^2/g, an average particle size of 80 μm, and an average pore size of 100 Å (42). These attributes allow for high recoveries of a wide range of analytes at flow rates in excess of 60 mL/min in certain column configurations.

The use of Carbonaceous adsorbents for the extraction of chemicals from water is one of the oldest forms of solid-phase extraction (25, 33, 34-41). Early carbon-based sorbents had problems of irreversible sorption, selective compound affinity, catalytic activity on the carbon surface and poor pressure resistance (34). Over time changes in the preparation of carbonaceous supports provided sorbents that were more homogeneous and lessened the problems of earlier sorbents. Graphitized carbon provides the unique

ability to extract acidic compounds along with basic and neutral compounds all in one step without pH adjustment of the sample due to the presence of positively charged active centers on the adsorbent's surface (39). It is important to note that large differences in sorption characteristics and performance can occur in the same sorbent family due to differences in the sorbent preparation.

Ambersorb carbonaceous adsorbents evaluated during this research included A-563, A-564, A-572, and A-1500. This family of adsorbents are spherical beads with a surface area up to 1100 m^2/g (43). Ambersorb adsorbents are produced by pyrolysis of a highly sulfonated styrene-divinylbenzene macroreticular ion-exchange resin with moderate surface area. Following pyrolysis, the adsorbent has a wide range of porosities, high capacities, and excellent physical integrity. The Ambersorb resins are available in varying surface polarities with the A-563 being the most non-polar and the A-1500 the most polar. The A-1500 allows the extraction of water samples without any pretreatment such as wetting with water-miscible organic solvents.

The initial studies involved a batch extraction of ethynylestradiol, atrazine and diazinon. For each compound, 200 mg of each resin was placed in a culture tube containing 10 mL of deionized water and the analyte of interest. The tubes were mixed several times using a Vortex-Genie™ mixer and then allowed to sit overnight. The tubes were then filtered through a glass wool fitted column keeping the effluent for analysis by liquid scintillation. The resin was eluted with 100 mL of 20/80 methanol/dichloromethane. Table 4 contains the recovery data for the batch extractions. For ethynylestradiol, the ENV+ resin had the overall highest recovery of 113.1% while all the others were less than 70%. The resin of choice for atrazine and diazinon was the A-1500 with total recoveries of 85.7% and 99.3% respectively.

Analyte	% Recovery	ENV+	A-563	A-564	A-572	A-1500
Ethynyl estradiol	% in water	31.8 ± 3.60	9.24 ± 2.15	34.2 ± 3.07	15.1 ± 2.44	7.00 ± 1.16
	% in resin eluate	81.3 ± 8.58	35.3 ± 2.34	35.1 ± 0.72	49.3 ± 9.47	51.3 ± 9.83
	Total %	113.1 ± 9.30	44.5 ± 3.18	69.3 ± 3.15	64.4 ± 9.78	58.3 ± 9.90
Atrazine	% in water	6.48 ± 1.32	25.6 ± 2.19	13.4 ± 1.29	11.6 ± 0.28	6.35 ± 0.97
	% in resin eluate	68.1 ± 1.10	51.5 ± 3.42	57.3 ± 2.18	70.6 ± 0.85	79.3 ± 9.48
	Total %	74.6 ± 1.72	77.1 ± 4.06	70.7 ± 2.53	82.2 ± 0.89	85.7 ± 9.53
Diazinon	% in water	44.3 ± 11.2	29.3 ± 1.90	22.1 ± 2.30	11.5 ± 2.00	13.2 ± 2.00
	% in resin eluate	42.1 ± 8.20	59.0 ± 5.40	62.2 ± 2.56	82.8 ± 3.29	86.1 ± 6.26
	Total %	86.4 ± 13.9	88.3 ± 5.72	84.3 ± 3.44	94.3 ± 3.85	99.3 ± 6.57

Table 4 – Batch Extraction of 5 Adsorbents. Values are percentage of analyte ± standard deviation (n=3).

Comparison of the known properties of each resin along with the results of the preliminary batch extraction, led to the selection of ENV+ and A-1500 for further study due to their ability to extract polar compounds from water. Total mass recovery for the analytes were not obtained for these two resins using the 20/80 methanol/dichloromethane solvent system, therefore, a study using six different solvent

mixtures with varied elutropic strengths was performed. For this study, mini-columns were constructed using glass pasteur pipettes containing 100 mg of resin secured between glass-fiber filter plugs. Ethynylestradiol and atrazine were chosen due to their high water solubility and difficulties in recovering these compounds once bound to the resins. The compounds were applied directly to the top of each column followed by a 5 mL DI water rinse to elute any analyte not retained by the resin. The rinse was collected and analyzed by LSC. The columns were then eluted with five 10 mL portions of solvent which were collected, reduced in volume and counted separately to determine when the bulk of the analyte was recovered. The six solvent combinations used were: 50/50 methanol/toluene; 100% methanol; 100% toluene; 100% dichloromethane; 50/50 dichloromethane/toluene; and 10/10/80 methanol/toluene/dichloromethane. Tables 5 and 6 list the data for ethynylestradiol recovery and Tables 7 and 8 are for atrazine.

Solvent*	% in water	1st 10 mL	2nd 10 mL	3rd 10 mL	4th 10 mL	5th 10 mL	Total % in Resin	Mass Balance
A	0.63 ± 0.10	89.1 ± 1.98	0.69 ± 0.01	0.52 ± 0.05	0.39 ± 0.05	0.83 ± 0.23	91.5 ± 1.99	92.2 ± 2.00
B	0.59 ± 0.12	89.3 ± 4.76	1.24 ± 0.47	0.82 ± 0.37	0.46 ± 0.15	0.73 ± 0.24	92.6 ± 4.81	93.1 ± 4.81
C	0.65 ± 0.13	56.0 ± 7.41	15.1 ± 6.57	3.61 ± 2.95	2.77 ± 1.11	1.20 ± 0.50	78.7 ± 10.4	79.3 ± 10.4
D	3.87 ± 1.25	85.4 ± 3.98	1.59 ± 0.40	0.35 ± 0.09	0.29 ± 0.06	0.25 ± 0.05	87.9 ± 4.00	91.8 ± 4.19
E	3.47 ± 0.68	76.4 ± 5.93	6.45 ± 1.18	1.88 ± 1.17	0.83 ± 0.43	0.39 ± 0.10	86.0 ± 6.17	89.4 ± 6.21
F	2.87 ± 0.78	97.7 ± 1.48	6.47 ± 1.02	0.17 ± 0.04	0.24 ± 0.02	0.19 ± 0.02	105 ± 1.80	107.6±1.96

Table 5 – Ethynylestradiol Recovery from Isolute ENV+. Values listed are percentage of analyte ± standard deviation (n=3). *solvent mixtures: A – 50/50 methanol/toluene; B – 100% methanol; C – 100% toluene; D – 100% dichloromethane; E – 50/50 dichloromethane/toluene; F – 10/10/80 methanol/toluene/dichloromethane

Solvent*	% in water	1st 10 mL	2nd 10 mL	3rd 10 mL	4th 10 mL	5th 10 mL	Total % in Resin	Mass Balance
A	0.68 ± 0.19	52.6 ± 10.9	5.72 ± 2.40	1.57 ± 0.37	1.02 ± 0.12	3.72 ± 1.26	64.6 ± 11.2	65.3 ± 11.2
B	0.80 ± 0.08	0.91 ± 0.27	0.57 ± 0.15	0.83 ± 0.64	0.67 ± 0.25	0.42 ± 0.05	3.40 ± 0.75	4.20 ± 0.76
C	0.89 ± 0.30	3.31 ± 1.54	2.40 ± 0.53	1.93 ± 0.24	2.15 ± 0.41	2.31 ± 0.97	12.1 ± 1.95	13.0 ± 1.98
D	0.70 ± 0.23	2.73 ± 0.64	0.81 ± 0.32	0.74 ± 0.38	0.74 ± 0.33	0.63 ± 0.16	5.65 ± 0.89	6.35 ± 0.92
E	0.83 ± 0.11	3.30 ± 0.32	1.86 ± 0.26	1.39 ± 0.12	1.34 ± 0.05	1.01 ± 0.06	8.90 ± 0.43	9.73 ± 0.45
F	0.92 ± 0.35	24.1 ± 3.21	0.13 ± 0.01	2.12 ± 0.23	1.68 ± 0.18	1.33 ± 0.13	29.4 ± 3.23	30.3 ± 3.24

Table 6 – Ethynylestradiol Recovery from Ambersorb 1500. Values listed are percentage of analyte ± standard deviation (n=3). *solvent mixtures: A – 50/50 methanol/toluene; B – 100% methanol; C – 100% toluene; D – 100% dichloromethane; E – 50/50 dichloromethane/toluene; F – 10/10/80 methanol/toluene/dichloromethane

Solvent*	% in water	1st 10 mL	2nd 10 mL	3rd 10 mL	4th 10 mL	5th 10 mL	Total % in Resin	Mass Balance
A	1.97 ± 0.09	86.6 ± 2.78	0.38 ± 0.03	0.18 ± 0.03	0.09 ± 0.01	0.07 ± 0.01	87.3 ± 2.78	89.3 ± 2.78
B	2.20 ± 0.05	73.1 ± 2.65	0.27 ± 0.01	0.14 ± 0.03	0.08 ± 0.02	0.07 ± 0.00	73.7 ± 2.65	75.9 ± 2.65
C	2.44 ± 0.76	46.2 ± 13.6	17.2 ± 5.15	7.06 ± 3.15	3.34 ± 2.44	1.53 ± 1.21	75.3 ± 15.1	77.8 ± 15.1
D	3.25 ± 0.66	83.0 ± 1.87	3.22 ± 0.77	0.51 ± 0.04	0.24 ± 0.02	0.18 ± 0.04	87.2 ± 2.02	90.4 ± 2.13
E	3.64 ± 0.85	72.9 ± 0.48	5.67 ± 1.87	3.83 ± 0.57	2.22 ± 0.58	0.63 ± 0.04	85.3 ± 2.10	88.9 ± 2.26
F	2.99 ± 1.20	97.1 ± 5.29	0.30 ± 0.11	0.22 ± 0.08	0.18 ± 0.04	0.13 ± 0.05	97.9 ± 5.29	100.9±5.43

Table 7 – Atrazine Recovery from Isolute ENV+. Values listed are percentage of analyte ± standard deviation (n=3). *solvent mixtures: A – 50/50 methanol/toluene; B – 100% methanol; C – 100% toluene; D – 100% dichloromethane; E – 50/50 dichloromethane/toluene; F – 10/10/80 methanol/toluene/dichloromethane

Solvent*	% in water	1st 10 mL	2nd 10 mL	3rd 10 mL	4th 10 mL	5th 10 mL	Total % in Resin	Mass Balance
A	0.05 ± 0.01	86.3 ± 2.25	1.52 ± 0.07	0.78 ± 0.03	0.39 ± 0.04	0.21 ± 0.03	89.2 ± 2.25	89.3 ± 2.25
B	0.05 ± 0.01	3.45 ± 0.47	25.7 ± 24.7	11.4 ± 4.06	15.8 ± 15.4	11.3 ± 8.23	67.7 ± 30.5	67.7 ± 30.5
C	0.05 ± 0.01	60.8 ± 1.84	7.23 ± 0.84	2.28 ± 0.31	1.13 ± 0.08	0.79 ± 0.09	72.2 ± 2.05	72.3 ± 2.05
D	0.28 ± 0.05	73.1 ± 0.67	2.26 ± 0.26	1.40 ± 0.07	0.94 ± 0.13	0.85 ± 0.46	78.6 ± 0.87	78.8 ± 0.87
E	0.40 ± 0.08	81.3 ± 0.91	3.01 ± 0.18	1.65 ± 0.01	1.35 ± 0.12	1.13 ± 0.07	88.4 ± 0.94	88.8 ± 0.94
F	0.57 ± 0.10	87.9 ± 1.33	2.26 ± 0.61	0.73 ± 0.08	0.52 ± 0.09	0.34 ± 0.03	91.8 ± 1.47	92.3 ± 1.47

Table 8 - Atrazine Recovery from Ambersorb 1500. Values listed are percentage of analyte ± standard deviation (n=3). *solvent mixtures: A – 50/50 methanol/toluene; B – 100% methanol; C – 100% toluene; D – 100% dichloromethane; E – 50/50 dichloromethane/toluene; F – 10/10/80 methanol/toluene/dichloromethane

Isolute ENV+ exhibited an excellent capacity for retaining ethynylestradiol and atrazine with less than 3.9% of the total analyte mass recovered in the deionized water rinses. For the recovery of ethynylestradiol the best solvent combination was 10/10/80 methanol/toluene/dichloromethane with a total mass recovery of 107.6%. Several of the other solvent combinations resulted in recoveries of greater than 90%. Atrazine was more difficult to recover using most solvent combinations, however, an excellent 100.9% mass balance was achieved using the 10/10/80 methanol/toluene/dichloromethane solvent system. Recovery of analytes from Ambersorb 1500 was not as successful. The resin did exhibit better retention of the analytes with less than 0.92% recovered from the rinses. Ethynylestradiol was strongly retained by the A-1500 with a best case of only 65.3% of the total mass recovered using 50/50 methanol/toluene. All other solvent combinations exhibited recoveries of 30% or less. Atrazine was easier to reclaim from the A-1500 with 92.3% recovered using 10/10/80 methanol/toluene/dichloromethane mixture. Recoveries of 89.3% and 88.8% were observed when using 50/50 methanol/toluene and 50/50 dichloromethane/toluene respectively. The recovery data has demonstrated that complete elution of ethynylestradiol and atrazine can be achieved when using Isolute ENV+ with the 10/10/80 methanol/toluene/dichloromethane solvent system. Low recoveries of

ethynylestradiol from the A-1500 discouraged further evaluation of this resin as an independent sorbent.

The affinity for sequestering polar organic compounds has been demonstrated for Isolute ENV+ and Ambersorb 1500. The Isolute ENV+ exhibited the capability for excellent recoveries but had a lower overall affinity for the polar organics. Ambersorb 1500 had a greater affinity for the analytes, but poor recoveries resulted due to problems in elution of the analytes.

To overcome the recovery problems of A-1500, dispersion of a small portion of the resin on a gel permeation styrene-divinylbenzene copolymer was attempted. An adaptation of the procedure by Huckins *et al.* involved dispersing finely ground A-1500 on S-X3 Bio Beads (44). The S-X3 Bio Beads are a member of the styrene-divinylbenzene copolymer series with 3% cross-linking of the polymer matrix. This degree of cross-linking results in the highest amount of adherence of carbon particles. The S-X3 dispersed A-1500 was prepared by admixing the materials in dichloromethane which acts as a swelling agent for the S-X3. The binding of the A-1500 to the S-X3 is not well understood, but it is believed that the phenomena is the result of electrostatic and covalent aromatic interactions between the two materials as well as partial inclusion of the A-1500 in the swelled gel matrix (44). The ground A-1500 particles should not be larger than 70 μm due to poor adherence of larger particles to the S-X3. Optimal particle sizes range from 0.1 to 50 μm. It was estimated that the A-1500 coated S-X3 had an average loading of 5% by weight of the A-1500 particles. A similar attempt to coat the Isolute ENV+ with the ground Ambersorb 1500 was unsuccessful. It is possible that the Isolute ENV+ has too small a particle size and/or is too highly cross-linked to allow any swelling or coating of the resin.

The S-X3 dispersed A-1500 exhibited the sorptive characteristics of the parent Ambersorb 1500 resin, while reducing the total carbon loading. Consequently, a mixture of the S-X3 dispersed A-1500 with Isolute ENV+ was employed in order to maximize the sequestering affinity and elution properties of each. An admixture of 20/80 (w/w) S-X3 dispersed A-1500/Isolute ENV+, hereafter referred to as "resin mix", was characterized using column extractions of each analyte.

Six mini-columns were constructed by placing 100 mg of the resin mix in a glass- fiber filter plugged pasteur pipet. Ethynylestradiol was added directly on column followed by a 5 mL DI water rinse. Ethynylestradiol was selected as the test compound due to the previous difficulty encountered in the sequestration and elution of this compound. The water rinse was collected and counted by LSC. The columns were subsequently eluted with 50 mL of the 10/10/80 methanol/toluene/dichloromethane solvent mixture hereafter referred to as "solvent mix". This experiment was repeated the following week in order to test the day-to-day variability of the method. Table 9 contains data for the trials. Uptakes of ethynylestradiol by the resin mix of 97.2% and 96.4% for Day 1 and 2 respectively demonstrate the usefulness of the resin mix for the sequestration and recovery of polar organic compounds.

Day 1			Day 2		
Trial	% in H_2O Rinse	% in Resin Eluate	Trial	% in H_2O Rinse	% in Resin Eluate
A	1.99	97.2	A	1.22	97.5
B	1.70	94.7	B	1.44	104.4
C	1.22	98.5	C	1.94	95.5
D	1.41	99.3	D	1.38	93.8
E	1.17	96.5	E	1.07	92.8
F	1.44	97.0	F	1.36	94.2
Average	1.49 ± 0.31	97.2 ± 1.60	Average	1.40 ± 0.30	96.4 ± 4.26
Total Mass Balance = 98.7 ± 1.54			**Total Mass Balance = 97.8 ± 4.27**		

Table 9 – Mini-columns with 100 mg of resin mix (20 mg dispersed A-1500, 80 mg ENV+) spiked with Ethynylestradiol. Mass balance reported as percent recovered ± standard deviation (n=6).

Due to the success of the resin mix for the sequestration and optimal recovery of ethynylestradiol, the experiment was repeated using atrazine and diazinon as the analytes. Results for the atrazine and diazinon trials are listed in Tables 10 and 11 respectively. The resin mix performed well for both analytes. Excellent day-to-day recoveries of 97.8% and 98.3% were observed for diazinon. The day-to-day recoveries of 87.7% and 88.7% for atrazine were lower than the observed recoveries of the other analytes however they were considered to be within acceptable limits. The extraction and recovery capabilities combined with a high degree of reproducibility demonstrated the suitability of this resin mix for environmental sampling.

Day 1			Day 2		
Trial	% in H_2O Rinse	% in Resin Eluate	Trial	% in H_2O Rinse	% in Resin Eluate
A	0.26	87.5	A	0.40	88.0
B	0.22	88.5	B	0.44	88.6
C	0.18	85.8	C	0.40	86.5
D	0.24	87.7	D	0.32	88.2
E	0.24	87.8	E	0.36	89.3
F	0.19	87.5	F	0.54	89.0
Average	0.22 ± 0.03	87.5 ± 0.90	Average	0.41 ± 0.08	88.3 ± 0.99
Total Mass Balance = 87.7 ± 0.90			**Total Mass Balance = 88.7 ± 0.99**		

Table 10 – Mini-columns with 100 mg of resin mix (20 mg dispersed A-1500, 80 mg ENV+) spiked with Atrazine. Mass balance reported as percent recovered ± standard deviation (n=6).

Day 1			Day 2		
Trial	% in H_2O Rinse	% in Resin Eluate	Trial	% in H_2O Rinse	% in Resin Eluate
A	0.10	96.4	A	0.25	97.7
B	0.11	98.3	B	0.22	97.8
C	0.10	93.9	C	0.30	97.1
D	0.15	98.2	D	0.25	98.7
E	0.16	101.2	E	0.21	100.2
F	0.19	98.1	F	0.23	97.1
Average	0.14 ± 0.04	97.7 ± 2.41	Average	0.24 ± 0.03	98.1 ± 1.18
Total Mass Balance = 97.8 ± 2.41			**Total Mass Balance = 98.3 ± 1.18**		

Table 11 – Mini-columns with 100 mg of resin mix (20 mg dispersed A-1500, 80 mg ENV+) spiked with Diazinon. Mass balance reported as percent recovered ± standard deviation (n=6).

True environmental water samples will require the extraction of one or more liters of water in order to achieve the analyte mass necessary for instrumental analysis. To test the ability of the resin mix to extract polar analytes from realistic quantities of water, the following experiments were performed. One liter DI water samples were fortified with ethynylestradiol, atrazine, and diazinon individually at high and low water concentrations. The water samples were poured through 1 cm (i.d.) glass chromatography columns containing 100 mg of the resin mix. The resin was eluted with 50 mL of the solvent mix. Aliquots of the column effluent along with the resin eluate were counted by LSC. The results for each analyte at two concentrations are listed in Table 12.

Analyte	Analyte Mass	% Column Effluent	% in Resin Eluate	Mass Balance
Ethynylestradiol	0.296 ng	2.87 ± 0.60	91.2 ± 0.49	94.1 ± 0.77
	1.00 mg	6.15 ± 1.74	91.8 ± 1.50	98.0 ± 2.30
Atrazine	1.33 µg	9.89 ± 3.82	79.4 ± 1.96	89.3 ± 4.29
	1.00 mg	16.8 ± 5.39	81.3 ± 2.95	98.1 ± 6.14
Diazinon	1.49 µg	1.77 ± 0.55	97.5 ± 0.23	99.3 ± 0.60
	1.03 mg	1.80 ± 1.36	96.3 ± 2.13	98.0 ± 2.53

Table 12 – Resin mix extraction of 1 L water samples fortified with high and low levels of Ethynylestradiol, Atrazine, and Diazinon. Values reported as percent of analyte ± standard deviation (n=3).

The extraction of the analytes at high and low water concentrations was successful. Greater amounts of the analytes were recovered from the column effluent, up to 16.8% for 1.00 mg/L atrazine, due to the breakthrough of this water-soluble analyte. The mass balance of 89% or greater for each trial further demonstrates the suitability of the resin mix for environmental sampling.

A final point of concern regarding the use of the resin mix as an integrative sampling media was the stability of the analytes on the resin over a period of up to 28 days. For the resin mix to be considered an effective sampling media, it must maintain the integrity of the analytes, minimize depuration of sequestered materials back into the surrounding environment and allow full recovery of these sequestered analytes following exposure. The Isolute ENV+ was believed to perform adequately with the only concern being loss of extracted materials over time. There was more concern with the S-X3 dispersed A-1500 over irreversible adsorption and catalytic degradation of the analytes which has been reported as a problem with some carbonaceous adsorbents (25, 33).

An experiment was designed to measure the stability of the resin mix over a 28 day period. Glass chromatography columns (1 cm i.d.) containing 100 mg of the resin mix were used to extract 5 sets in triplicate, 1 L portions of DI water containing ~1 µg of either ethynylestradiol, atrazine, or diazinon. Aliquots of the column effluent were counted as the columns were left to "age". The resin mix was left moist with a water level ~1 cm above the sorbent bed to simulate the conditions of the resin mix during use in a sampling device. At regular intervals throughout the 28 day period, a set of three columns for each analyte were analyzed. The remaining column water was drained and collected prior to resin elution with 50 mL of the solvent mix. Recovery data for each analyte is listed in Table 13.

Analyte	Days Aged	% Column Effluent	% in Resin Eluate	Mass Balance
Ethynylestradiol	2	3.19 ± 1.77	89.2 ± 1.37	92.4 ± 2.24
	7	3.73 ± 1.65	85.0 ± 3.07	88.7 ± 3.49
	14	N/A*	N/A*	N/A*
	21	5.71 ± 2.88	84.6 ± 1.79	90.3 ± 3.39
	28	4.34 ± 3.38	84.5 ± 3.98	88.9 ± 5.15
Diazinon	2	3.35 ± 1.42	91.6 ± 2.16	95.0 ± 2.58
	7	0.97 ± 0.60	93.1 ± 1.50	94.1 ± 1.62
	14	3.75 ± 2.91	92.6 ± 3.23	96.4 ± 4.35
	21	3.55 ± 1.09	91.8 ± 2.46	95.4 ± 2.69
	28	2.64 ± 2.48	92.0 ± 1.76	94.6 ± 2.80
Atrazine	2	12.6 ± 2.53	85.0 ± 2.65	97.6 ± 3.66
	7	9.06 ± 1.97	82.8 ± 1.95	91.9 ± 2.77
	14	9.50 ± 1.17	79.6 ± 2.73	89.1 ± 2.97
	21	13.1 ± 3.91	79.6 ± 4.69	92.7 ± 6.11
	28	8.49 ± 1.89	80.6 ± 1.78	89.1 ± 2.60

Table 13 – Resin mix "aging" over 28 days. Ethynylestradiol, Diazinon, and Atrazine were applied at 1.0 µg/L, 1.49 µg/L, and 1.33 µg/L respectively. Values reported as percent recovered ± standard deviation (n=3). * no measurements were taken on this day.

The results of the resin aging study indicate that sequestered polar organic contaminants can be adequately recovered up to 28 days after initial uptake. Following 28 days, a mass balance of at least 88.9% was achieved for each analyte. The analytes were not depurated from the resin mix as no more than 0.01% of any analyte was recovered from the water which was left in the columns while aging. With only about a 10% loss in recovery, which can be mostly accounted for in replicate variability and expected loss from solvent evaporations and solution transfers, the resin mix appears not to be limited by any potential shortcoming.

Membrane Evaluation:

The membrane enclosure acts as a semipermeable barrier between the sequestering media and the surrounding environment. It is desirable that the membrane allows compounds of interest to pass through essentially uninhibited, but that larger materials such as particulate matter, colloids and biogenic mass are selectively excluded. Introduction of these excluded materials into the sequestering media could result in monopolization of active binding sites on the sequestering media necessary for contaminant uptake and potential interferences during instrumental analysis. Fouling of the membrane's exterior due to periphytic growth can affect the uptake rates of contaminants. It is important that the membrane selected has some resistance to biofouling in order to allow for extended sampling periods.

Membranes can be roughly classified via their structure as nonporous and porous membranes. Separations involving a nonporous membrane require the dissolution of the solute in the membrane in order for the solute to diffuse thorough the membrane. This results in a membrane that is highly selective to solute permeation. Porous membranes are largely non-selective, open to transport of any compound of appropriate size by simple diffusion through the pores. Although separations through a porous membrane are mainly determined by size, dissolution of the solute molecules into the membrane matrix is often observed (45). The polymeric membrane materials studied in this research were a combination of porous and nonporous membranes.

The selection of a suitable membrane, which will allow the uptake of polar organic compounds, involved the evaluation of several different membrane materials. Low density polyethylene (LDPE), polyvinylidene fluoride, regenerated cellulose, an acrylic copolymer, nylon, hydrophilic polypropylene, and polyethersulfone were the membranes studied. These membranes differ in their degree of hydrophobicity, thermal and mechanical properties, resistance to degradation, and commercially available formats. An ideal membrane is one that maintains the mechanical and thermal properties of a hydrophobic membrane such as LDPE but would be easily wettable (have lower contact angles with water) allowing the permeation of the more polar organic compounds (46).

Huckins *et al.* described the use of LDPE in the construction of SPMDs (47, 48). LDPE is a nonporous hydrophobic polymer which allows diffusion through transient cavities formed by random thermal motions of the polymeric chain. These transient cavities (~10 Å in diameter) are similar in size and function to the pores in biomembranes such as the gill epithelium of fish thereby allowing the SPMD to mimic the bioconcentration process

of aquatic organisms. LDPE is a rugged, commercially available thermoplastic amenable to customized construction and formation of molecular welds by heat sealing.

SPMDs (46 cm long PE tube, 0.5 mL of triolein), made from LDPE as described by Huckins (47), were exposed to ethynylestradiol in 1-liter microcosms for one month. Three exposures were performed with the water buffered to pH's of 4, 6, and 8 respectively. Different pH's were used to determine if there was any ionization occurring with ethynylestradiol that may result in the lack of uptake into SPMDs. Ionization of the phenolic hydroxide at the 3 position would prevent the diffusion through the LDPE membrane. Following exposure, the SPMDs were dialyzed with two 75 mL portions of 10/90 isopropanol/hexane over 24 hours. Table 14 contains the exposure data for this study. Changes in pH seemed to have little positive effect on uptake as less than 4.5% was recovered from the SPMDs in all trials.

pH	% in Membrane[a]	% in Dialysate	Total %
4	1.87 ± 1.12	2.63 ± 2.15	4.50 ± 2.42
6	1.91 ± 0.90	1.62 ± 1.19	3.53 ± 1.49
8	1.56 ± 0.10	1.25 ± 0.21	2.81 ± 0.23

Table 14 – One month SPMD exposure to Ethynylestradiol at various pHs. Values reported as percent ethynylestradiol recovered ± standard deviation (n=3). [a] percent remaining in the SPMD following dialysis.

The polyethylene used in SPMDs was further evaluated for the uptake of ethynylestradiol by varying the sequestering media inside the layflat tubing. Four different configurations were exposed to ethynylestradiol in 500 mL of water for 1 week. The exposure vessels were shaken at 100 rpm and maintained at room temperature. The four configurations include: normal SPMD (46 cm long PE tube, 0.5 mL triolein), PE only (46 cm long sealed tube, no sequestration media), PE/H_2O (46 cm long PE tube, 0.5 mL DI water), and PE/Resin (46 cm long PE tube, 0.5 mL DI water + 100 mg resin mix). Following exposure, the SPMDs were dialyzed in two 75 mL portions of 10/90 isopropanol/hexane over 24 hours. The dialysate was reduced in volume and transferred to LSC vials for counting. The contents of the membrane, if any, were placed into vials as was the dialyzed membrane for counting. The PE/Resin devices were cut open after dialysis and the resin was transferred into a glass 1 cm (i.d.) chromatography column. Subsequent elution followed with 50 mL of the solvent mix which was reduced in volume and counted by LSC. Results of the study are listed in Table 15. The high percentages (>73%) of ethynylestradiol remaining in the exposure water after 1 week indicate the PE tubing is unsuitable for use when sampling polar organic compounds.

Configuration	% in Water	% Membrane[a]	% in Contents[b]	% Dialysate	Mass Balance
Normal SPMD	84.1 ± 8.07	2.32 ± 0.40	1.00 ± 0.32	2.28 ± 1.70	89.7 ± 8.26
PE only	100.6 ± 18.3	3.00 ± 1.78	0.44 ± 0.26[c]	2.53 ± 0.89	106.6 ± 18.4
PE / H_2O	87.3 ± 6.50	3.58 ± 0.58	0.22 ± 0.02	3.87 ± 2.39	95.0 ± 6.95
PE / Resin	73.7 ± 9.28	3.18 ± 0.94	0.55 ± 0.06	8.18 ± 0.35	85.6 ± 9.33

Table 15 – One week exposure of various PE membrane configurations to Ethynylestradiol. Reported values represent percent recovered ± standard deviation (n=3). [a] percent of analyte recovered from membrane following dialysis. [b] sequestration media inside the PE tube. [c] percent in the residual dialysis solvent which recovered from inside the PE tube.

The Spectra/Por® Type F polyvinylidene fluoride (PVDF) membrane was selected due to its availability as layflat tubing, strength, and ability to be heat sealed. PVDF is a highly resistant material, compatible with most organic solvents as well as aqueous acids and bases (49). PVDF is a nonporous membrane and is slightly more hydrophilic than polyethylene. The transport cavities in the PVDF membrane are rated with a molecular weight cut-off of 80,000 Daltons. It was believed that polar molecules would pass through the large transport corridors present in the polymer matrix.

PVDF membranes (6.4 cm long) were filled with 100 mg of resin mix and 0.25 mL of DI water. The devices were exposed in 500 mL of water fortified with either diazinon or ethynylestradiol and shaken at 100 rpm for 18 hours. Following exposure, the devices were analyzed according to the procedure used for the PE/Resin devices in the polyethylene study. Results from the exposures are given in Table 16. The PVDF devices demonstrated little uptake of the compounds, especially ethynylestradiol. The amounts of diazinon and ethynylestradiol recovered from the devices as a whole were 20.9% and 2.08% respectively. Apparently the hydrophobic nature of the membrane was a strong factor limiting the uptake of the hydrophilic compounds regardless of the transport corridor size.

Analyte	% in Water	% Membrane[a]	% Resin Mix[a]	% Dialysate	Mass Balance
Diazinon	76.4 ± 7.77	0.77 ± 0.10	0.17 ± 0.09	20.0 ± 2.82	97.3 ± 8.27
Ethynylestradiol	92.5 ± 1.17	0.26 ± 0.10	0.73 ± 0.06	1.09 ± 0.42	94.6 ± 1.25

Table 16 – 18 hour exposure of PVDF membranes containing 100 mg of resin mix to Diazinon and Ethynylestradiol. Values reported as percent recovered ± standard deviation (n=3). [a] percent analyte recovered from the membrane and resin mix eluate following dialysis.

Spectra/Por® 6 regenerated cellulose (molecular weight cut-off of 1,000 daltons) was selected due to its availability as layflat tubing and its common use as a dialysis membrane. The membrane is composed of cellulose reconstituted from cotton linters (49). Regenerated cellulose membranes carry no fixed charge and do not absorb most solutes. Cellulose membranes are one of the most hydrophilic membranes available.

Potential drawbacks to the use of cellulose membranes as environmental samplers are its sensitivity to acid or alkaline hydrolysis and biological degradation (50).

Pieces of the cellulose tubing were cut 7.6 cm long , filled with 100 mg of the resin mix and 0.25 mL of DI water. Cellulose is not a thermoplastic and cannot be heat sealed therefore a dialysis bag clip was used to enclose the resin mix. The devices were placed in 500 mL of water spiked with analyte and shaken at 100 rpm for 18 hours. Following exposure, the devices were analyzed in the same manner as the PVDF membranes. Table 17 contains uptake data for the exposures. The cellulose devices exhibited a limited uptake of 16.3% for both diazinon and ethynylestradiol. The hydrophilicity of cellulose was intriguing, however, the limitations of the potential for biological degradation of the membrane during exposure in the environment eliminated it from further consideration.

Analyte	% in Water	% Membrane[a]	% Resin Mix	% Dialysate	Mass Balance
Diazinon	80.0 ± 3.84	0.11 ± 0.05	8.10 ± 2.53	8.13 ± 0.83	96.3 ± 4.67
Ethynylestradiol	73.2 ± 9.09	0.48 ± 0.18	10.4 ± 2.79	5.44 ± 3.10	89.5 ± 10.0

Table 17 – 18 hour exposure of Cellulose membranes containing 100 mg resin mix for Diazinon and Ethynylestradiol. Values reported as percent recovered ± standard deviation (n=3). [a] percent analyte recovered from the membrane following dialysis.

The Versapor® acrylic copolymer is a hydrophilic membrane which is commercially available in disk form (47 mm diameter, 0.2 μm pore size). The acrylic copolymer is on a nonwoven support which adds to the strength and durability of the disk. The disks were designed as filtration media to be used in applications such as ambient air and industrial hygiene monitoring and ground and surface water sampling requiring tensile strengths in excess of 3000 psi (51).

Attempts to form heat seals were unsuccessful as any seals made were very brittle. Exposure studies were performed by enveloping 100 mg of the resin mix in two of the Versapor disks which were secured in the stainless steel ring supports. The devices were exposed for 24 hours in 1-liter of water fortified with either diazinon or ethynylestradiol. The microcosms were magnetically stirred throughout the experiment. Exposure data is listed in Table 18. Marginal uptake was observed for the two analytes. After 24 hours, 81.2% and 80.4% of the diazinon and ethynylestradiol remained in the water.

Analyte	% in Water	% Membrane	% Resin Eluate	Mass Balance
Diazinon	81.2 ± 5.27	0.61 ± 0.06	12.0 ± 1.86	93.8 ± 5.59
Ethynylestradiol	80.4 ± 3.78	1.18 ± 0.05	10.3 ± 1.16	91.9 ± 3.95

Table 18 – 24 hour exposures with Acrylic membrane + 100 mg of resin mix for Diazinon and Ethynylestradiol. Values reported as percent recovered ± standard deviation (n=3).

Nylaflo™ nylon 6,6 membrane disks (47 mm diameter, 0.2 μm pore size) were evaluated due to their hydrophilic nature. The carboxylic acid amide groups in the matrix hydrogen bond with one another increasing the strength and heat resistance of the polymer (52). Nylaflo membrane disks are designed to be used for the filtration of HPLC samples and mobile phases and should not contribute any significant artifacts to subsequent analyses.

The membrane lacked the tensile strength of some of the other membranes studied. Attempts to heat seal the disks also failed. Devices were constructed using the ring supports as described for the Versapor disks. Devices with only membrane disks and ones with membrane plus 100 mg of the resin mix were exposed for one week to water fortified with ethynylestradiol. The 1-liter microcosms were magnetically stirred during the exposure. Water concentrations of 66.8% and 34.2% for the nylon only and nylon + resin devices, respectively, demonstrated an excellent uptake for ethynylestradiol. Although the uptake of polar chemicals using this membrane was promising, this membrane lacked the durability necessary for prolonged exposures in the environment.

Two final membranes were selected for evaluation due to their availability and physical characteristics. These are Supor® polyethersulfone (PES) and GH Polypro® hydrophilic polypropylene (Hy-PP). Both are commercially available as filtration membranes with various pore sizes in the form of precut disks or flat sheets. It is desirable to use membranes with the smallest diameter pore size possible which will allow contaminants of interest to pass through but will retard the passage of unwanted macromolecules. The available pore sizes of 0.1 μm for polyethersulfone and 0.2 μm for hydrophilic polypropylene were selected. Disks (47 mm diameter) were chosen to be used in the stainless steel ring holders.

The polyethersulfone membrane is hydrophobic as the bulk polymer but becomes inherently hydrophilic as the polymerized membrane (46). Polyethersulfone is resistant to most organic solvents with the exception of chlorinated hydrocarbons. The 0.1 μm polyethersulfone membrane is prepared by casting a mixture of 15% polyethersulfone, 18% dimethylformamide, 66.5% polyethyleneglycol and 0.5% glycerine (46). Variations of these proportions will result in membranes of different pore sizes. The polyethyleneglycol and the glycerine, most of which is removed during the final stages of membrane production, aid in formation of the hydrophilic membrane. The polyethersulfone membranes have been designed for rapid filtration of biological, pharmaceutical, and environmental samples requiring a high throughput and low protein binding. Studies have demonstrated that PES outperforms nylon and PVDF membranes of equivalent pore sizes in terms of flow rate and total throughput (51). This membrane also exhibits superior strength compared to many of the other membranes studied.

The hydrophilic polypropylene has undergone a proprietary chemical alteration to increase the wettability of this inherently hydrophobic polymer. Hydrophilic polypropylene was designed as an all purpose filtration membrane compatible with essentially all aqueous and organic solvents (51). Like PES, Hy-PP exhibits a low protein binding and can be utilized for various operations. Both of these membranes

formed very weak heat seals, requiring the use of the ring holders to form a compression seal.

The polyethersulfone and hydrophilic polypropylene membranes were evaluated by exposing the membranes for 24 hours and 1 week to atrazine, diazinon, and ethynylestradiol in both stirred and non-stirred conditions. Recovery data for devices with membrane only and ones with membrane plus 100 mg of the resin mix exposed for 24 hours with stirring are presented in Tables 19 and 20 respectively. Tables 21 and 22 contain data for the 1 week stirred exposure of membrane only and membrane + resin devices. Comparison of PES and Hy-PP devices with the resin mix under non-stirred conditions for 24 hours and 1 week are listed in Tables 23 and 24. In one set of experiments, both types of membrane were used to construct devices containing the resin mix and were exposed for 24 hours with constant stirring. Following exposure, the whole device was taken through a dialysis process. This was attempted to possibly reduce the number of procedural steps in analysis as the exposed membrane layers already undergo a dialysis step to remove any sequestered analyte. Dialysis of the whole devices was performed using 100 mL of solvent per step. Following dialysis, the device was disassembled, membrane counted and resin eluted in a chromatography column as before. Table 25 contains the dialysis data.

		Membrane Only			
Polyethersulfone		% Water	% Dialysate	% Membrane[a]	Mass Balance
	Atrazine	94.6 ± 6.67	8.94 ± 0.22	0.29 ± 0.01	103.8 ± 6.67
	Diazinon	54.4 ± 2.86	35.0 ± 1.32	0.69 ± 0.10	90.1 ± 3.15
	Ethynylestradiol	54.4 ± 6.76	30.9 ± 3.00	0.92 ± 0.15	86.2 ± 7.40
Hydrophilic Polypropylene		% Water	% Dialysate	% Membrane[a]	Mass Balance
	Atrazine	100.8 ± 4.56	0.39 ± 0.03	0.13 ± 0.01	101.3 ± 4.56
	Diazinon	96.7 ± 3.09	3.34 ± 0.05	0.12 ± 0.01	100.2 ± 3.09
	Ethynylestradiol	79.6 ± 12.5	2.45 ± 0.31	0.34 ± 0.03	82.4 ± 12.5

Table 19 – 24 hour comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane Only devices under stirred conditions. Reported as percent recovered ± standard deviation (n=3).
[a] % remaining in membrane following dialysis.

		Membrane + Resin Mix			
Polyethersulfone		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	73.1 ± 6.90	4.83 ± 0.61	14.8 ± 1.71	92.7 ± 7.13
	Diazinon	65.4 ± 2.73	11.6 ± 0.55	12.8 ± 0.68	89.8 ± 2.87
	Ethynylestradiol	59.5 ± 6.77	18.9 ± 0.81	5.30 ± 0.60	83.7 ± 6.84
Hydrophilic Polypropylene		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	83.9 ± 16.3	0.67 ± 0.07	20.2 ± 2.68	104.8 ± 16.5
	Diazinon	68.6 ± 3.66	2.80 ± 0.58	18.9 ± 1.65	90.3 ± 4.06
	Ethynylestradiol	59.7 ± 4.21	1.48 ± 0.23	17.1 ± 1.97	78.3 ± 4.65

Table 20 – 24 hour comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane with 100 mg of Resin Mix devices under stirred conditions. Reported as percent recovered ± standard deviation (n=3).

		Membrane Only			
Polyethersulfone		% Water	% Dialysate	% Membrane[a]	Mass Balance
	Atrazine	84.7 ± 1.18	15.7 ± 0.38	0.40 ± 0.04	100.8 ± 1.24
	Diazinon	42.3 ± 1.78	51.0 ± 0.50	1.66 ± 0.20	95.0 ± 1.86
	Ethynylestradiol	27.6 ± 1.80	62.1 ± 1.61	1.68 ± 0.16	91.4 ± 2.42
Hydrophilic Polypropylene		% Water	% Dialysate	% Membrane[a]	Mass Balance
	Atrazine	N/A	N/A	N/A	N/A
	Diazinon	N/A	N/A	N/A	N/A
	Ethynylestradiol	N/A	N/A	N/A	N/A

Table 21 – 1 week comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane Only devices under stirred conditions. Reported as percent recovered ± standard deviation (n=3).
[a] % remaining in membrane following dialysis. N/A = exposures were not performed due to degradation of the Hydrophilic Polypropylene membrane over time.

		Membrane + Resin Mix			
Polyethersulfone		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	40.0 ± 11.2	8.03 ± 1.58	45.7 ± 5.75	93.7 ± 12.7
	Diazinon	20.7 ± 1.35	31.5 ± 1.15	36.9 ± 0.75	89.1 ± 1.93
	Ethynylestradiol	19.9 ± 2.21	34.1 ± 3.29	27.0 ± 3.32	81.0 ± 5.17
Hydrophilic Polypropylene		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	N/A	N/A	N/A	N/A
	Diazinon	N/A	N/A	N/A	N/A
	Ethynylestradiol	49.9 ± 7.42	0.99 ± 0.15	35.3 ± 6.29	86.2 ± 9.73

Table 22 - 1 week comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane with 100 mg of Resin Mix devices under stirred conditions. Reported as percent recovered ± standard deviation (n=3). N/A = exposures were not performed due to degradation of the hydrophilic polypropylene membrane over time.

		Membrane + Resin Mix			
Polyethersulfone		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	94.2 ± 13.2	1.45 ± 0.16	3.55 ± 0.46	99.2 ± 13.2
	Diazinon	89.5 ± 4.87	5.14 ± 0.30	0.74 ± 0.12	95.4 ± 4.88
	Ethynylestradiol	88.9 ± 4.39	4.78 ± 0.07	1.14 ± 0.07	94.8 ± 4.39
Hydrophilic Polypropylene		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	90.5 ± 2.48	0.21 ± 0.02	5.67 ± 0.24	96.4 ± 2.49
	Diazinon	94.6 ± 1.26	0.74 ± 0.07	5.28 ± 0.30	100.6 ± 1.30
	Ethynylestradiol	100.0 ± 7.93	0.54 ± 0.09	3.54 ± 0.38	104.1 ± 7.94

Table 23 – 24 hour comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane with 100 mg of Resin Mix devices under non-stirred conditions. Reported as percent recovered ± standard deviation (n=3).

		Membrane + Resin Mix			
Polyethersulfone		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	66.2 ± 0.81	7.66 ± 1.00	17.5 ± 2.69	91.4 ± 2.98
	Diazinon	63.7 ± 5.01	22.1 ± 1.06	8.09 ± 0.52	93.9 ± 5.15
	Ethynylestradiol	64.0 ± 7.45	18.9 ± 2.69	5.71 ± 1.14	88.6 ± 8.00
Hydrophilic Polypropylene		% Water	% Membrane	% Resin Eluate	Mass Balance
	Atrazine	73.0 ± 3.50	0.21 ± 0.04	23.4 ± 1.62	96.6 ± 3.86
	Diazinon	66.0 ± 1.26	1.05 ± 0.05	26.8 ± 0.64	93.9 ± 1.41
	Ethynylestradiol	66.9 ± 3.22	0.37 ± 0.03	15.0 ± 0.64	82.3 ± 3.28

Table 24 – 1 week comparison of Polyethersulfone and Hydrophilic Polypropylene Membrane with 100 mg of Resin Mix devices under non-stirred conditions. Reported as percent recovered ± standard deviation (n=3).

		Membrane + Resin Mix				
Polyethersulfone		% Water	% in Dialysate	% in Membrane[a]	% in Resin[a]	Mass Balance
	Atrazine	77.8 ± 3.56	21.1 ± 1.48	0.73 ± 0.21	2.43 ± 0.77	102.1 ± 3.94
	Diazinon	57.6 ± 4.22	35.8 ± 1.70	0.73 ± 0.07	2.06 ± 0.38	96.2 ± 4.57
	Ethynylestradiol	52.3 ± 6.37	37.1 ± 0.50	0.69 ± 0.07	3.86 ± 0.57	94.0 ± 6.42
Hydrophilic Polypropylene		% Water	% in Dialysate	% in Membrane[a]	% in Resin[a]	Mass Balance
	Atrazine	75.9 ± 5.54	20.6 ± 0.50	0.34 ± 0.03	2.37 ± 0.27	99.2 ± 5.57
	Diazinon	68.8 ± 2.64	25.2 ± 2.23	0.20 ± 0.02	2.24 ± 0.40	96.4 ± 3.48
	Ethynylestradiol	60.6 ± 4.44	7.16 ± 1.76	1.52 ± 0.29	1.19 ± 0.20	70.5 ± 4.79

Table 25 – Dialysis of PES and Hy-PP Membrane with 100 mg of Resin Mix devices following a 24 hour exposure under stirred conditions. Reported as percent recovered ± standard deviation (n=3). [a] membrane and resin were analyzed after dialysis.

For the Membrane Only exposures, polyethersulfone exhibited a greater overall uptake for each analyte than hydrophilic polypropylene. With the addition of the resin mix into the device, the uptake rates were approximately equal for all analytes between the two membranes. The PES membrane retained up to 20 times the amount of the analyte as the Hydrophilic PP which allows most of the analyte to pass through to the resin. Ethynylestradiol exhibited the most rapid uptake in all cases with diazinon closely following. Atrazine's uptake was very slow, in some cases half that of ethynylestradiol and diazinon. The addition of the resin mix into the device caused the uptake of atrazine

to nearly double. Atrazine is not retained as strongly in the membrane as is observed for diazinon and ethynylestradiol. The lower observed uptake of atrazine may be attributed to a resistance in the analyte partitioning into the membrane.

The major disadvantage of hydrophilic polypropylene is its durability. Prior to exposure, the Hydrophilic PP had approximately the same strength as PES. This durability degrades rapidly during limited exposures in water, as short as a few days, regardless of water movement. On several of the 1 week exposures, the membranes tore with a slight touch while taking apart the devices. This type of degradation is not evident with the PES membranes. Exposures were conducted lasting in excess of 2 months during the uptake and depuration experiments (see *28 Day Uptake and Depuration Studies* in the following text). During these experiments, the PES membranes retained their durability. Due to this lack of sustained resistance to degradation, hydrophilic polypropylene, although appealing in other aspects, was deemed unusable for this project.

28 Day Uptake and Depuration Studies:
Experiments to this point consisted of exposures lasting 24 hours to 1 week. In reality, true environmental exposures would last up to 28 days in order to maximize the mass of analyte sequestered. Another point of interest is how much of the sequestered analytes will be released back to the surrounding water during prolonged exposures. To achieve a true time-weighted average water concentration, the device must retain sequestered analytes regardless of changes in environmental water concentrations. This becomes important in cases where there is a short-lived pulse of contamination due to a spill or a surface runoff event resulting in an increased concentration of contaminants entering the water system that is rapidly reduced back to a nominal concentration over short duration. The following studies involved exposing polyethersulfone devices to atrazine, diazinon, and ethynylestradiol for 28 days and then monitoring the depuration of sequestered analytes back into fresh water for a second 28 day period.

Variations of the sampling device were exposed in 1-liter of water fortified individually with each analyte under stirring conditions for 28 days. During this uptake period, 5 mL aliquots of the exposure water were measured by LSC at regular intervals to monitor the decrease in water concentration and by inference, the uptake. Upon conclusion of the initial 28 day period, the devices were transferred into 1-liter of analyte-free water and stirred for an additional 28 days. As during the uptake phase, aliquots of the water were counted to monitor analyte levels (i.e., to estimate depuration).

The variations of the devices used were PES Only, PES + Resin Mix, and PES + Empore. The PES Only devices consisted of two 47 mm PES disks contained in the stainless steel ring holders. Following uptake, one of the PES disks was sacrificed for analysis while the other remained in the holder during the depuration stage. The PES + Resin devices contained two PES disks enveloping 100 mg of the resin mix. One of the triplicate devices was taken for analysis following uptake while the other two were used for estimating depuration. The PES + Empore devices consisted of an Empore SDB-RPS Sulfonated disk between two PES disks. Following uptake, one of the triplicate devices was sacrificed for analysis with the other two used for depuration. Comparison of the

uptake of each device variation is depicted in Figures 3, 4, and 5 for ethynylestradiol, diazinon, and atrazine respectively. The subsequent depuration of ethynylestradiol, diazinon, and atrazine is displayed in Figures 6, 7, and 8, respectively.

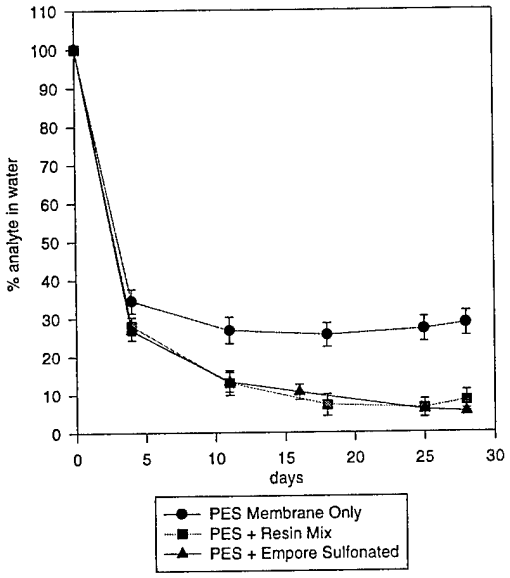


Figure 3- 28 Day Uptake of Ethynylestradiol by various PES device configurations.

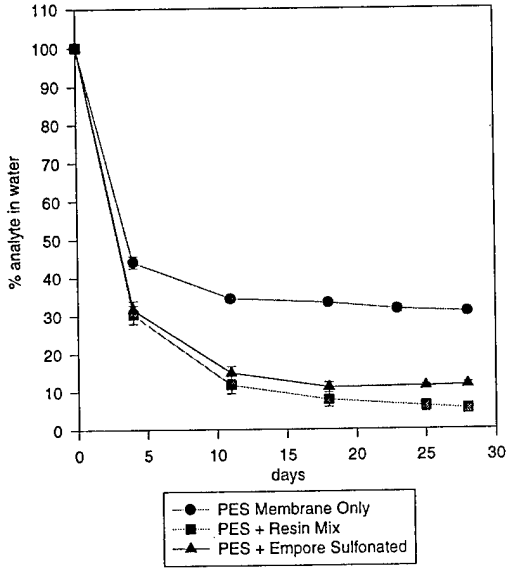


Figure 4 – 28 Day Uptake of Diazinon by various PES device configurations.

The initial uptake of ethynylestradiol through the first 4 days of exposure was similar for all device configurations with 34.5% remaining in the water for the PES Only devices, 28.0% for the PES/Resin devices and 26.8% for the PES/Empore devices. There was slightly more variation in the uptake of diazinon between the PES Only and the Resin and Empore containing devices with 44.1%, 30.3% and 31.6% remaining in the water respectively. Atrazine exhibited the greatest differences in uptake over 4 days with 83.7% remaining in the water for the PES Only devices, 47.6% for the PES/Empore devices and 23.5% for the PES/Resin devices. After 11 days of exposure, the PES Only

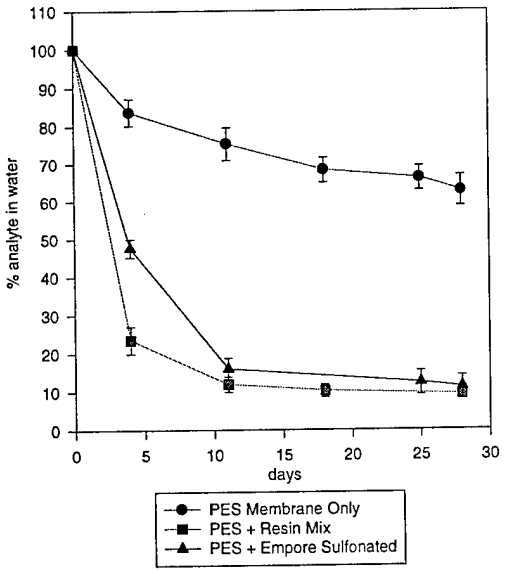


Figure 5 – 28 Day Uptake of Atrazine by various PES device configurations.

devices approach a steady-state equilibrium with the surrounding environment with approximately 28% of ethynylestradiol and 32% of diazinon remaining in the water. The PES Only uptake of atrazine occurred much slower with 62.8% remaining in the water after 28 days. Devices containing either the Resin Mix or the Empore Sulfonated disk exhibited a much greater uptake for each analyte when compared to the PES Only devices with less than 16% remaining in the water after 11 days. There is essentially no difference in the uptake of ethynylestradiol and diazinon for the Resin or Empore containing devices. The same is true for the uptake of atrazine after 11 days of exposure.

Comparison of the PES Only and the Resin and Empore containing devices indicates initial analyte uptake involves partitioning into the membrane matrix which is followed by sequestration by the internal media. After a short exposure period of 10 days or less, the PES Only devices reach equilibrium whereas the devices containing a sequestration media act as an infinite sink and continue to sample throughout the 28 day period. Atrazine exhibited little uptake by the membrane alone. Observations suggest that

atrazine uptake occurred largely via passage through the membrane's pores to the sequestration media instead of actual partitioning into the membrane matrix as appeared to be the case with ethynylestradiol and diazinon. Table 26 contains data on the distribution of analyte in the various device configurations following 28 days of exposure.

	Uptake	% water (28 days)	% in PES membrane	% Resin Eluate or Empore[a]	Total % in Device	Mass Balance
Ethynylestradiol	PES Only	28.5	59.2	N/A	59.2	87.7
	PES + Resin	8.35	***	***	46.4	54.8
	PES + Empore	5.33	4.38	76.5	80.9	86.2
Diazinon	PES Only	30.9	63.4	N/A	63.4	94.3
	PES + Resin	5.36	25.7	54.1	79.8	85.2
	PES + Empore	11.6	22.9	51.4	74.3	85.9
Atrazine	PES Only	62.8	30.4	N/A	30.4	93.2
	PES + Resin	9.27	5.21	59.1	64.3	73.6
	PES + Empore	11.1	5.75	50.1	55.8	67.0

Table 26 – 28 Day Uptake by various PES device configurations. Data is based on analysis of 1 of 3 devices taken prior to depuration. *** PES + Resin devices were dialyzed during analysis, therefore individual membrane and resin recoveries are not available. [a] Empore recoveries based on % of analyte in Empore eluate and % of analyte remaining in the disk following solvent extraction.

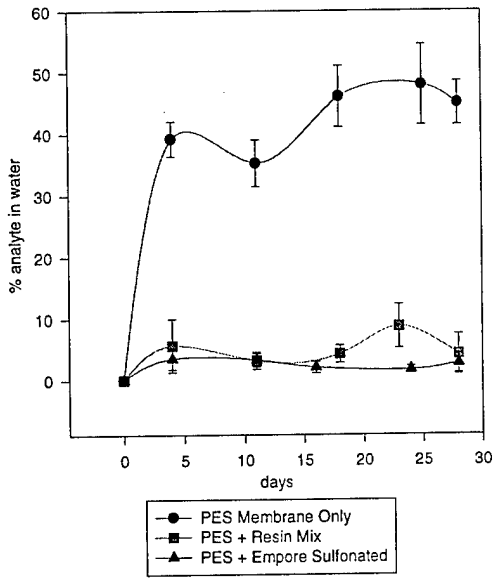


Figure 6 – 28 Day Depuration of Ethynylestradiol by various PES device configurations.

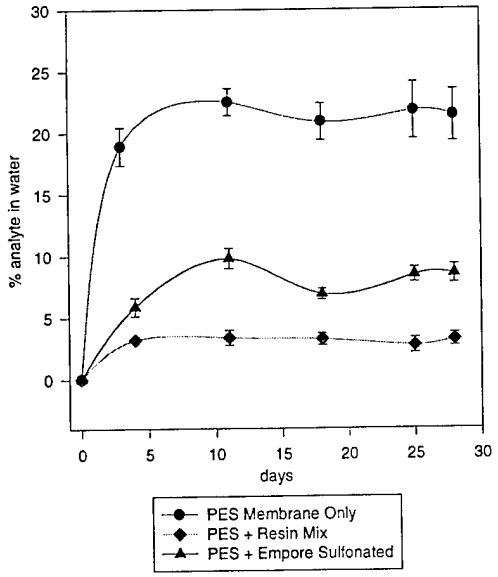


Figure 7 – 28 Day Depuration of Diazinon by various PES device configurations.

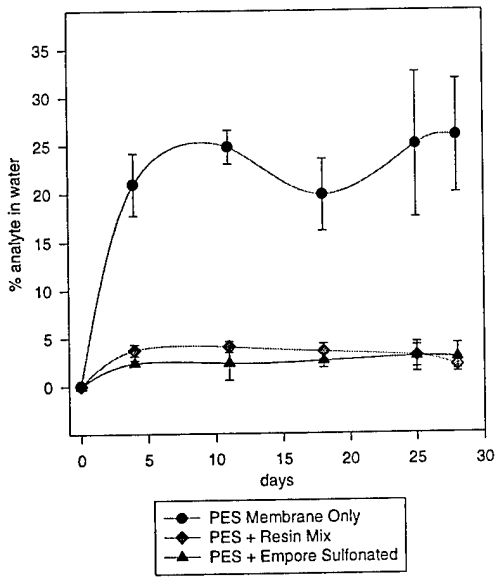


Figure 8 – 28 Day Depuration of Atrazine by various PES device configurations.

The amount of analyte lost during the depuration phase varied greatly depending on the configuration of the device. The PES Only devices exhibited the greatest depuration, losing 39.2%, 18.9%, and 20.9% of the sequestered ethynylestradiol, diazinon, and atrazine respectively within the first 4 days. Over the same time period the PES + Resin devices lost less than 5.7% and the PES + Empore devices depurated less than 5.9% into the surrounding water. Data suggests that over time, most of the sequestered analyte partitions from the outer membrane into the inner resin or Empore layer. Once in the resin or Empore disk, the analyte is effectively trapped and any depuration observed is likely residual analyte left in the outer membrane enclosure. Fresh water was not replenished during the experiment resulting in an apparent equilibrium attained for each device after approximately 4 days. It is believed that repeating the experiment as a static renewal or in a flowing system would result in continued depuration of analyte partitioned in the outer membrane. As was observed during the uptake experiments, there is little difference in the performance of the Resin and Empore containing devices with the lone exception for the depuration of diazinon where the PES/Empore devices had a slightly higher degree of depuration than the PES/Resin devices. Table 27 presents data on the distribution of analyte following the 28 day depuration.

	Depuration	% water (28 days)	% in PES membrane	% Resin Eluate or Empore[a]	Total % in Device	Mass Balance
Ethynylestradiol	PES Only	45.0 ± 3.54	50.2 ± 4.05	N/A	50.2 ± 4.05	95.2 ± 5.38
	PES + Resin	4.19 ± 3.26	***	***	97.6 ± 4.38	101.8 ± 5.46
	PES + Empore	2.67 ± 1.53	5.62 ± 3.93	83.5 ± 3.93	89.1 ± 5.56	91.8 ± 5.77
Diazinon	PES Only	21.4 ± 2.10	77.3 ± 3.37	N/A	77.3 ± 3.37	98.7 ± 3.97
	PES + Resin	3.20 ± 0.53	27.8 ± 0.35	63.2 ± 0.71	91.0 ± 0.79	94.2 ± 0.95
	PES + Empore	8.52 ± 0.74	24.0 ± 3.04	57.4 ± 2.33	81.4 ± 3.83	89.9 ± 3.90
Atrazine	PES Only	25.9 ± 5.90	75.3 ± 5.48	N/A	75.3 ± 5.48	101.2 ± 8.05
	PES + Resin	2.05 ± 0.41	11.8 ± 0.07	58.3 ± 1.41	70.1 ± 1.41	72.2 ± 1.47
	PES + Empore	2.85 ± 1.50	8.60 ± 1.04	77.1 ± 7.99	85.7 ± 8.06	88.6 ± 8.20

Table 27 – 28 Day Depuration by various PES device configurations. Data is based on analysis 2 of 3 devices taken through depuration. *** PES + Resin devices were dialyzed during analysis, therefore individual membrane and resin recoveries are not available. [a] Empore recoveries based on % of analyte in Empore eluate and % of analyte remaining in the disk following solvent extraction.

Final Selection of POCIS Materials:

As discussed in the *Resin Characterization* and *Membrane Evaluation* sections, several materials under consideration for use in the POCIS were studied in detail. A cursory examination of the resin mix and the Empore Sulfonated disk for use as the sequestration medium indicates comparable performance of the two materials. The disk are an attractive alternative to the resin mix, however, problems exist in the complete elution of analytes from the Empore disk. In some cases, up to 15 % of the total analyte mass was determined to be unrecoverable from the disk. Similar problems of incomplete analyte recovery from the resin mix were not encountered in any experiment.

The data presented demonstrates the usefulness of the polyethersulfone membrane as a membrane barrier and the resin mix as the sequestration phase in the POCIS. This combination has been demonstrated to be effective for the uptake and retention of polar organic chemicals. Further evaluation of this configuration of the POCIS, including determination of uptake sampling rates is described in subsequent sections.

Sampling Rate Determinations:

The sequestration of polar organic chemicals from an aqueous environment by the POCIS has been demonstrated and reported in the previous sections. At this point, the only valid information that is provided by the POCIS is confirmation of the presence or absence of a chemical in the water. No information involving the actual environmental water

concentration of a particular chemical is available. To determine the actual water concentrations, the POCIS must be calibrated (i.e., sampling rate experimentally determined) for each analyte of interest. Once enough experimental data is collected, it may become possible to develop models to estimate the uptake of chemicals based on their octanol-water partition coefficient (K_{ow}), chemical class, molecular size, or chemical functionality.

The experimental determination of sampling rates involve exposing the device to a chemical under nearly constant flow conditions and analyte water concentrations. The sampling rate is defined as the equivalent volume of water from which the analyte is quantitatively extracted per unit time, usually expressed as liters per day (L/d) (47). In other words, in the present research the sampling rate is a measure of the number of liters of water which are cleared of a certain analyte in one day. For the POCIS to be considered an integrative sampler, the sampling rate must remain relatively constant over an extended period generally greater than 28 days. Another consideration for an integrative sampler is that the sampling rate is independent of concentration. This is important to allow for the estimation of the time-weighted average concentration of an analyte in the water over the time interval for which the device was deployed. Factors affecting the sampling rate and device performance will be addressed throughout this chapter and later sections describing modeling approaches.

Previous experiments involved exposures where the water concentration decreased over time. In these cases the only information on the sampling rate that could be obtained was from the slope of the uptake curve during the initial phase of the exposure. After this phase, the device approached a steady-state equilibrium with the surrounding water and the sampling rate decreased until it equaled the depuration rate. In order to maintain a constant sampling rate, the water concentration must remain fairly constant over the entire exposure period. This was accomplished by renewing the exposure water with fresh analyte-fortified water at regular intervals (i.e., a static renewal exposure). Studies described in the following sections involve exposures of membrane only and membrane + resin devices to ethynylestradiol, diazinon, and atrazine under non-stirred static and stirred static conditions.

Non-Stirred Static Renewal Exposures:
The exposure of the devices under non-stirred conditions in the laboratory is representative of a field deployment in quiescent waters. In this set of experiments, PES Only devices containing 2 polyethersulfone disks and PES/Resin devices containing 2 PES membrane disks plus 100 mg of the resin mix were exposed to ethynylestradiol, diazinon, and atrazine in one liter of DI water. The water was spiked with the appropriate analyte and mixed to ensure homogenous distribution of the analyte prior to addition of the sampling device. Each Monday and Friday morning, the sampler was removed and immediately placed in a freshly prepared microcosm. The previously sampled microcosm was then stirred and two 5.0 mL aliquots were taken and measured by LSC to determine the water concentration. From the concentration of analyte remaining in the water, the sampling rate of the device in liters per day could be calculated. Graphic representations of the static renewal of ethynylestradiol, diazinon, and atrazine are

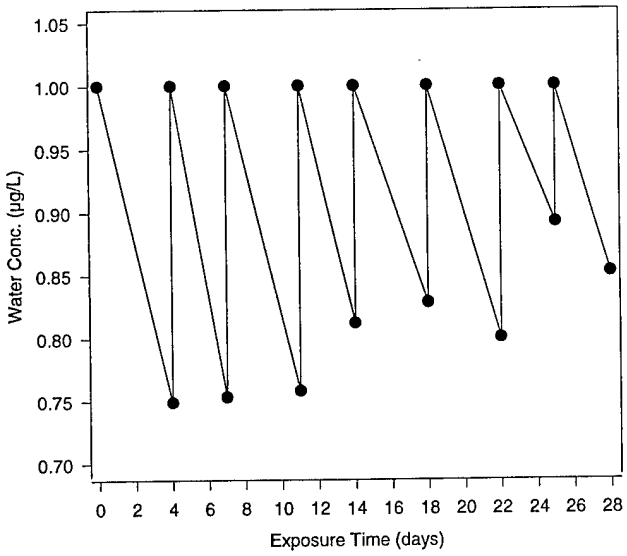


Figure 9 – PES Only Non-Stirred Static Renewal Exposure to Ethynylestradiol.

depicted in Figures 9, 10, and 11 for the PES Only devices and Figures 12, 13, and 14 for the PES/Resin devices. A summary of the experimentally determined sampling rates is presented in Table 28.

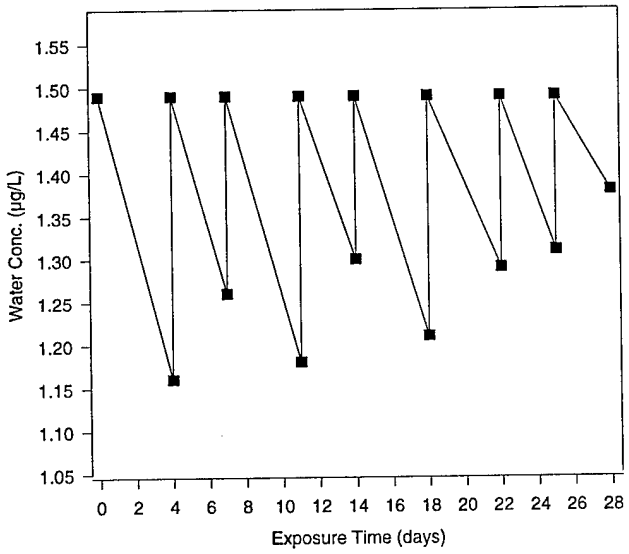


Figure 10 – PES Only Non-Stirred Static Renewal Exposure to Diazinon

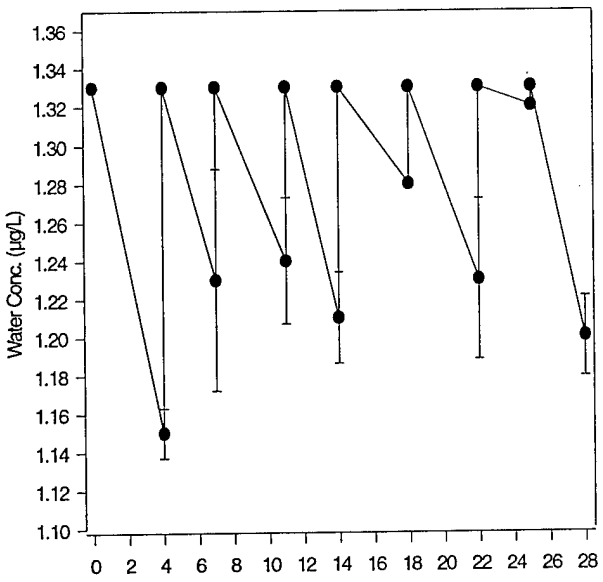


Figure 11 – PES Only Non-Stirred Static Renewal Exposure to Atrazine.

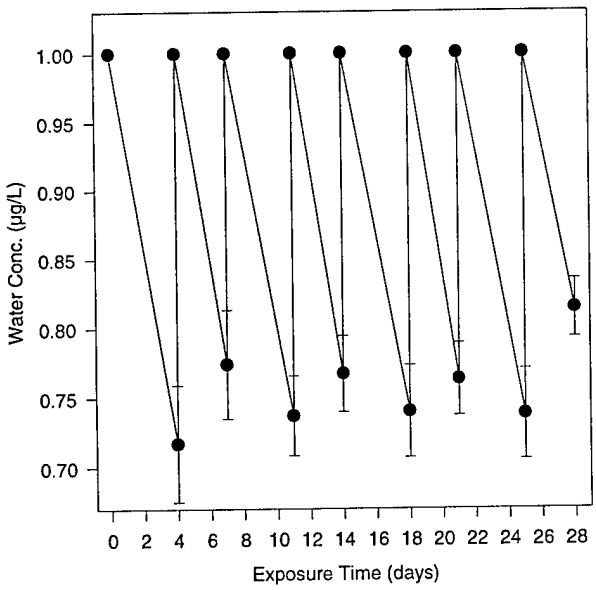


Figure 12 – PES/Resin Non-Stirred Static Renewal Exposure to Ethynylestradiol.

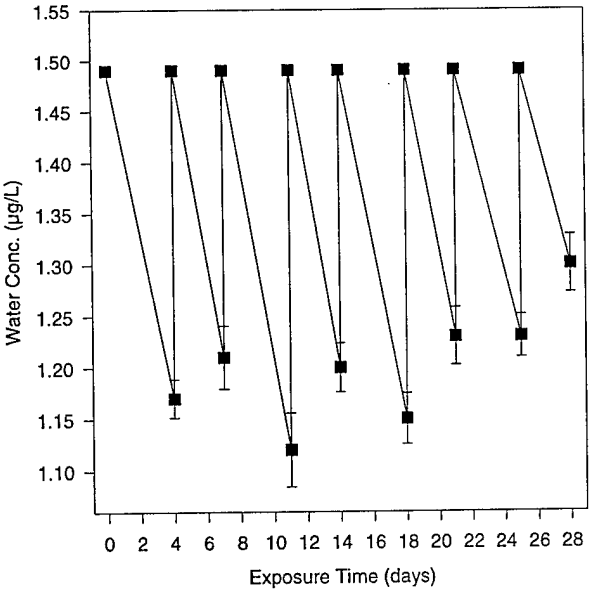


Figure 13 – PES/Resin Non-Stirred Static Renewal Exposure to Diazinon.

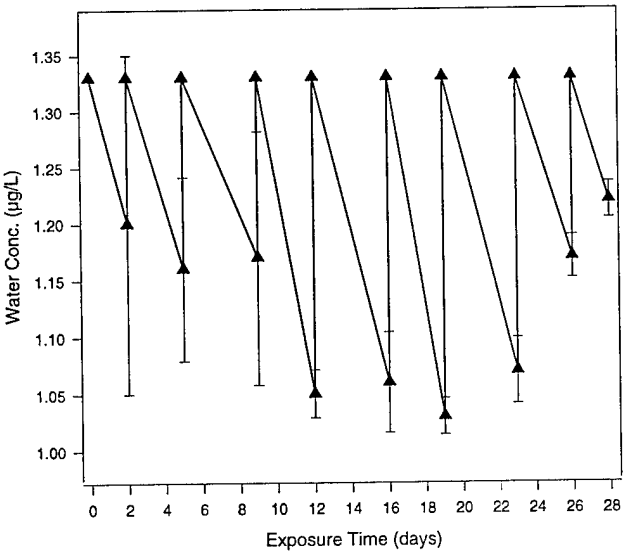


Figure 14 – PES/Resin Non-Stirred Static Renewal Exposure to Atrazine.

Analyte	PES Only (L/d)	PES/Resin (L/d)
Ethynylestradiol	0.055 ± 0.016	0.070 ± 0.006
Diazinon	0.046 ± 0.007	0.056 ± 0.008
Atrazine	0.026 ± 0.007	0.050 ± 0.014

Table 28 – Calculated Sampling Rates in Liters of Water Cleared of Analyte per Day for the Non-Stirred Static Renewal Exposures. Reported values are ± standard deviation (n=8).

The sampling rate was calculated from the analyte concentration remaining in the water following each exposure. The measured analyte water concentration subtracted from the original analyte water concentration, divided by the original water concentration, multiplied by the volume of water in liters and then divided by the number of days the device was exposed in the water provides the sampling rate in the units of liters per day. For example, 1 L of water was originally spiked with 1.49 µg of diazinon. Following exposure for 3 days, there was 1.24 µg remaining in the water. The sampling rate for this exposure is calculated as follows:

$$\frac{\dfrac{(1.49\mu g - 1.24\mu g)}{1.49\mu g}}{3\,\mathrm{d}}(1\,\mathrm{L}) = 0.056\ \mathrm{L/d}$$

The calculated sampling rate for each renewal is then used to find the average sampling rate of that particular analyte.

The assumption that the introduction of a sequestration media, such as the resin mix, would increase the sampling rate over that of the membrane alone was validated. The resin-containing devices have a greater overall sampling rate than the membrane only devices and for atrazine a significant increase of 0.026 to 0.050 L/d was observed. A less pronounced increase was observed for ethynylestradiol and diazinon, 0.055 to 0.070 L/d and 0.046 to 0.056 L/d respectively.

Sampling rates remained fairly constant in each case thereby satisfying one of the constraints of an integrative sampler. Using the average sampling rates for each chemical, a single POCIS configured as described previously, deployed in quiescent waters over 28 days, would clear 2.0, 1.6, and 1.4 liters of water of ethynylestradiol, diazinon, and atrazine respectively. The use of several samplers at the deployment site would increase the total volume of water cleared, providing a greater cumulative analyte mass available for analysis.

Stirred Static Renewal Exposures:

The exposure of the devices under stirring conditions in the laboratory is representative of a field deployment in turbulent waters such as a flowing stream or river. As in the non-stirred static renewal experiments, PES Only and PES/Resin devices were exposed to ethynylestradiol, diazinon, and atrazine in one liter of DI water. The water was fortified with the appropriate analyte and allowed to mix via a magnetic stir plate to ensure a homogeneous distribution of the analyte prior to addition of the sampling device. Each weekday morning, the device was removed and immediately placed in a freshly prepared microcosm. Two 5.0 mL aliquots were taken from the previously sampled microcosm and measured by LSC to determine the water concentration and therefore calculate the sampling rate of the device as liters of water cleared per day. Graphic representations of the stirred static renewal of ethynylestradiol, diazinon, and atrazine are presented in Figures 15, 16, and 17 for the PES Only devices and Figures 18, 19, and 20 for the PES/Resin devices. A summary of the experimentally determined sampling rates is given in Table 29.

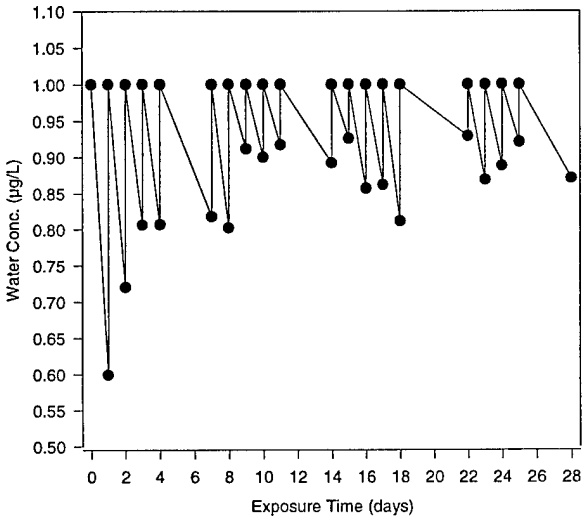


Figure 15 – PES Only Stirred Static Renewal Exposure to Ethynylestradiol.

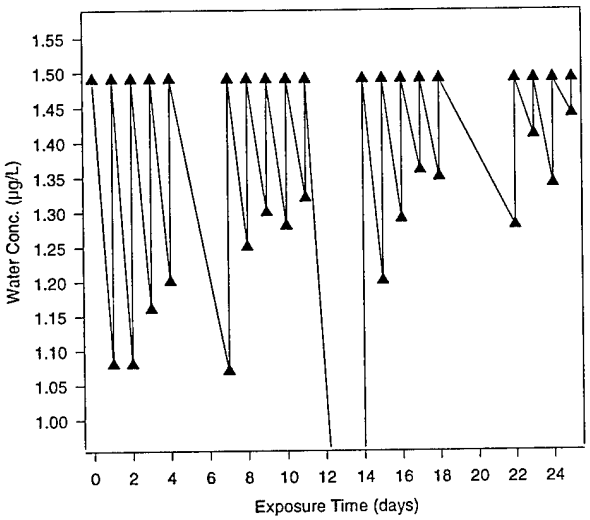


Figure 16 – PES Only Stirred Static Renewal Exposure to Diazinon.

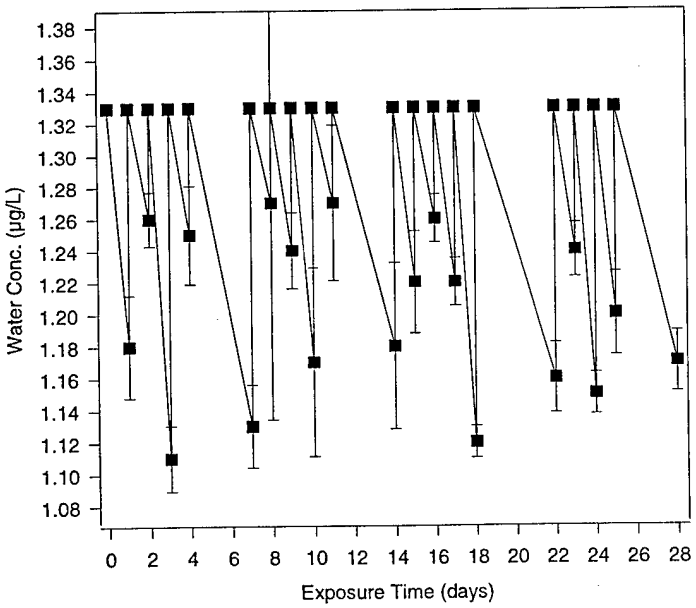


Figure 17 – PES Only Stirred Static Renewal Exposure to Atrazine.

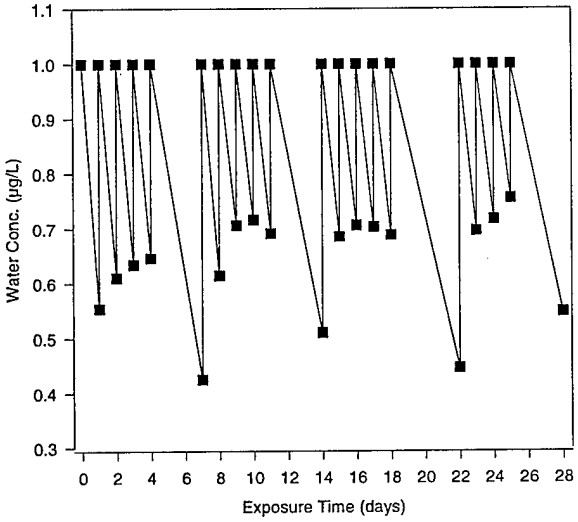


Figure 18 – PES/Resin Stirred Static Renewal Exposure to Ethynylestradiol.

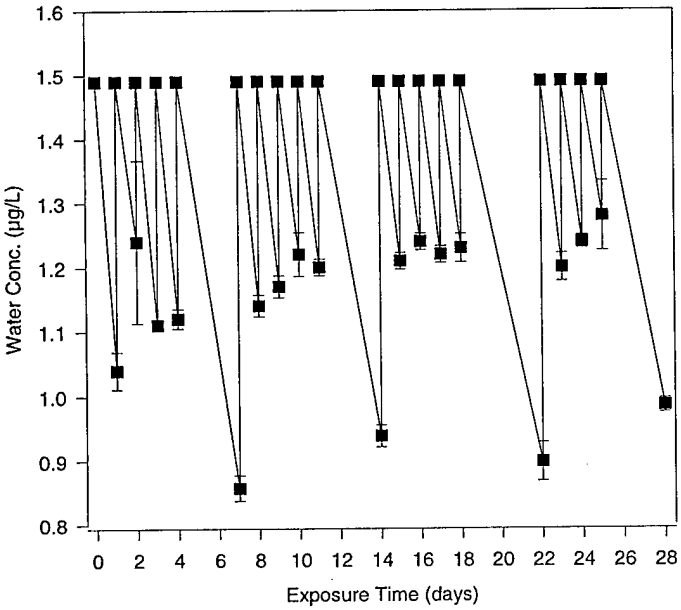


Figure 19 – PES/Resin Stirred Static Renewal Exposure to Diazinon.

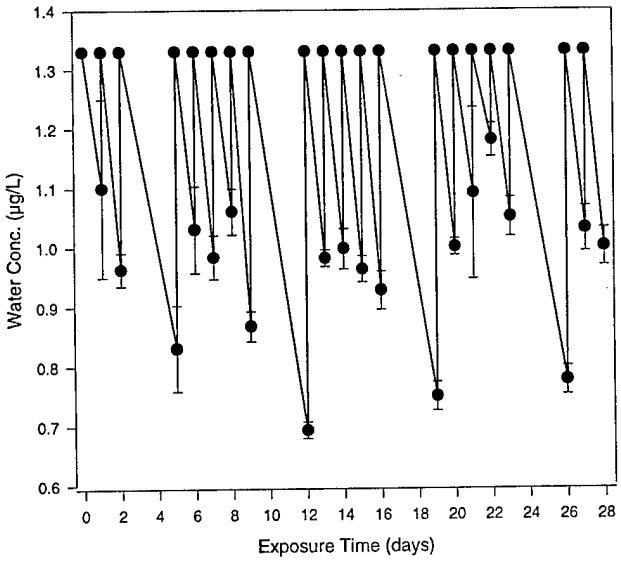


Figure 20 – PES/Resin Stirred Static Renewal Exposure to Atrazine.

Analyte	PES Only (L/d)	PES/Resin (L/d)
Ethynylestradiol	0.121 ± 0.043	0.302 ± 0.034
Diazinon	0.113 ± 0.046	0.186 ± 0.025
Atrazine	0.088 ± 0.036	0.240 ± 0.056

Table 29 – Calculated Sampling Rates in Liters of Water Cleared of Analyte per Day for the Stirred Static Renewal Exposures. Values are ± standard deviation (n=12).

Sampling rates were calculated for the stirred static renewals in the same manner as for the non-stirred static renewals. Values corresponding to the weekend exposures were omitted from the computation of the average sampling rate for each analyte. Sampling during the weekend periods extended beyond the linear portion of uptake curve, resulting in values which were significantly lower than observed values for 24 hour renewals. Under stirring conditions, a sampling rate of 0.2 to 0.3 L/d will greatly decrease the concentration of the water in a microcosm in 24 to 48 hours resulting in variable sampling rates.

Comparison of sampling rates for the PES Only and PES/Resin supports observations from the *28 Day Uptake and Depuration Studies* section. The overall sampling rates for the PES/Resin devices were greater than the rates for the PES Only devices.

Ethynylestradiol had an increase in the sampling rate from 0.121 to 0.302 liters of water per day. Diazinon and atrazine exhibited an increase, 0.113 to 0.186 L/d and 0.088 to 0.240 L/d respectively.

A single POCIS deployed in a turbulent aquatic system for 28 days could reach clearance volumes of up to 8.5, 5.2, and 6.7 liters of water for ethynylestradiol, diazinon, and atrazine respectively. As discussed in the non-stirred static renewal section, increasing the number of samplers deployed will greatly increase the cumulative analyte mass sequestered.

Membrane and/or Resin Pretreatment:
Visual inspection of the graphical data from the stirred static renewal exposures reveals increased sampling occurring for both the PES Only and PES/Resin devices in the first 4 to 6 renewals (or 1 to 8 days). This increased sampling could be due to an increased flux of water crossing the membrane in an attempt to solvate or wet the polymer. The increased contact with water would result in the sequestration of additional analyte. It may be possible to alleviate this initial period of increased sampling by presoaking the membranes in water or some other wetting agent prior to device construction and deployment. A second consideration is that the prewetting of the membrane and/or the resin mix may increase the overall sampling rate for the device. Several experiments were designed with variations in the treatment of the membrane and resin to determine the effects on sampling rates.

Various membrane pretreatment regimes involved the use of water, methanol, and isopropanol. Ethynylestradiol was selected as the test chemical for all exposures. The initial attempt to prewet the membrane involved immersing the membrane disks in DI water for a period of 10 days prior to device construction. The samplers with the water-soaked membranes and 100 mg of the resin mix were used in a stirred static renewal exposure for 28 days. Figure 21 depicts the sampling of the PES+H_2O/Resin devices.

All data points after day 1, excluding the weekends, indicate a fairly stable sampling rate of 0.307 L/d which is essentially equal to the 0.302 L/d observed in the later stages of the PES/Resin study. The increased sampling occurring on the first exposure is most likely due to the sharp concentration gradient between the membrane and the surrounding water.

Conditioning of the membranes with an organic solvent was attempted by soaking the membrane in methanol and isopropanol for 2 days. Following the soaking period, the membranes were used in PES Only devices for the stirred static renewal exposure to ethynylestradiol over 1 week. Results for the organic solvent conditioning study are shown in Figure 22.

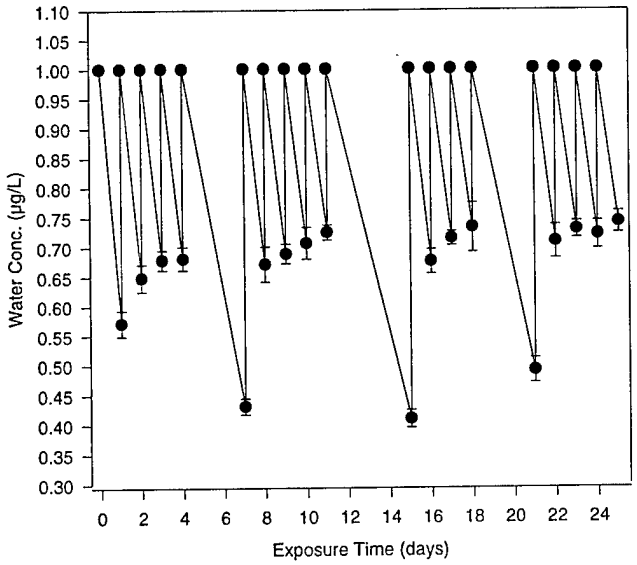


Figure 21 – PES+H_2O/Resin Stirred Static Renewal Exposure to Ethynylestradiol. Membrane presoaked in DI water.

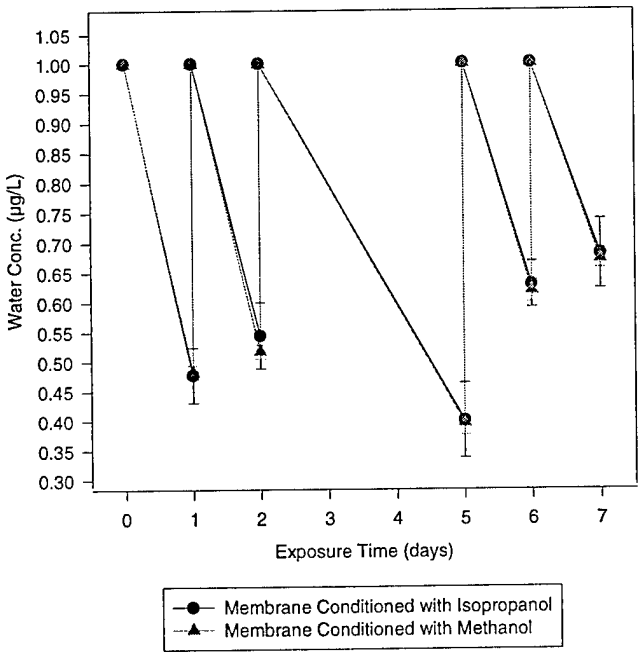


Figure 22 – Methanol and Isopropanol Conditioning of PES Only devices. Stirred Static Renewal Exposure to Ethynylestradiol.

The conditioning solvents were evaporated to a final volume of less than 1 mL following soaking of the membranes. The isopropanol remained clear whereas the methanol became turbid with a white precipitate along the walls of the tube. This precipitate is most likely plasticizers or some material from the polymerization process in the membrane's manufacture. It was also noted that the methanol conditioned membranes became rigid after soaking, but there was no observable change in the isopropanol conditioned membranes.

There was no discernible difference between the sampling rates for the methanol and isopropanol conditioned membranes after one week. Increased sampling rates of 0.428 and 0.418 L/d for the methanol and isopropanol conditioned membranes, respectively, were intriguing, however, there were not enough data points to determine if the sampling rates would remain at those levels over extended time periods. Due to the unknown consequences of conditioning with organic solvents, it was decided that conditioning the membranes with DI water prior to exposure was the preferable option.

An attempt to condition the resin mix in hopes of increasing the sampling rate was performed by the addition of methanol to the resin mix during device construction. Methanol was added dropwise (8-12 drops) until the resin became moist. The devices were used in the stirred static renewal exposure to ethynylestradiol for 28 days. A slight increase in the average sampling rate (0.355 L/d) was observed. As there was not a significant increase in the sampling rate due to conditioning of the resin, it can be concluded that the sampling rate is likely controlled by the analyte flux through the membrane and not the extraction capacity of the resin. The use of methanol for resin conditioning may produce undesirable membrane effects as previously discussed when it inevitably comes in contact with the membrane surface. This exposure is depicted in Figure 23.

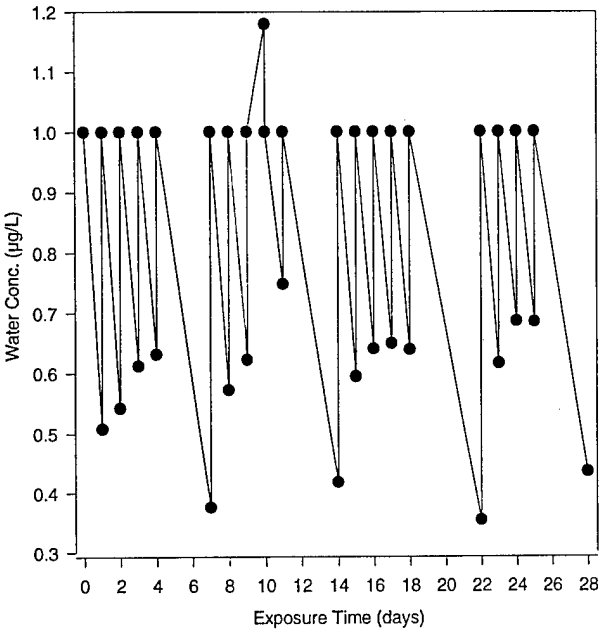


Figure 23 – PES/Resin Stirred Static Renewal Exposure to Ethynylestradiol. Resin conditioned with Methanol prior to device construction.

Membrane Fouling Effects on Sampling Rate:

One purpose of the membrane barrier is to prevent suspended solids, colloids, macromolecules, and microorganisms from reaching the sequestration media. However the buildup of such materials on the membrane surface could alter the performance of the device by clogging the pores and the formation of biofilms across the membrane surface. Biofilms form at a phase transition interface with the accumulation of microorganisms by diffusion, gravitational settling, or bulk fluid convection (50). Huckins *et al.* reported a reduction in the sampling rates of priority pollutant PAHs caused by periphytic growths and particulates on the surface of SPMD membranes (53). Sampling rates were observed to decrease 20-70% for heavily fouled membranes as the K_{ow} of the analytes increased. It should be expected that the formation of a biofilm would retard uptake of nonpolar compounds, however, this may not necessarily be true for polar compounds. Biofilms and periphytic growths may include polar functionalities which could increase the sampling of such compounds by making the membrane inherently more polar.

The extent to which biofouled PES membranes enhanced or retarded the sampling of ethynylestradiol was experimentally determined. Six devices with PES membrane only were suspended in a control pond at CERC for 30 days from June to July, 1999. Following exposure in the pond, the devices were retrieved and examined for the presence of membrane fouling. Surprisingly, there was very little fouling of the membrane compared to the degree of fouling of polyethylene SPMD membranes in the same pond. Essentially no biofilm was observed, only some discoloration of the membrane due to adhesion of suspended particulate matter to the surface. The pond itself did undergo a period of biological growth as algae grew in the pond throughout the exposure.

The membranes allowed to foul in the pond were used in the construction of PES Only and PES/Resin devices to be used in non-stirred and stirred static renewal studies. As before, the non-stirred static exposures were renewed every Monday and Friday and the stirred exposures every weekday morning. The sampling curves for the non-stirred static renewal studies are depicted in Figure 24 and for the stirred static renewal studies in Figure 25.

Removal of the fouling from the membrane was attempted following the renewal studies. One of the two membranes in each sampler was scrubbed with water and a toothbrush to remove as much surficial material as possible. The other membrane was swabbed with a cotton swab and methanol to remove any ethynylestradiol that may have adhered to any surficial biofilm. Less than 0.4% of the ethynylestradiol was recovered from the swabbing of the membrane surface indicating that cleaning of the membrane surface will not result in loss of analyte. Neither membrane surface became totally clean as some of the particulate matter had apparently embedded in the pore structure of the membrane. The membrane survived the cleaning process with no ill effects, further demonstrating its durability.

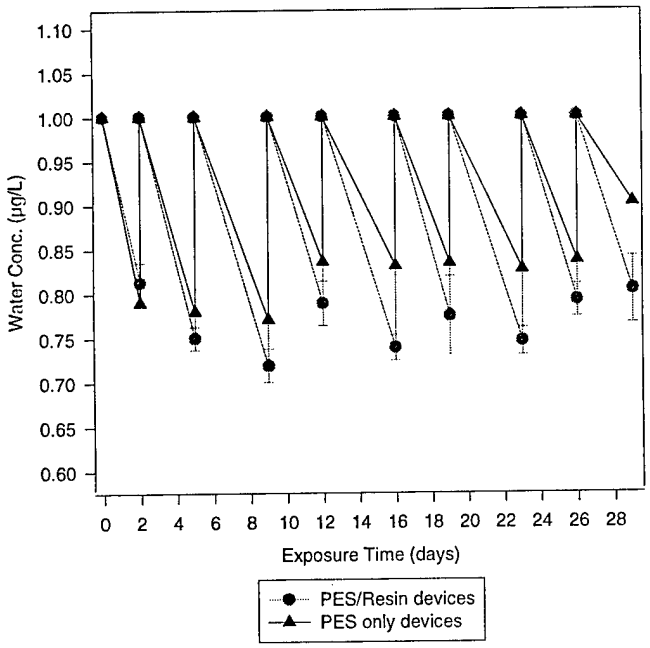


Figure 24 – Effects of Membrane Fouling on the Sampling Rate of Ethynylestradiol during a non-stirred Static Renewal exposure.

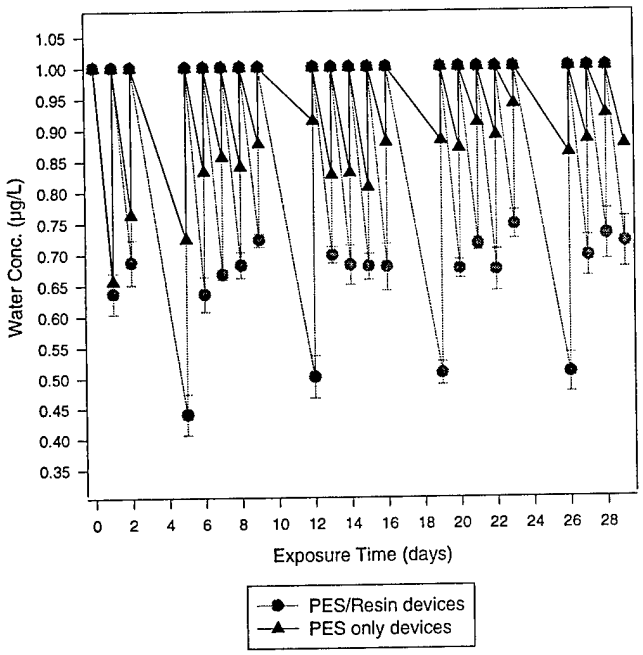


Figure 25 – Effects of Membrane Fouling on the Sampling Rate of Ethynylestradiol during a Stirred Static Renewal exposure.

The sampling rates for fouled membranes were essentially identical to sampling rates for non-fouled membranes. In the non-stirred static renewal study, sampling rates of 0.052 and 0.070 L/d were observed for PES Only and PES/Resin devices. This is compared to 0.055 and 0.070 L/d for the non-fouled PES Only and PES/Resin devices. This trend was also present for the stirred exposure with fouled sampling rates of 0.131 and 0.307 L/d for PES Only and PES/Resin devices compared to 0.121 and 0.302 L/d for non-fouled PES Only and PES/Resin devices. This commonality between the sampling rates of fouled and non-fouled devices indicates the ability of the device to be used in various environments regardless of biological growth.

Estimation of Ambient Water Concentrations:

Using passive sampling devices, the estimation of ambient water concentrations can be inferred from integrative sampling rates (provides time-weighted average concentrations), equilibrium steady-state partitioning coefficients, and exchange kinetics. Selection of the appropriate method to use is dependent on exposure duration and performance of the sampling device. A plot depicting the analyte concentration in the

sampling device versus exposure time, indicates sampling starts in the integrative (linear) phase, followed by curvilinear and equilibrium partitioning phases (Figure 26).

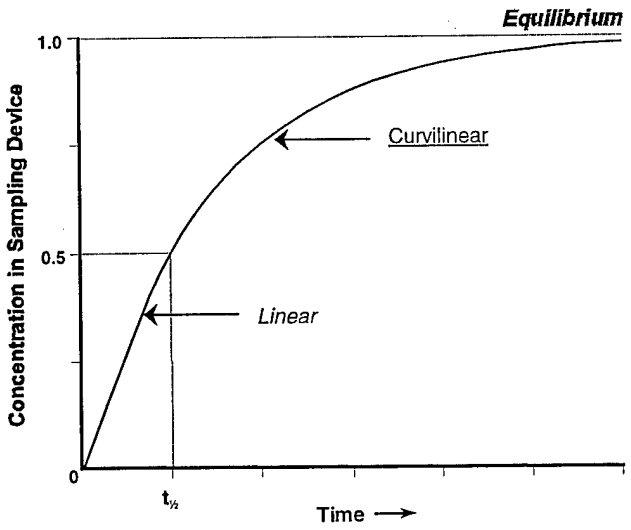


Figure 26 – Plot of the three phases of sampling device uptake. The time axis is given in half-times ($t_{1/2}$) to equilibrium.

In the integrative sampling phase, the sampling device acts as an infinite sink for contaminants of interest and analyte uptake is linear during the entire exposure period. Thus the rate constants for the analyte loss are very small and the times to reach equilibrium are very large. Since, integrative sampling represents the linear phase of an overall first-order kinetic process, the uptake rate constant (k_u) and sampling rate (R_s) are independent of environmental concentration and the sampling rate is expressed as the volume of water cleared of an analyte per unit time.

To use the equilibrium partitioning method for estimating ambient water concentrations, the sampling device must approach or reach equilibrium with the surrounding water within the exposure period. Equilibrium partitioning simplifies the modeling required to estimate environmental concentrations from the analyte concentrations accumulated in the device but does not necessarily reflect ambient analyte concentrations during the entire exposure period. Also, residue from episodic events may not always be retained during longer exposures. The time required for a passive sampler to reach equilibrium for many chemicals (under typical environmental conditions) can be large when the sampling device contains a sequestration media with a high analyte capacity. These limitations often reduce the utility of the equilibrium partitioning approach in environmental sampling.

Using the model compounds selected for this research, exposure studies indicate that POCIS remains in the linear uptake phase for at least 28 days, with no indication of when sampling becomes curvilinear. Thus, for the purposes of modeling the performance of POCIS, the sequestration media (resin mix) is assumed to act as an infinite sink for contaminants and linear uptake kinetics are used.

The following is a mathematical expression for the overall uptake curve in Figure 26:

$$C_W = C_S / K_{SW} (1 - \exp [-k_e t]) \quad (1)$$

where C_W is the analyte concentration in the water, C_S is the analyte concentration in the sorbent, K_{SW} is the equilibrium sorbent-water sorption coefficient, t is time in days and k_e is an exchange coefficient or rate constant (t^{-1}) for both overall uptake and depuration. To permit the use of the one-compartment model, the term k_e is expressed [60] as

$$k_e = k_W A / K_{SW} (V_S + K_{MS} V_M) = k_M K_{MW} A / K_{SW} (V_S + K_{MS} V_M) \quad (2)$$

where k_W and k_M are the mass transfer coefficients through the aqueous diffusion layer and the membrane, respectively, A is the surface area of the sampling device, V_S and V_M are the volumes of the sorbent and membrane, respectively, K_{MS} is the membrane-sorbent partition coefficient, and K_{MW} is the membrane-water partition coefficient. When $k_e t$ is very small ($<<1$) or $C_S/C_W << K_{SW}$, the chemical uptake is linear or integrative, equation 1 can be simplified as follows (54).

$$C_W = C_S V_S / R_S t \quad (3)$$

The precise form of this mathematical expression of the sampling rate can change based on whether the uptake is under boundary layer control or membrane control (see *Barriers to Analyte Mass Transfer*). Under boundary layer control, $R_S = (D_W/1_W) A$, where D_W is the diffusion coefficient in water and 1_W is the thickness of the aqueous boundary layer. Under membrane control, $R_S = (D_M / 1_M) K_{MW} A$, where D_M is the diffusion coefficient in the membrane and 1_M is the thickness of the membrane. For equation 3 to be valid, certain assumptions need to be made. These include: a relatively constant temperature and flow regime; establishment of a steady-state flux of chemical into the sampler; and linear uptake of analytes (i.e., the sampling period must be < 1 half-time.) Figure 26 shows that uptake will remain in the linear phase until half the capacity of the sorbent is reached.

The mass transfer of chemicals through a nonporous membrane such as polyethylene has been assumed to occur by a dissolution-diffusion mechanism in the polymer. However, analyte transfer in porous membranes, such as the polyethersulfone used in the sampling devices, can be more complex. Mass transfer through a porous membrane can follow a biphasic mechanism with analyte transport through both water-filled pores and the polymer matrix.

The analyte flux (quantity transported per unit time per unit area) through the POCIS membrane is a function of the flux through two mechanistic pathways. One mass transfer mechanism represents analyte movement through the aqueous boundary layer and the water-filled pores in the membrane. The other mass transfer mechanism represents analyte dissolution into the polymer matrix and diffusion through the membrane matrix to the enclosed sequestration phase. The contributions of each pathway to the overall flux are assumed to be additive.

Barriers to Analyte Mass Transfer:

The rate of mass transfer of an analyte through a membrane into a sequestration medium is potentially limited by several barriers. These rate-limiting barriers include the aqueous diffusion layer including stagnant water-filled membrane pores, any particulate matter or biofilm on the membrane surface, and the polymer matrix of the membrane (Figure 27). The aqueous diffusion layer (or boundary layer) is a thin hydrodynamically complex region at the membrane-water interface. Water layers can have an effective resistance to mass transfer, often resulting in a steep concentration gradient. The analyte concentration in the bulk water is much greater than the concentration at the membrane-water interface and the water inside the membrane. The effective thickness (l_W) of the aqueous diffusional layer in various aquatic systems is largely unknown, however, estimates suggest a range of as low as 10 μm for fast-moving, turbulent water to as high as 1000 μm for the quiescent sediment-water interface of deep stratified lakes or the deep ocean (55). Reduction in the thickness of the aqueous diffusion layer in turbulent systems results in increased sampling rates, as observed in the stirred and non-stirred static renewal studies.

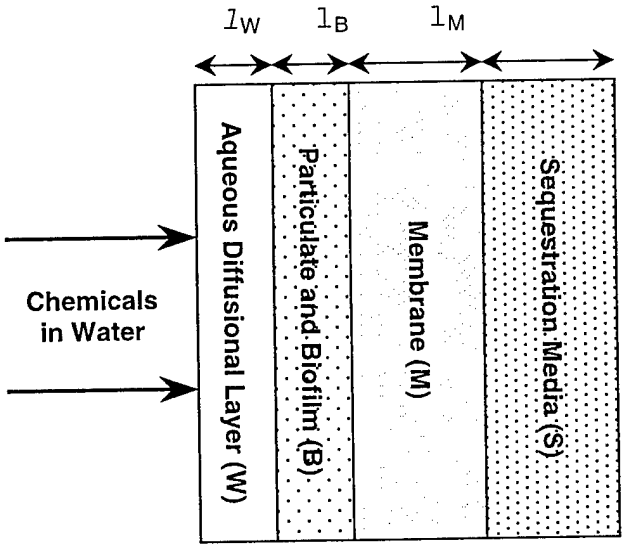


Figure 27 – Potential barriers to analyte mass transfer into a POCIS. The hypothetical thickness of each respective barrier is denoted by l.

The buildup of a biofilm on a membrane surface is another potential barrier to analyte mass transfer. The thickness of a biofilm (l_B) will vary from exposure to exposure depending on the environmental conditions. Observations of polyethylene SPMD membranes after extended deployments indicate regions of biofilm up to 1 mm thick grow during extended warm water (>18°C) exposures (54). For SPMDs, the presence of a biofilm can cause significant decreases in the sampling rate of hydrophobic compounds. As reported in the *Membrane Fouling Effects on Sampling Rate* section, polyethersulfone membranes exhibited an apparent resistance to biofilm development and the uptake of ethynylestradiol was not significantly impeded by the presence of any particulate or biofilm layer. This is because resistance to mass transfer in the biofilm is greatest for very hydrophobic compounds whereas hydrophilic compounds encounter little resistance.

The membrane itself can contribute a significant resistance to mass transfer. As previously discussed, the analyte flux through the membrane occurs by a biphasic mechanism of transport in water-filled pores and diffusion in the polymeric matrix. The polyethersulfone disks used in POCIS construction were estimated to be 76.5% open pores from empirical mass and polymer density information. Analyte diffusion through the polymeric matrix of the membrane is constant at a given temperature. The water in the membrane pores will remain essentially stagnant, regardless of the flow conditions of the surrounding bulk water, resulting in a constant resistance to analyte flux through the pores. Therefore it was concluded that if the sampling rate through the polymeric matrix and the water-filled pores is essentially constant under different experimental conditions, changes in the sampling rate is controlled by the aqueous boundary layer. Boundary layer control is supported by experimental data indicating an increase in R_s under more turbulent conditions (i.e., turbulent thinning of the boundary layer).

The overall resistance to the mass transfer or uptake of analytes is a combination of all the aforementioned barriers. The contribution of these barriers are additive and are independent of one another. The overall resistance (R_o) to analyte mass transfer is given by the reciprocal of the overall mass transfer coefficient (k_o).

$$R_o = 1 / k_o = l_W / D_W + l_B / D_B K_{BW} + l_M / D_M K_{MW} \quad (4)$$

where l is the layer or barrier thickness, D is the diffusivity in a particular region, K is the partition coefficient, and the subscripts W, B, and M, represent the aqueous diffusion layer and pore water, the biofilm, and the membrane, respectively. In a laboratory setting, a biofilm layer should theoretically not exist, therefore $l_B = 0$ and equation 4 is reduced to

$$R_o = 1 / k_o = l_W / D_W + l_M / D_M K_{MW} \quad (5)$$

Equation 5 can be used to determine if the path of greatest resistance (i.e., rate-limiting step) to analyte uptake is through the water or through the polymeric membrane matrix. Evaluation of the resistances requires the description of each variable. The diffusion coefficient in the water (D_W) can be calculated by the Hayduk and Laudie method (56).

The diffusion coefficient in the membrane (D_M) is unknown, but it is widely accepted that diffusion in a solid (membrane) is 10^3 to 10^4 times slower than diffusion in water (57). Herein, the resistance is calculated using D_M values that are 10^3, 10^4, and 10^5 times less than D_W in order to determine model sensitivity to D_M and to estimate the path of least resistance. The thickness of the membrane (l_M) is 152 μm. In this exercise, the thickness of the water (l_W) includes the aqueous boundary layer plus the membrane pore water. The aqueous boundary layer was assumed to be 50 μm under stirred water conditions and the membrane pore length, although torturous, was assumed to be approximately the same as the thickness of the membrane (l_M ~150 μm), therefore the total l_W was ~200 μm. The membrane-water partition coefficients (K_{MW}) was experimentally determined for ethynylestradiol, diazinon, and atrazine (see *Calculation of the Membrane-Water Partition Coefficient* section).

The Hayduk and Laudie method [62] for estimating the diffusion coefficient of organic compounds in water is based on the following equation:

$$D_W = \frac{13.26 \times 10^{-5}}{\eta_W^{1.14} V_B'^{0.589}} \qquad (6)$$

where η is the viscosity of water (assumed a constant temperature of 20°C) and V_B' is the molal volume of the analyte. The molal volumes for each analyte were calculated using the LeBas method of summing volume incremental values for the individual components of the molecule. Calculated molal volumes for ethynylestradiol, diazinon, and atrazine were 343.1, 314.8, and 239.8 cm^3/mol, respectively. These values were in agreement with reported values for diazinon and atrazine (58). The resulting D_W values were then calculated to be 4.25×10^{-6}, 4.47×10^{-6}, and 5.25×10^{-6} cm^2/s for ethynylestradiol, diazinon, and atrazine, respectively.

The resistance to mass transfer was calculated for the water and membrane phases individually using the values given earlier. For ethynylestradiol, the resistance due to the water was 4710 s/cm and resistance due to the membrane was 293, 2930, and 29300 s/cm for D_Ms of 10^{-3}, 10^{-4}, and 10^{-5} times the D_W, respectively. A resistance from the water component of 4470 s/cm and from the membrane component of 84, 840, and 8400 s/cm for each D_M was calculated for diazinon. Atrazine had a water resistance of 3810 s/cm and membrane resistances of 184, 1840, and 18400 s/cm for each D_M. These calculated resistance values indicate that for each model compound studied, the phase with the greatest resistance to analyte mass transfer is the water surrounding the membrane and water-filled pores in the membrane except in the unlikely case in which $D_M = 10^{-5} \cdot D_W$. This conclusion is supported in part by the experimental results of increased sampling rates for stirred versus non-stirred exposures. It should be understood that although thinning of the aqueous boundary layer reduces the overall resistance (i.e., increases R_s), the boundary layer is < 25% of the total l_W (boundary layer and membrane pore water) and only contributes a portion of the resistance in the water. A proportional relationship does not exist between the aqueous boundary layer thickness and sampling rates. Observed sampling rates changed 4 to 5-fold from stagnant to more turbulent water

conditions compared to a possible 10-fold change in the thickness of the aqueous boundary layer.

Calculation of the Membrane-Water Partition Coefficient:
The membrane-water partition coefficient (K_{MW}) is a ratio of the concentration of analyte in the membrane (C_M) versus the concentration of analyte in the water (C_W) at equilibrium. The K_{MW} can often be a measure of an analyte's affinity or lack thereof for a membrane. This term is used in the determination of rate constants (polymer matrix control) as well as the overall resistance to analyte mass transfer.

The K_{MW} for each analyte was calculated from data from the 28 day depuration studies using PES Only devices. In each of these studies (see *28 Day Uptake and Depuration Studies*), an apparent steady-state was reached after 7 to 10 days. An average concentration in the membrane and in the water was attained across all data points in the equilibrium period. Using these average concentrations, the K_{MW} for ethynylestradiol, diazinon, and atrazine was calculated. The membrane-water partition coefficients for ethynylestradiol, diazinon, and atrazine were 12200, 40500, and 36800, respectively.

The calculated K_{MW}s for ethynylestradiol and diazinon fit the experimental data. In the 28 day studies, both ethynylestradiol and diazinon were rapidly sampled by the PES Only devices. During the depuration stage, ~40% of the sequestered ethynylestradiol was eliminated from the membrane. In comparison, only ~20% of the sequestered diazinon was lost. The higher K_{MW} of diazinon indicates a stronger affinity for the membrane than the surrounding water, which was observed. The K_{MW} of atrazine only partially fits the experimental data. The 28 day depuration data supports the calculated K_{MW} with ~25% of the sequestered atrazine dissipated from the membrane. The uptake data contradicts the calculated K_{MW} as there was less than a 35% uptake of atrazine after 28 days when a 60-70% uptake would have been predicted from the K_{MW}. It is possible that there is a problem with either the uptake or depuration data or some unforeseen phenomenon is occurring.

Proof-of-Concept Field Deployment:
The final stage of this research involved a proof-of-concept deployment at a site with environmental relevance. The area selected for this study was a system of wetlands used as a final treatment of the City of Columbia's treated wastewater. Following secondary treatment of wastewater, the city's treatment facility uses three wetland units to act as a final mode of treatment for the wastewater. The water leaving the third wetland unit is pumped into the nearby Eagle Bluffs Conservation Area. The City of Columbia and the Missouri Department of Conservation have a cooperative agreement allowing treated wastewater from the city to be used as a constant source of water for the Eagle Bluffs wetland system. Additional water can be provided from the Missouri River to supplement the city's water supply to maintain the area's needs (Figure 28).

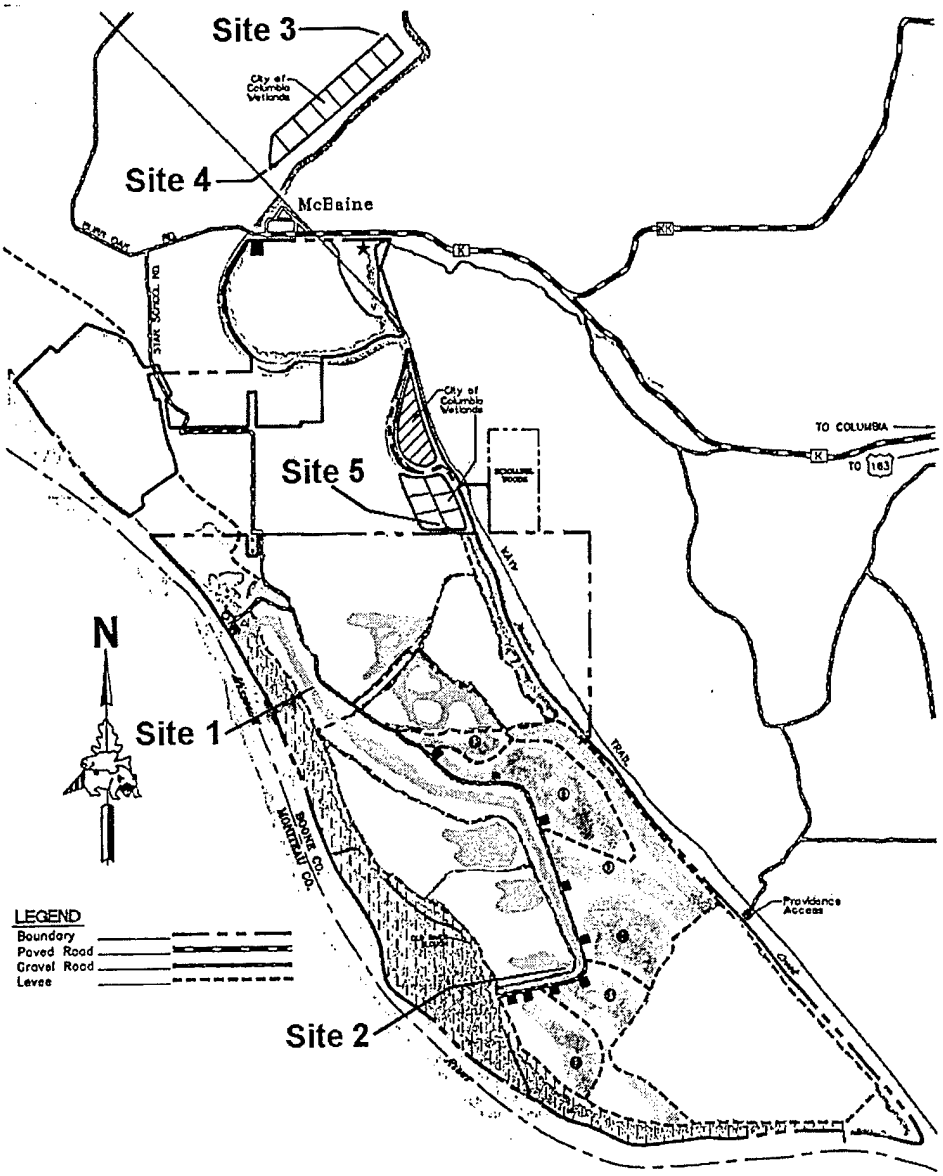


Figure 28 – Locations of Deployment Sites in the City of Columbia and Eagle Bluffs Wetland Complex. Map courtesy of the Missouri Department of Conservation.

The City of Columbia's wetland complex, finished in 1994, was built to supplement the city's existing wastewater treatment facility which was having difficulty in keeping up with the increasing water usage of the continually growing city population. The complex was designed as a series of three connected, although independent, wetland cells (construction of a fourth began in 1999) to allow for maintenance without disruption of the whole complex. Each constructed wetland unit is lined with a minimum of 12 inches of compacted clay to prevent wastewater leaching into the groundwater. The clay liner is covered with 6 to 12 inches of topsoil and planted with cattails (*Typha latifolia*) (60). Cattails were favored due to their natural abundance in the region and their ability to

grow rapidly into a dense vegetative mat. The wetland units are surrounded by flood control berms for protection from the Missouri River. The city's wetland units handle approximately 12 millions gallons of wastewater per day with maximums of up to 18 million gallons. The residence time of water in each wetland unit is approximately 24 hours.

The Eagle Bluffs Conservation Area, maintained by the Missouri Department of Conservation (MDC), is a natural wetland area bordered on the west by the Missouri River and on the east by Perche Creek. The MDC acquired the area in 1989 as part of a statewide wetland restoration and management program. Eagle Bluffs' 13 wetland pools and 1250 acres of seasonal and emergent marshes provide a year-round habitat for migrating and wintering birds and permanent resident wildlife (60). A constant supply of water is provided by the City of Columbia's wetland complex with additional water supplied from the Missouri River as needed. Water in Eagle Bluffs is directed from the northern to southern end of the area by means of distribution channels, levees, water control structures and pumps, where it is eventually released back into the Missouri River.

Overview of Wetland Treatment:
The notion of using of constructed wetlands as a means of wastewater treatment began in the 1970s as people observed water flowing from a natural wetland was of higher quality than water flowing into the wetland (61, 62). Over one thousand constructed and natural wetlands are used worldwide, treating municipal, industrial, agricultural, and urban runoff wastewaters (63). Wetlands offer a low cost, low maintenance, and practical means of supplementing current treatment systems for communities without the budget to upgrade existing systems. Wetlands not only provide an additional means of wastewater treatment, but due to the water and growing plants, provide the essential basis of an ecological foodweb resulting in the presence of a wide array of wildlife (63).

Wetlands improve the overall water quality by the following mechanisms: sedimentation, filtration, chemical precipitation and adsorption, microbial interaction, and uptake of chemicals by vegetation (61). The major mechanism for organic contaminant removal is by microbial metabolism. Wetland plants are able to transport oxygen to their roots providing both aerobic and anaerobic conditions for bacterial diversity (61, 62). This bacterial diversity allows for continuous wastewater treatment regardless of changes in the types and/or quantities of contaminants in the influent, irregular flow rates and seasonal or climatic variations. The bacteria responsible for the degradation of organic contaminants reside on the surfaces of the plants as a biofilm. These biofilms have a greater capacity to metabolize organic substrates than the free-floating planktonic bacteria (61). The wetland degradation of phenol and m-cresol (60-90% reduction in concentration after 24 hours), aromatic compounds (81-99% reduction) and aliphatics (49-93% reduction) have been reported (61).

Preparation of POCIS Components:
Prior to the construction of the POCIS needed for deployment, the individual components (i.e., resin and membranes) were cleaned to remove potentially interfering compounds

with regards to subsequent POCIS analysis. The preparation of each component was as follows:

Isolute ENV+
Ten grams of the Isolute ENV+ resin was placed in a 4 cm (i.d.) glass chromatography column and washed with 2-liters of the solvent mix. The resin was then eluted with an additional 50 mL of the solvent mix which was collected, reduced in volume to 1 mL and screened on a GC using both ECD and FID methods of detection (see Figure 29 for chromatograms). At this point the Isolute ENV+ was considered acceptable for use, allowed to air dry on solvent rinsed aluminum foil, and stored in an air-tight container.

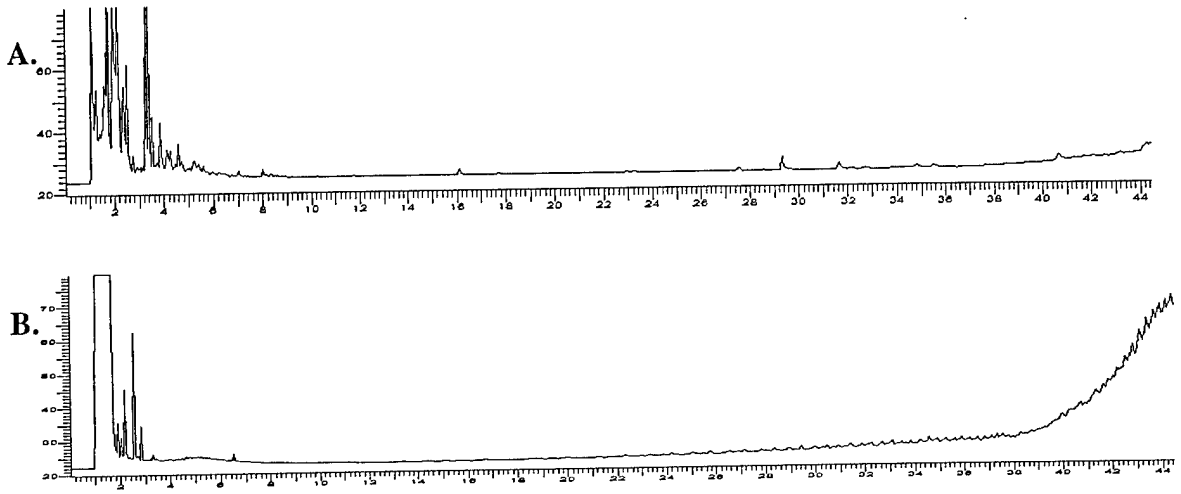


Figure 29 – GC screening of "cleaned" Isolute ENV+ resin. A - ECD detection. B - FID detection.

S-X3 dispersed A-1500
Five grams of the S-X3 dispersed A-1500 was prepared. The S-X3 dispersed A-1500 was placed in a 4 cm (i.d.) glass chromatography column and cleaned by washing with 2-liters of the solvent mix. The resin was then eluted with an additional 50 mL of the solvent mix which was collected, reduced in volume to 1 mL and screened on a GC using both ECD and FID methods of detection (see Figure 30 for chromatograms). At this point the S-X3 dispersed A-1500 was considered acceptable for use, allowed to air dry on solvent rinsed aluminum foil, and stored in an air-tight container.

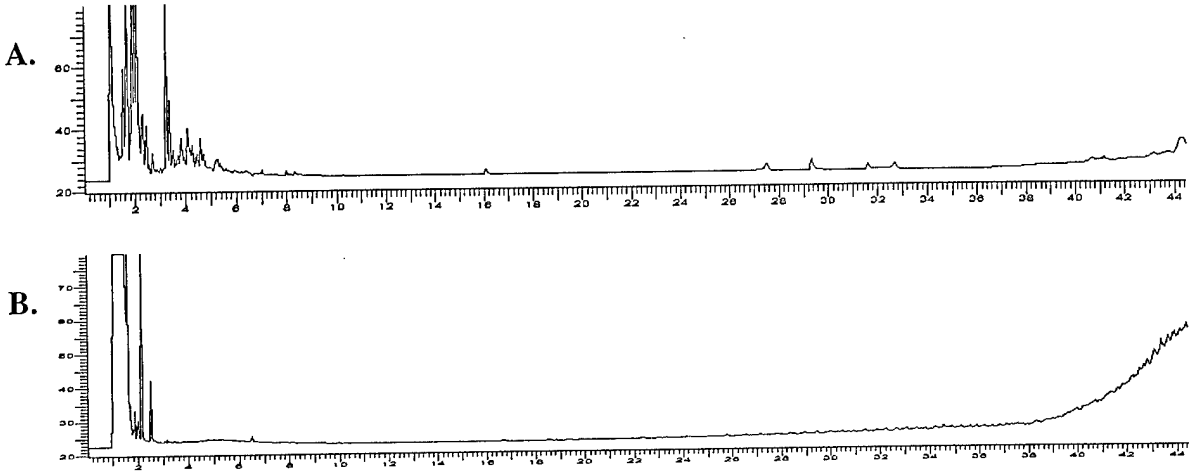


Figure 30 – GC screening of "cleaned" S-X3 dispersed A-1500. A – ECD detection. B – FID detection.

Polyethersulfone Membrane Disks

Approximately 150 membrane disks were prepared by placing the disks into six canning jars for dialysis, and adding 200 mL of 10/90 hexane/isopropanol to each jar. The jars were sealed with foil-lined caps and were placed in the dialysis incubator at 18°C for 24 hours. Following the initial dialysis period, the dialytic solvent was exchanged at 7 am and 4 pm for two days, resulting in a total of 5 solvent exchanges. After the last dialysis period, the membrane disks were removed from the solvent and allowed to air dry on solvent rinsed foil. The final portions of the dialytic solvent were reduced in volume to 1 mL by rotary evaporation and screened by GC-ECD and GC-FID (see Figure 31 for chromatograms).

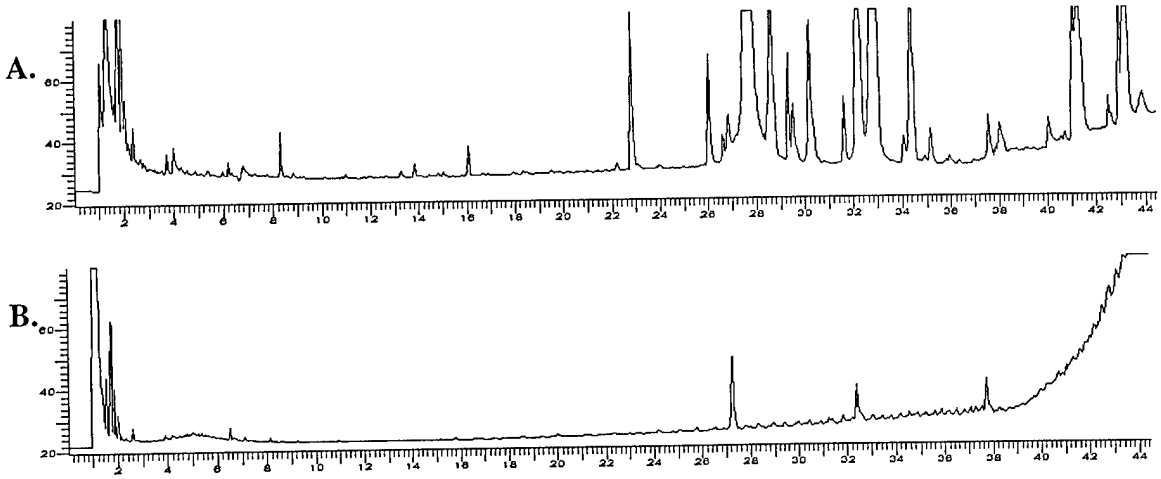


Figure 31 – GC screening of "cleaned" PES membranes. A – ECD detection. B – FID detection.

The large number of peaks present in the ECD chromatogram for PES membrane screening, suggest an alternative method of cleaning the membrane may be required. For

the purpose of this proof-of-concept deployment, the membranes were used as is. It was decided that only the chemicals sequestered by the resin mix would be analyzed and the membrane disks would be discarded prior to POCIS processing. The laboratory exposures previously discussed have shown that after exposure periods of 28 days, the majority of the sequestered material resides in the resin mix and not in the membrane.

Development of the Deployment Canister:
A deployment canister was designed by modifying an SPMD canister developed by Dr. Harry Prest (Long Marine Laboratory, U.C. at Santa Cruz, CA). Dr. Prest's canister involved 10 threaded support rods (2 sets of 5) connecting 2 circular plates which formed the top and bottom. A metal screen was used to enclose the sides of the cylinder portion of the canister. All parts were made of stainless steel to prevent corrosion. In the original design, 4 SPMDs were woven back and forth through the support rods and were protected by the metal screen.

In modifying this design, 6 of the support rods were removed to facilitate canister construction. A hole was drilled in the top and bottom plate, offset from the center, to allow for the placement of a single rod. This rod was used to support 4 POCIS, using five 7/8" coupling nuts to evenly space the samplers. The rod containing 4 samplers was fastened to one end of the canister with a lock washer and nut. Each POCIS was then staggered on the rod to allow maximal water contact. The metal screen was placed around the 4 support posts and the lid was fastened with washers and nuts. The prototype canister was now ready for deployment (Figures 32 and 33).

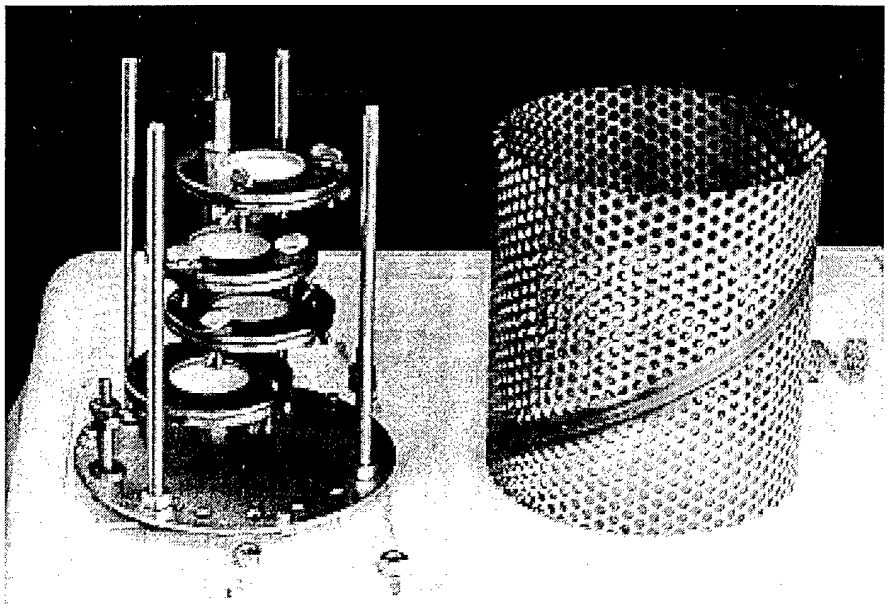
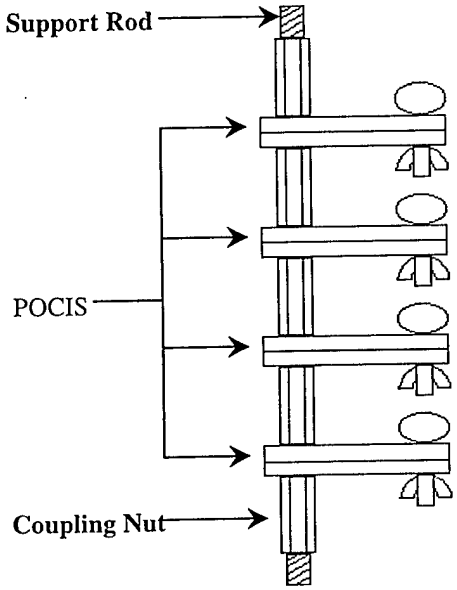
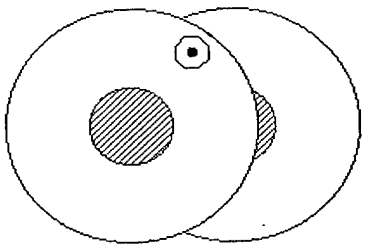


Figure 32 – Four POCIS supported in a field deployment canister adapted from an SPMD canister designed by Dr. Harry Prest (Long Marine Laboratory, U.C. at SantaCruz, CA).



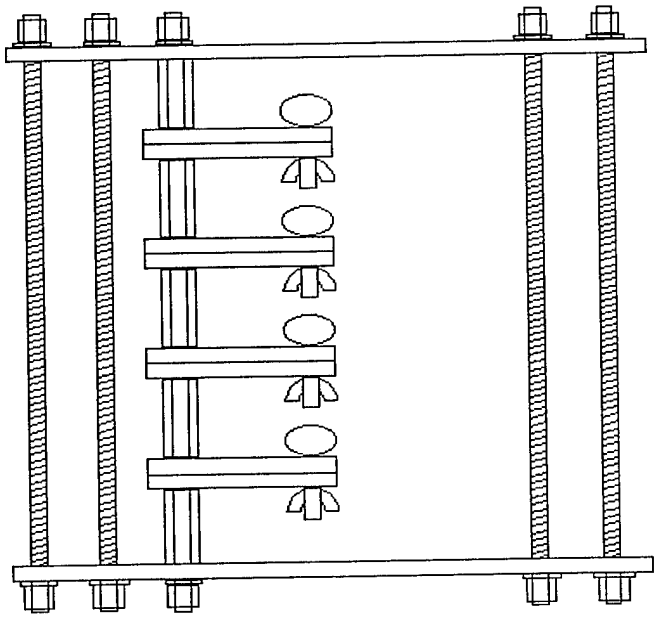
Side View

4 POCIS on Support Rod



Top View

POCIS staggered on Support Rod



Side View

Prototype Deployment Canister with 4 POCIS. Protective Metal Screen

Figure 33 – Schematic Drawing of a Prototype POCIS Deployment Canister used in the Proof-of-Concept Deployment.

Construction of POCIS for Deployment:
Prior to POCIS construction, all stainless steel parts and hardware were solvent rinsed to remove any residual contaminants, oils, etc. The individual samplers were constructed with 100 mg of the 20% S-X3 dispersed A-1500 / 80% Isolute ENV+ resin mix. Each POCIS was secured with two thumb screws, leaving the third hole free for attachment into the deployment canister.

Four POCIS were placed on a threaded rod using 7/8" coupling nuts as spacers. Once secured, the rod and 4 samplers were placed in a jar filled with DI water. The water-filled jars were used to store the devices until the moment of deployment when the rod with 4 samplers would be removed and inserted into the deployment canister. As previously discussed, it is preferable to pre-solvate the membranes prior to use in order to achieve a steady sampling rate. Storing the POCIS in DI water during shipment prior to deployment satisfies the presolvation requirement. At the deployment site, the rod with 4 samplers was removed from the storage water and fastened into the canister, ready for deployment.

Field blanks (or Trip blanks) were prepared along with the POCIS for deployment for use as a quality control measure. One field blank (1 support rod containing 4 POCIS) was prepared for each day of deployment. The field blanks were stored in DI water and taken to the deployment site just as were the actual samplers. During the time the samplers were removed from the storage water until they were placed in the deployment site, the lid on the field blank jar was removed allowing contact with the surrounding air. This method allows for the background correction of any compounds that may be picked up during the act of deploying the samplers and not actually due to exposure at the site.

Deployment Site Description:
The sampling devices were deployed at five sites throughout the City's and Eagle Bluffs wetland system. The samplers were deployed at two sites in Eagle Bluffs on September 2, 1999 and at the three sites in the city wetlands on September 3, 1999 (Figure 34).

The samplers were retrieved on September 30, 1999. During retrieval, the support rod with 4 POCIS was removed from each canister and placed in a sealed jar (without water) for transport back to the laboratory. Once at the laboratory, the samplers were stored at -15°C until processing to minimize the possibility of additional sampling (i.e., airborne contaminant sampling).

Figure 34 – Sampling device canisters deployed at Site 3 . The deployment team included (left-to-right) David Alvarez, Jim Petty, Rusty Sutterlin, and Bill Brumbaugh.

Site 1 – Eagle Bluffs Main River Supply Channel
The river supply channel provides additional water into the Eagle Bluffs complex. The previous day (9/1/99) there was no water in the channel therefore supply pumps were activated to allow for the additional flooding of the area for the upcoming waterfowl hunting season. At the time of deployment, water depth in the channel was 2-2.5 feet with mild flow. The selection of this site provides information on the introduction of chemicals into the Eagle Bluffs complex from the Missouri River.

Two POCIS canisters were deployed on aircraft cable secured to the shore by a trailer anchor. A field blank was used at this site. The anchor was placed on the east side of the channel near a bend in the road. It was in a clearing between a large and small clump of trees just at the south edge of the larger clump of trees. The cable was placed in the water nearly perpendicular to the bank. Water temperature was not taken at time of deployment but was estimated to be approximately 26°C.

The water level at this site rose significantly following deployment. The support anchor on the bank to which the cable was attached was under 3 feet of water at retrieval. Periodic observations at the site during the exposure period noted the water level and flow had remained fairly constant after the first few days following deployment. The surfaces of the deployment canisters and the POCIS were covered with a carbonate precipitate scale. The field blank used during deployment was again exposed to the surrounding environment during POCIS retrieval at this site. The water temperature at retrieval was 18.4°C.

Site 2 – Eagle Bluffs Pool 9
Pool 9 is located just to the north of the main distribution channel near the southern end of the wetland complex. This point represents the longest residence time of water in the system before it empties back into the Missouri River. At the time of deployment the water in Pool 9 was primarily from the city effluent. Although this site receives its water from the main distribution channel which is connected to the main river supply channel, it is believed that water conditions at this site remained nearly constant throughout the exposure period. It would take a considerable amount of time for the river water to fill the empty pools and flood marshes as it travels the distance to Pool 9. Selection of this site provides information on the effectiveness of treatment of primarily city wastewater effluent.

Two POCIS canisters were deployed on aircraft cable secured to the shore at a water control structure. A field blank was not used at this site. The water control structure is located just to the east of the main pool. This point was selected due to its accessibility and its water depth (~3 feet at deployment with mild flow). The water was too shallow and the bottom too soft to allow deployment in the main body of the pool. The cable was attached to the end of a metal railing just underwater. The cable was strung out at a 10-20° angle to the right (northeast) of the railing to avoid deeper water at the structure inlet. Water temperature was not taken at time of deployment but was estimated to be approximately 26°C.

At retrieval, the water level at Site 2 rose ~6 inches following deployment. The canisters were filled with a very fine, silt-like material representative of the bottom sediment. As much of the sediment as possible was washed off the POCIS and canisters at the site. The water temperature at retrieval was 16.0°C.

Site 3 – City of Columbia Inflow into Wetland Unit 1
Wetland Unit 1 is the first in a series of wetland units utilized by the city as a means of wetland wastewater treatment. Wastewater from the city's treatment plant flows into this unit through a 72 inch pipeline at the northern end of the unit. From here the water traverses several individual wetland cells within the unit before it exits via an outflow pipe at the southern end of the wetland. This site represents the secondary treatment effluent from the wastewater treatment plant prior to any additional treatment in the wetland system.

Three POCIS canisters were deployed on aircraft cable secured to the shore by a trailer anchor. A field blank was used at this site. The anchor was placed approximately 10 yards to the southwest of the inlet with the cable thrown out from shore at ~70° angle from the bank. Water depth at this point was 5-5.5 feet with constant flow. There were a large amount of solids in the water at this point. Water temperature at time of deployment was 24.9°C.

The water at retrieval appeared to contain less solids than during the initial deployment. The samplers were relatively clean except for the blood worms and other organisms that had attached to the surfaces. Two of the POCIS had "ballooned" during the exposure

possibly due to gas buildup within the membranes. As during deployment, a field blank was exposed to the environment during POCIS retrieval at this site. At the time of retrieval, the water temperature was 21.5°C. The water temperature was higher at this site than others due to recent processing at the wastewater treatment facility.

Site 4 – City of Columbia Outflow from Wetland Unit 1
Water flows through the wetland cells to the outflow pipe by means of an 18 inch downward gradient from the inflow pipe. At this outflow point, the water travels through a pipe into Wetland Unit 2 where it undergoes additional treatment. Site 4 represents the wastewater from the treatment plant after being subjected to wetland treatment in Unit 1.

Three POCIS canisters were deployed on aircraft cable secured to the shore at the outflow structure. No field blanks were used at this site. The cable was attached to the end of the metal railing on the east side of the concrete outflow structure. To keep the samplers in the deepest part of the channel and lowest amount of flow, the cable was strung along the concrete wall adjacent to the outflow pipe. Water depth at this point was 2-3 feet with constant flow. There were less solids in the water at this point than was observed at the inflow point. Water temperature at time of deployment was 24.9°C.

The POCIS canisters were nearly half full of sediment and other solid materials at retrieval. This site had the highest degree of water flow which may have resulted in the trapping of solid materials inside the canisters. As with Site 3, all surfaces were covered with blood worms and other organisms. Retrieval water temperature was 16.5°C.

Site 5 – City of Columbia Effluent Pump Station at Wetland Unit 3
Following the final stage of treatment in the city's wetland system, the wastewater from the third wetland unit is pumped into Eagle Bluffs. At the end of the city's system is a Effluent Pump Station which was selected as the site representing the culmination of the city's wastewater treatment process. The Pump Station cycles approximately every 20 minutes pumping water out of a holding canal into Eagle Bluffs.

Three POCIS canisters were deployed on aircraft cable secured to the shore at the end of the pump station canal. A field blank was not used at this site. The cable was attached to the end of the metal railing on the east side of the concrete canal north of the pump station. To keep the samplers in the deepest part of the canal, the cable was placed in the water at the end of the pump cycle before the canal began to refill. Water depth varies from a minimum of 1 foot to several feet deep depending on the pump cycle. Water temperature at time of deployment was 26.0°C.

This site underwent heightened biological growth following deployment. An algal bloom had covered one side of the concrete canal and the deployment canisters. Both the inside and outside of the canisters were covered with the growth. As observed at Sites 3 and 4, a large population of blood worms and other organisms covered all surfaces. The water temperature at retrieval was 14.9°C.

Processing of Retrieved POCIS:

The samplers and quality control blanks were processed over five consecutive days. The quality control samples included the field blanks from Eagle Bluffs and City Wetland deployments as well as process blanks and solvent blanks. The field blanks were carried through the same process as the exposed samplers. The process blank (or resin blank) was the elution of 400 mg of clean resin mix, the equivalent of 1 deployment canister or 4 POCIS. The process blank provides information on potentially interfering compounds that are due to the resins used in the samplers. A solvent blank was 200 mL of the solvent mixture, equivalent to solvent usage for 4 POCIS, which provides information on any compounds due to the solvent. All blanks underwent the same evaporation and solvent exchange steps as the actual exposure samples.

Prior to processing of the POCIS, they were removed from the freezer and allowed to equilibrate to room temperature. They were then rinsed under warm running water to remove any materials adhering to the surfaces. The POCIS were removed from the support rod and the resin mix transferred into individual 1 cm (i.d.) glass chromatography columns for subsequent elution. The transfer occurred by carefully opening the POCIS and rinsing the resin mix into the column with methanol. The methanol rinse was collected in the same collection flask which was used for the eluting solvent. Following the rinse, the membrane disks were discarded. The resin mix was eluted with 50 mL of the 10/10/80 methanol/toluene/dichloromethane solvent mix. The rinse and eluate was evaporated to ~1 mL by rotary evaporation. This concentrated extract was then passed through mini-columns constructed from disposable pasteur pipets containing anhydrous sodium sulfate (2 cm bed volume) to remove any residual water. The dried extract along with several isopropanol rinses were collected in culture tubes and evaporated to ~1 mL under nitrogen. Two evaporations under nitrogen to <1 mL with the addition of excess isopropanol were performed to remove most of the remaining toluene. The final extracts were combined into 2 to 4 POCIS equivalents for each site. Portions of the extracts, equivalent to 4 POCIS, were ampulated and sent to Dr. Colleen Rostad, Dr. Ed Furlong, and Tom Leiker (USGS, Denver, CO) for mass spectral screening.

The POCIS from all 5 sites survived exposure remarkably well. The membranes showed no evidence of damage and very little biofouling was observed. The devices from Site 1 were difficult to dissemble due to the buildup of a carbonate scale on the threads of the thumb screws. The extracts from the city wetland sites had a distinct sewage odor. The extracts from all sites, at a 1 mL volume, were dark green in color, possibly from chlorophyll. Some components were difficult to keep in solution in 1 mL of cold isopropanol (sample extracts were stored in a freezer at -15°C). A green film would form at the bottom of the tube. This film could be placed back into solution by agitation of the tube. For testing, both in-house and at the USGS Denver laboratory, the supernatant liquid was removed from the green film and used in subsequent analysis. The green film was kept for possible later study. No attempt was made to "cleanup" or remove potential interferences from the samples in fear of removing desired unknown analytes.

POCIS Analysis by GC/MS:

A portion of the Site 5 extract sent to the USGS Denver laboratory was analyzed using GC/MS. A 100 μL aliquot of the original 1 mL sample was taken for analysis. The gas chromatographic separations were performed using a 60-meter DB-5 column with the following temperature program: 50°C for 3 min, then increase 20°C/min to 150°C, followed by 2.5°C/min to 250°C, then 20°C/min to 300°C and hold for 10 min. Detection was by a magnetic sector mass spectrometer with electron impact ionization providing positive molecular ions as well as extensive fragmentation of the analyte into daughter ions. Ionization occurs by bombardment of the analyte molecule with electrons generated from a heated tungsten or rhenium filament (64). The positive molecular and daughter ions formed are accelerated through plates with varying potentials into the mass analyzer. Identification of analytes was performed by a routine mass spectral library search. Analytes which matched library spectra were ranked due to their degree of fit to the library spectra and to the purity or lack of extraneous peaks in the spectra. The purity of the spectra is considered due to the complexity of the obtained spectra. As a conservative estimation, values for the purity and fit >700 were considered a tentative identification (per Tom Leiker). All other matched spectra with values <700 provide less evidence supporting the suggested identification. Tables 30 and 31 list the tentatively identified and suggested identification analytes, respectively.

Tentative ID	Purity	Fit	Use
Ibuprofen*	639	975	pain reliever
Alkyl phenols (Nonyl phenol)*	-----	-----	surfactant degradation product
Ephedrine Artefact Acetate 1	872	969	metabolite of decongestant
Nicotinic Acid	763	846	vitamin
Oxindole	793	974	treat viral infections, intestinal disorders
4-hydroxyindole	763	967	fragrance
5-phenylhydantoin	726	872	metabolite of phenytoin (anti-convulsant)
Tri-2-butoxyethyl phosphate*	681	955	impurity from B-D Vacutainers
Phenelzine*	681	781	anti-depressant
Mephenytoin*	561	692	anti-convulsant
Ethotoin*	531	686	anti-convulsant

Table 30 – List of Tentatively Identified Compounds from Site 5 by GC/MS. * These compounds have purity values below 700, however, Tom Leiker was confident in their identification.

Suggested ID	Purity	Fit	Use
Dihydrocarveol	610	828	mint fragrance
β-Terpineol	571	857	perfumes, soaps
Isopulegol	564	895	perfumes, soaps
1-Terpineol	539	822	perfumes, soaps
Hedycaryol	529	872	
large range of phthalates	483-650	895-973	various uses in manufacturing
Guaiol	572	875	wood oil
Longcyclene	518	834	
β-Patchoulene	513	803	
Valencene	511	826	perfumes
δ-Selinene	495	891	
Aromadendrene	475	894	
α-Gurjunene	473	915	
β-Gurjunene	483	919	
α-Muurolene	461	832	
trans-2-Tridecenal	559	793	
trans-2-Dodecenal	516	736	
cis-Sabinenehydrate	531	787	
trans-Sabinenehydrate	502	773	
β-Terpinyl acetate	504	758	
Ethyl-3-phenylpropanoate	572	646	
Hydrocinnamic acid – methyl ester	557	708	
Hydrinantin	528	653	reagent for amino acid determinations
Apiol	514	756	oil of dill or parsley

Table 31 – List of Suggested Analyte Identifications at Site 5 determined by GC/MS.

The number of identified compounds in Tables 30 and 31 is small compared to the total number of mass spectra generated during analysis. Most spectra were too complex for the mass spectral library to make an identification. A full scan total ion current chromatogram along with a representative mass spectra are shown in Figures 35 and 36, respectively.

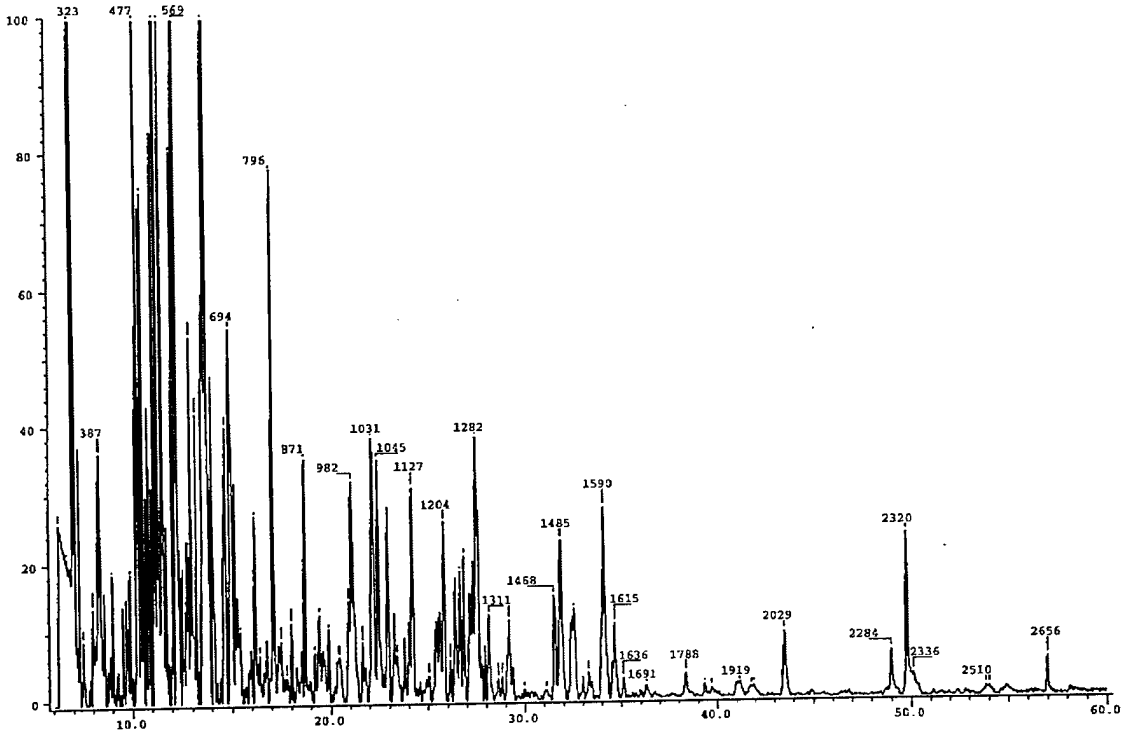


Figure 35 – Total Ion Current Chromatogram from GC/MS analysis of Site 5.

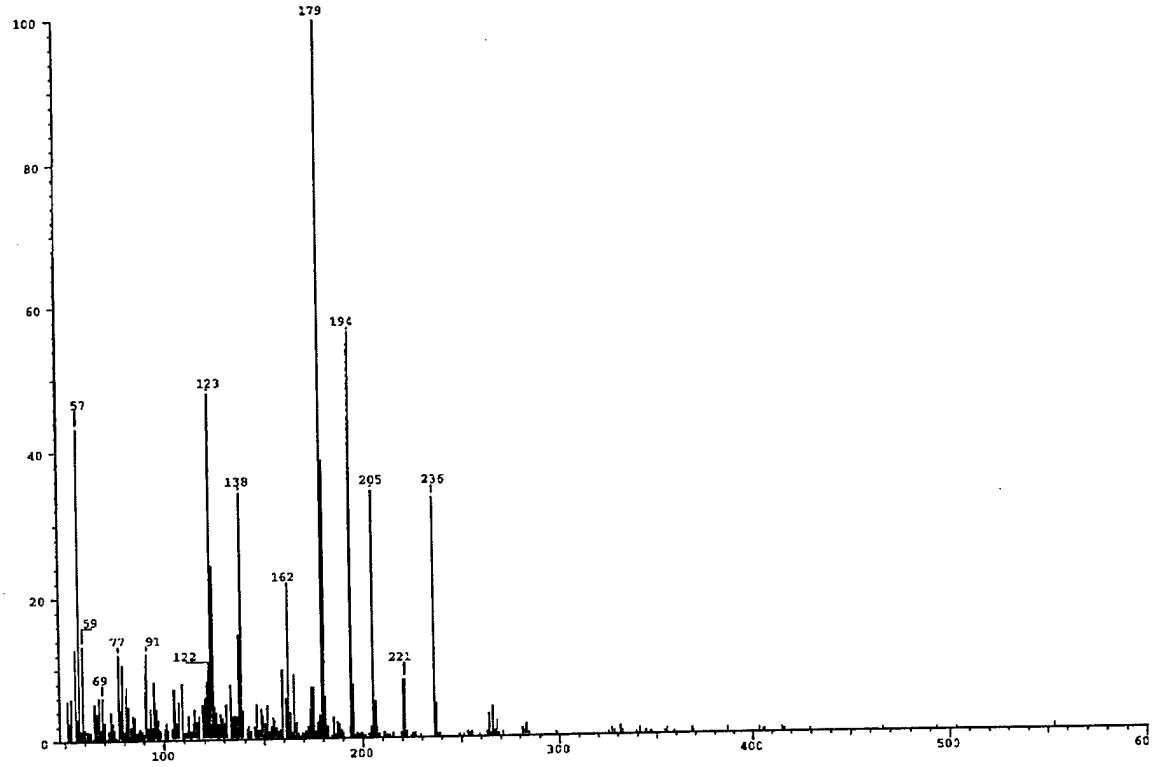


Figure 36 – Representative mass spectra from GC/MS analysis of Site 5.

POCIS Analysis by LC/MS:
Portions of sample extracts from each exposure site and quality control blank were analyzed by LC/MS. A 20 μL aliquot from each sample diluted with 100 μL of water was used in the analysis. The formation of both positive and negative molecular ions was achieved with an electrospray ionization interface, connecting the LC to the mass spectrometer.

Electrospray ionization occurs at atmospheric pressure and is suited for the analysis of nonvolatile and thermally labile analytes such as high molecular weight compounds and biopolymers (65). An electrospray is produced by applying a high electric field to the LC column effluent which slowly flows (1-10 μL/min) through a capillary tube (66). The electric field disrupts the liquid surface, forming a stream of highly charged liquid droplets. Desolvation of the effluent occurring by collision and thermal means results in the formation of analyte ions. The ability to form ions of high molecular weight compounds is due to the formation of multiply charged ions which appear in lower mass/charge ranges.

Electrospray is a mild ionization process, generally producing only molecular ions. Not all compounds will produce both positive and negative ions. Compounds that are very acidic may not produce positive ions and very basic compounds may not produce negative ions (67). The produced ions are generally analyzed by conventional quadrupole mass spectrometers.

Analyte identification was performed by matching the LC retention time with retention times from a group of target analytes of interest per the USGS National Water Quality Laboratory (NWQL) Schedule 9060. For the positive ion electrospray, 53 current-use pesticides, herbicides, and compounds of interest were selected as a standard target mixture. In the negative ion electrospray, 17 target compounds were screened.

The full scan total ion current chromatograms for each site were very complex. Only a few compounds matched the target analytes at each site for the positive ion electrospray (Table 32). The signals from the negative ion electrospray were less complex than the positive ion with zero matches to the target list.

	Compound Detected	MS Response	Sample Concentration[a]
Site 1	Hydroxyatrazine	329434	<MQL
	Atrazine	654220	22600 µg/L
Site 2	Desethyl-Deisopropyl Atrazine	30657	<MQL
	Hydroxyatrazine	143342	<MQL
	Atrazine	131577	<MQL
Site 3	Desethyl-Deisopropyl Atrazine	63988	<MQL
	Caffeine	430522	<MQL
Site 4	Desethyl-Deisopropyl Atrazine	51770	<MQL
Site 5	Desethyl-Deisopropyl Atrazine	115454	<MQL
	Caffeine	99596	<MQL
	Propoxur	74991	<MQL
Field Blank	No target analytes present	-----	-----
Process Blank	No target analytes present	-----	-----
Solvent Blank	No target analytes present	-----	-----

Table 32 – Identified Compounds from LC/MS analysis. [a] Analyte concentration in the original 1 mL sample corrected for any dilutions. <MQL = below the minimum quantification limit.

In the Missouri River distribution channel (Site 1) atrazine and its major metabolite, hydroxyatrazine, were detected. This was expected due to the major use of atrazine in corn production in areas through which the Missouri River and the river's tributaries flow. Atrazine at this site was the only identified analyte at a concentration above the minimum quantification limit of the instrument. Calculation of the ambient water concentration of atrazine was performed using the predetermined sampling rate data under flowing conditions. The entire sample, equivalent to 4 POCIS, contained 22.6 µg atrazine. Dividing the sequestered amount by the combined sampling rate (R_s for 1 POCIS multiplied by 4) and the exposure time in days, provides the ambient water concentration.

$$\frac{22.6\ \mu g}{(4 \times 0.240\ L/d)(28\ d)} = 0.84\ \mu g/L = 0.84\ ppb$$

Atrazine concentrations in this region of the Missouri River typically maintain a consistent year-round level of 1-3 ppb (68). At times of increased use and/or increased surface runoff, the level may reach 4-6 ppb. During years, such as 1999, with little rainfall to serve as a transport mechanism, atrazine levels will fall below the typical levels. Data from the USGS National Stream Water Quality Network (NASQAN) gave an atrazine concentration of 1.16 ppb at Hermann, MO (60-70 miles downstream from Eagle Bluffs) on June 6, 1999 (70). Yearly data indicates decreasing atrazine water concentrations following the spring crop application. The estimated level of 0.84 ppb

from the POCIS deployment is in excellent agreement with the reported atrazine levels. The addition of a cleanup step in the processing of the samplers theoretically would reduce the number of interfering peaks and simplify the spectra, resulting in a more accurate estimation of water concentration.

At Site 2, the atrazine response levels had decreased by a factor of 5, whereas the hydroxyatrazine decreased only by a factor of 2. Desethylatrazine and deisopropylatrazine, two other metabolites of atrazine, were detected. The slower rate of disappearance of hydroxyatrazine in the wetland suggests that atrazine is primarily metabolized into hydroxyatrazine which is in turn being metabolized. The response levels of the metabolites compared to the parent compound, indicate the wetland is working properly by degrading organic contaminants.

The City of Columbia wetlands (Sites 3, 4, and 5) provided the most complex ion current chromatograms due to the increasing number of unidentified compounds present. At all three sites, both desethylatrazine and deisopropylatrazine was identified. It is likely that atrazine and hydroxyatrazine were present in the samples, but were not identified due to the complexity of the chromatograms. Propoxur, an insecticide for ants, cockroaches, flies, and mosquitoes, was detected at Site 5. A common contaminant in municipal wastewater effluent, caffeine, was identified at Sites 3 and 5. The lack of detection of caffeine at Site 4 is possibly due to the buildup of sediment inside the deployment canisters which may have limited the sampling ability of the POCIS. The level of caffeine decreased by a factor of 4 between the influent and effluent of the city's wetland complex. This decrease is indicative of a working wetland system.

No target analytes were identified in any of the blanks (field, process, or solvent). The overall ion response of the blanks were 1 to 2 orders of magnitude less than the actual samples. The positive ion electrospray total ion current chromatograms for Sites 1 and 5 are depicted in Figures 37 and 38 respectively.

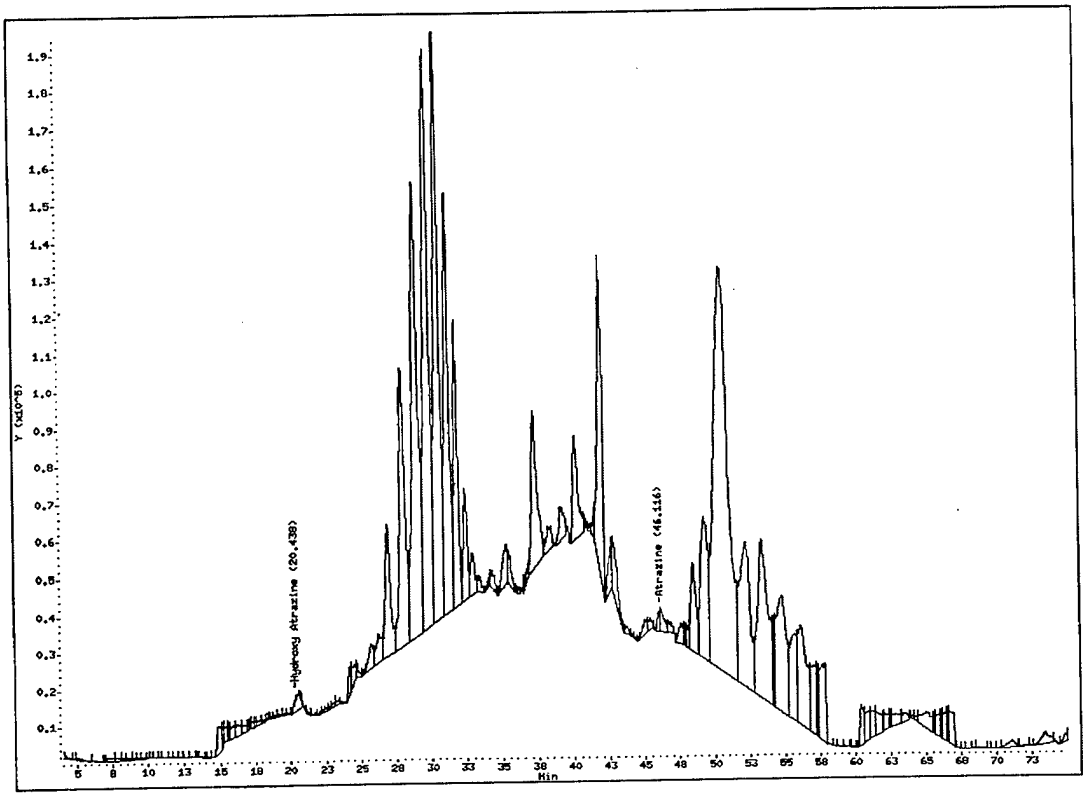


Figure 37 – Positive ion electrospray LC/MS total ion current chromatogram for Site 1.

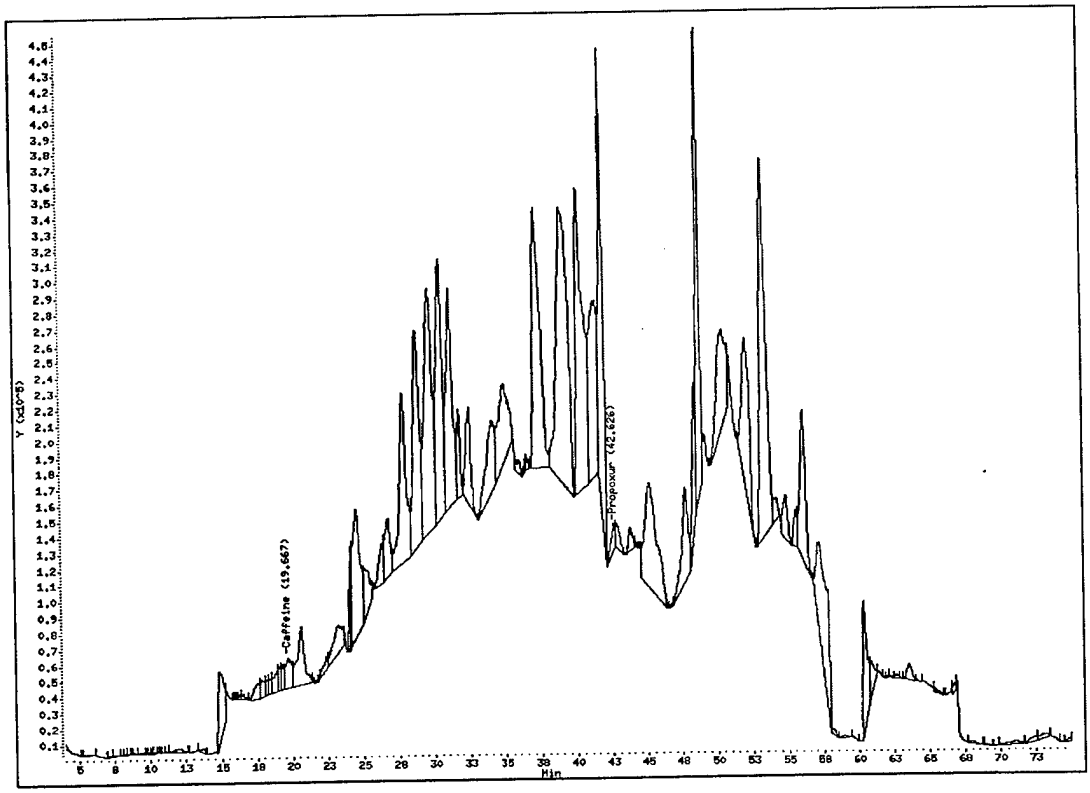


Figure 38 – Positive ion electrospray LC/MS total ion current chromatogram for Site 5.

POCIS Analysis by FIA-MS:
Portions of the extracts from each site and blank were also analyzed by Flow Injection Analysis (FIA) coupled to a mass spectrometer with both positive and negative electrospray ionization. A 100 μL aliquot of the sample diluted with 100 μL of water was used in all determinations. No automated attempt to identify analytes was performed either by matching with target analytes or a mass spectral library.

A flow injection system consists of a sample injector similar to ones used in HPLC, a solvent delivery system or pump, and a detector. Early FIA systems were used for the colorimetric determination of inorganic ions (64). The technology is now being applied to automated extractions, on-line reactions, and sample introduction. As applied to sample introduction into a mass spectrometer, FIA involves the flow of a carrier solvent where samples were injected into the flow at one minute intervals. The carrier solvent prevents mixing of the individual samples by the formation of discrete segments (solvent-sample-solvent) in the flow. In this manner, multiple samples can be introduced into the ion source of a mass spectrometer in sequential order.

Electrospray ionization techniques were used to produce positive and negative ions for mass spectral detection. Gentle ionization conditions were used therefore only the molecular ions were formed. Tentative identification of analytes was performed by a manual inspection of the spectra. A list of possible analytes of interest was compiled and the masses of their positive and negative molecular ions were determined. Positive ions form by the addition of a sodium ion (M+Na) therefore 23 was added to the molecular mass of the analyte. Negative ions lose a hydrogen (M-H), so 1 was subtracted from the analyte's molecular mass. The positive and negative masses were searched for in the spectra. If a mass was found, the blanks were examined to determine if the mass was present as part of the processing or was sequestered from the environment. A match of both the positive and negative ion masses supported a tentative identification. Matching only one of the ions presented evidence towards identification. Tables 33 and 34 list the tentatively identified and evidence towards identification of analytes respectively. Representative mass spectra for the positive and negative electrospray ionization of Site 3 and the Field Blank are depicted in Figures 39-42.

	Compound Detected	Positive Ion (M+Na)	Negative Ion (M-H)
Site 1	β-Estradiol-3-sulfate	does not exist	351
	Oxytetracycline	483	459
Site 2	Sulfamerazine	287	263
	Sulfadiazine	273	249
Site 3	Ethynylestradiol	309	295
	Vitamin A	309	285
Site 4	Sulfadimethoxine	333	309
	Oxytetracycline	483	459
	Vitamin A	309	285
Site 5	Vitamin A	309	285

Table 33 – Tentatively identified compounds by match of both the positive and negative molecular ions from the FIA-MS analysis.

	Compound Detected	Positive Ion (M+Na)	Negative Ion (M-H)
Site 1	Chlorpyrifos		350
	Sulfamethazine		277
	Sulfamerazine		263
	Sulfadiazine		249
	Tetracycline	445	
	Tiamulin fumerte		609
Site 2	Ethynylestradiol	319	
	Metolachlor	307	
Site 3	Sulfamerazine		263
	Sulfadimethoxine		309
	Metolachlor	307	
	Alachlor or Acetochlor	293	
	Vitamin D_2		396
Site 4	Sulfamethazine		277
	Sulfamerazine		263
	Sulfadiazine		249
	Metolachlor	307	
	β-Estradiol		271
	Vitamin D_2		396
	Vitamin E acetate	496	
Site 5	Sulfamethazine		277
	Sulfamerazine		263
	Sulfadiazine		249
	Ethynylestradiol		295
	Metolachlor	307	
	Alachlor	293	
	Vitamin D_2		396
	Vitamin E		454

Table 34 – Compounds with evidence towards a possible identification due to the match of either the positive or negative molecular ion. Analysis by FIA-MS.

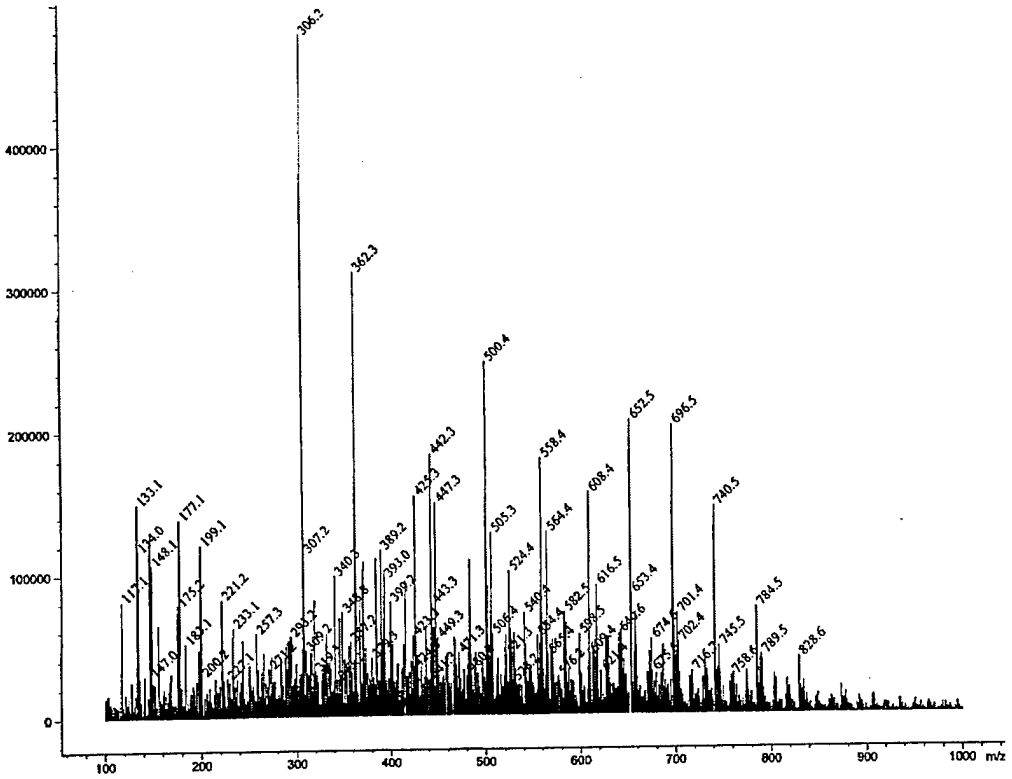


Figure 39 – Positive ion electrospray of Site 3 from FIA-MS analysis.

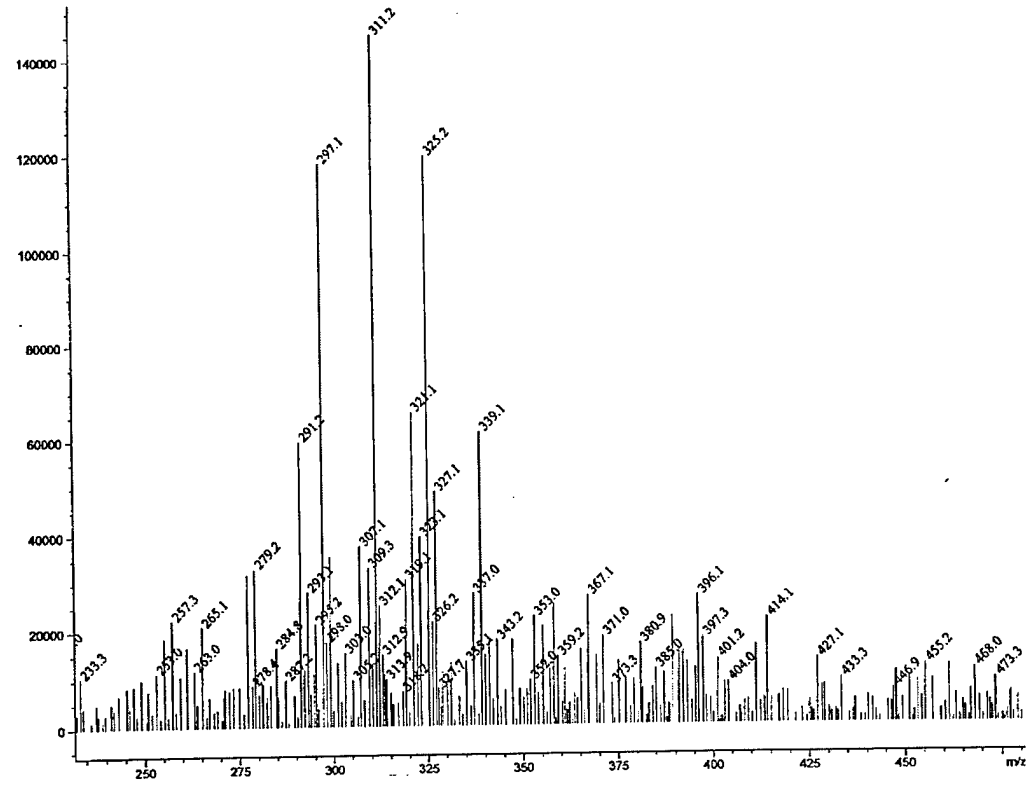


Figure 40 – Negative ion electrospray of Site 3 from FIA-MS analysis.

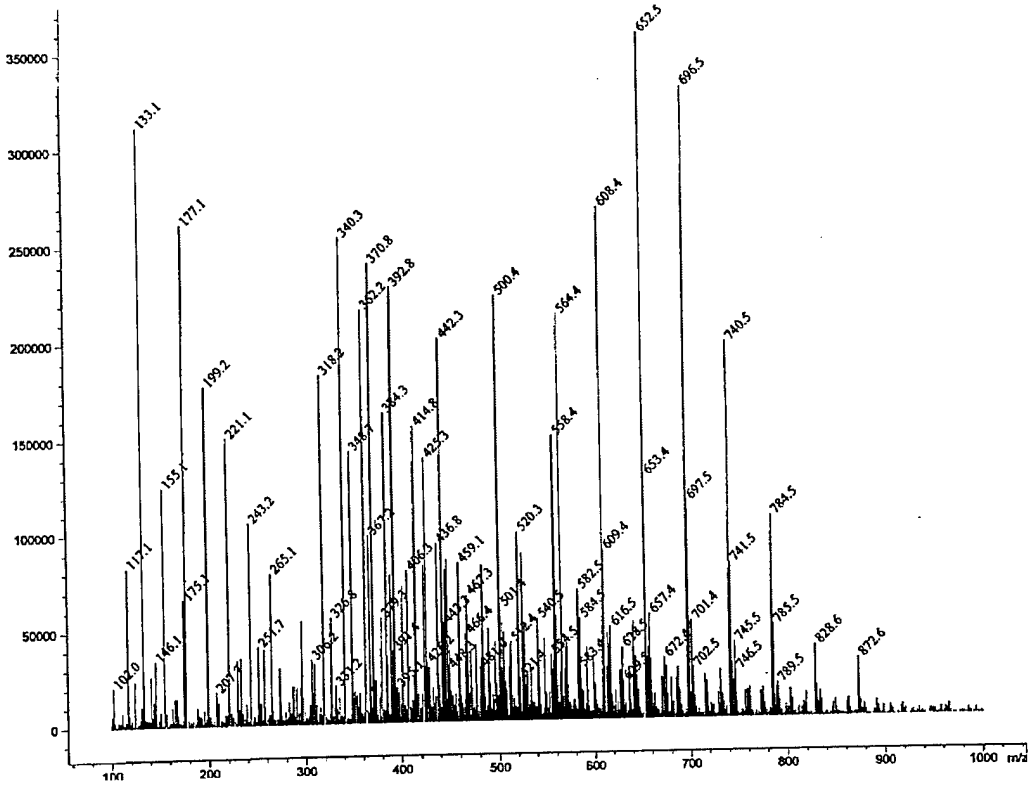


Figure 41 – Positive ion electrospray of the Field Blank from FIA-MS analysis.

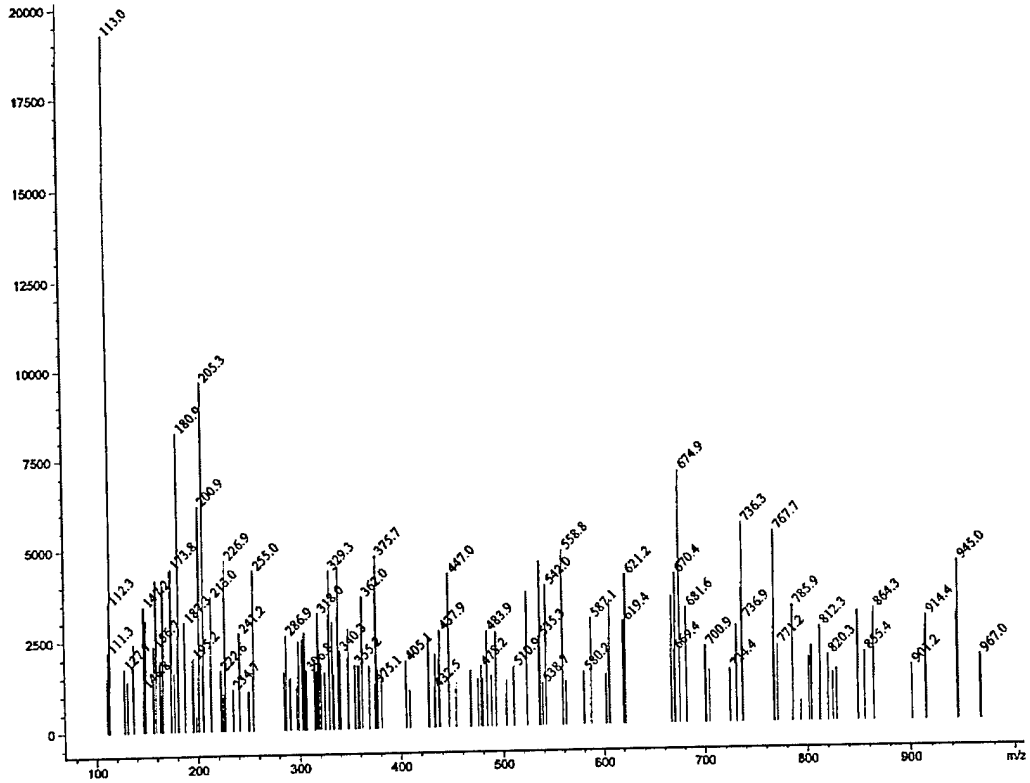


Figure 42 – Negative ion electrospray of the Field Blank from FIA-MS analysis.

Due to the complexity of the mass spectra, only a cursory interpretation could be performed. Identification of the individual peak masses was limited by the number of peaks selected by the mass spectrometer's data analysis software. An increased number of ions matched in the negative ion spectra which was much less complex than the positive ion spectra. It is highly likely that many of the compounds with only the positive or negative ion found were actually present with the accompanying positive or negative ion hidden by surrounding peaks. The list of compounds identified should by no means be considered complete. These selected compounds were chosen due to their likelihood of being present at the deployment sites. They suited the purpose of demonstrating the ability of the POCIS to sample environmental contaminants of interest.

Conclusions:

The Polar Organic Chemical Integrative Sampler, or POCIS, was developed for the sampling of hydrophilic contaminants over extended time periods. The prototype sampler consisting of a polymeric resin sequestration media enclosed within a hydrophilic membrane barrier has been demonstrated to sample model polar organic contaminants such as 17α-ethynylestradiol, diazinon, and atrazine.

The development of the POCIS involved the selection of a suitable sequestration media and membrane material to facilitate the uptake of polar organic environmental contaminants. Extensive studies of various solid-phase extraction resins, resin-embedded teflon (Empore) disks, and thin gels embedded with polymeric resins were performed to determine the optimal configuration. An admixture of two solid-phase extraction resins, Isolute ENV+ polystyrene divinylbenzene and S-X3 dispersed Ambersorb 1500, was selected in order to combine the high capacity of the Ambersorb 1500 with the excellent recoveries achieved with Isolute ENV+. This "resin mix" is a rugged material, capable of reproducible results as demonstrated by the week-to-week comparisons for the extraction and recovery of the model compounds. Greater than 87% recovery was achieved for ethynylestradiol, diazinon, and atrazine for all trials. The performance of the resin was independent of water concentration. Extraction of water samples containing analytes with at least a 3-fold difference in concentration exhibited >89% mass balance in all cases. The results of a resin aging study indicate that sequestered polar organic contaminants can be adequately recovered up to 28 days after initial sequestration. Following 28 days, a mass balance of at least 88.9% was achieved for each analyte. The analytes were not released from the resin mix into the surrounding water as no more than 0.01% of any analyte was recovered from the water left in the columns during aging. Considering the overall performance of the resin mix, it was determined to be a suitable sequestration media for use in the sampling devices.

The membrane barrier is an essential component of the POCIS as it selectively allows compounds of interest to pass through to the sequestration media and excludes larger materials such as particulate matter, colloids, and biogenic material which could interfere with subsequent analysis. Polymeric membranes, differing in their chemical and physical properties, were evaluated by exposure to the model compounds under controlled conditions. Polyethersulfone was selected for use in the POCIS due to its amenability to polar organic analyte uptake and excellent durability.

Exposure of the POCIS to ethynylestradiol, diazinon, and atrazine in 1-liter of water for 28 days demonstrated its sampling capability for these compounds. After 7 days, ~85% of the original analyte had been sequestered by the POCIS. Following uptake, the samplers were placed in fresh water for an additional 28 days to monitor analyte depuration. Less than 4.2% of the sequestered analytes was lost due to depuration into the fresh water. The ability of the sampler to retain sequestered analytes is necessary for the determination of a time-weighted average water concentration.

Calibration of the POCIS, from experimentally determined sampling rates of the model compounds, allows for the estimation of ambient water concentrations. The experimental determination of sampling rates involve exposing the device to a chemical under constant conditions and water concentrations. The sampling rate is a measure of the number of liters of water which are cleared of a certain analyte in one day. For the POCIS to be considered an integrative sampling device, the sampling rate must remain relatively constant over an extended period generally greater than 28 days. Another stipulation of integrative sampling is that the sampling rate is independent of analyte concentration. This is important to allow for the estimation of the time-weighted average concentration of an analyte in the water over the time interval for which the device was deployed. In order to maintain a constant sampling rate, the water concentration during the calibration exposures must remain fairly constant over the entire exposure period. This was accomplished by renewing the exposure water with freshly fortified water at regular intervals. Studies were performed exposing the POCIS to ethynylestradiol, diazinon, and atrazine under non-stirred and stirred conditions.

The analyte uptake into the devices was monitored by measuring the decrease in the surrounding water concentration. A sampling rate (in L/d) was calculated for each renewal and an average sampling rate was determined at the end of the exposure period for the non-stirred and stirred exposures. Sampling rates for model compounds in the non-stirred static renewal studies ranged from 0.050 to 0.070 L/d. The stirred static renewal studies exhibited sampling rates from 0.186 to 0.302 L/d. Using the average sampling rates for each chemical, a single POCIS deployed in quiescent waters over 28 days, would clear 2.0, 1.6 and 1.4 liters of water of ethynylestradiol, diazinon, and atrazine, respectively. In a turbulent aquatic system, clearance volumes of up to 8.5, 5.2, and 6.7 liters for ethynylestradiol, diazinon, and atrazine, respectively, could be attained over 28 days. The use of several samplers at the deployment site would increase the total volume of water cleared, providing a greater cumulative analyte mass available for analysis.

Inspection of the data from the stirred static renewal exposures reveals increased sampling occurring for the POCIS in the first 4 to 6 renewals (or 1 to 8 days). This increased sampling could be due to an increased flux of water crossing the membrane in an attempt to solvate or wet the polymer. The increased contact with water would result in the sequestration of additional analyte. Once the wetting or solvation of the membrane is complete then the uptake is controlled by the flux through the water-filled pores and the membrane as expected. Presolvation of the membranes with water, methanol, and

isopropanol eliminated the long initial period of increased uptake observed in the non-presolvated exposures. The use of organic solvents as a means of presolvation was discontinued due to potential detrimental effects on the membrane matrix. Presolvation of the membranes with water prior to device construction was employed resulting in a consistent sampling rate throughout the entire calibration exposure.

One purpose of the membrane barrier is to prevent suspended solids, colloids, macromolecules, and microorganisms from reaching the sequestration media. However the buildup of such materials on the membrane surface could alter the performance of the device by clogging the pores and the formation of biofilms across the membrane surface. The extent to which biofouled polyethersulfone membranes enhanced or retarded the sampling of ethynylestradiol was experimentally determined. Six devices with PES membrane only were suspended in a control pond at CERC for 30 days from June to July, 1999. Following exposure in the pond, the devices were retrieved and examined for the presence of membrane fouling. Surprisingly, there was very little fouling of the membrane compared to the degree of fouling of polyethylene SPMD membranes in the same pond. The presence of a biofilm was not observed, only some discoloration of the membrane due to adhesion of suspended particulate matter to the surface. The pond itself did undergo a period of biological growth as algae grew in the pond throughout the exposure. The fouled polyethersulfone membranes were then used in the construction of six POCIS which were subsequently used in the stirred and non-stirred renewal exposures to ethynylestradiol. The sampling rates for fouled membranes were essentially identical to sampling rates for non-fouled membranes. In the non-stirred renewal study, an average sampling rate of 0.070 L/d was observed for the fouled device compared to 0.070 L/d for the non-fouled device. This trend was also observed for the stirred exposure with an average fouled sampling rate 0.307 L/d compared to 0.302 L/d for the non-fouled device. This commonality between the sampling rates of fouled and non-fouled devices indicates the ability of the device to be used in various environments regardless of biological growth.

Experimental data indicates POCIS samples integratively for at least 28 days. Analyte uptake by a POCIS occurs by a biphasic mechanism of transport through water-filled pores and partitioning into the polymeric matrix. Calculations of the resistance to mass transfer contributed by each barrier (i.e., aqueous boundary layer, biofilm, membrane) indicate the region of maximum resistance or region of sampling rate control to be transport through the water.

The ability of the POCIS to perform under actual environmental conditions was demonstrated by a proof-of-concept field deployment. Samplers were deployed at five sites for 28 days in a wetland complex consisting of treated effluent from a municipal wastewater treatment facility and water from the Missouri River. Upon retrieval, the resin mix was removed from the samplers and eluted. The eluate from each POCIS was qualitatively screened in order to identify some of the sampled compounds. Highly complex mass spectral patterns indicating the presence of large numbers of compounds were observed by LC/MS. Tentatively identified compounds include among many others: atrazine, hydroxyatrazine, ibuprofen, ethynylestradiol, caffeine, sulfamerazine, and

sulfadiazine. Atrazine in the Missouri River distribution channel (Site 1) in Eagle Bluffs was estimated to be present at 0.84 ppb using calculated sampling rates. This level is in agreement with a value of 1.16 ppb from a NASQAN study reported 4 months earlier.

A review of the work performed during the development of the POCIS indicates it is a viable option as a sampler for hydrophilic contaminants. It is preferable to standard sampling regimes as it can be deployed for extended time periods of at least 28 days, it requires no maintenance and can be deployed by inexperienced personnel without compromising the sampler. Extracts for the sampler can be analyzed by nearly any instrumental technique as well as employed in bioindicator tests to determine chemical mixture toxicity.

The culmination of this work has just scratched the surface of the potential of the POCIS as a tool for environmental sampling. The range of compounds that may be sequestered and types of rigorous environments suitable for deployment can only be speculated. Continued investigation into the development of the POCIS may lead to a revolution in standard environmental monitoring techniques.

Research Area 5-

Bioindicator Assessment of Extracts from Field Deployed SPMDs:
During the first reporting period of this project, extracts of SPMDs deployed at two sites in the Missouri River basin, were used in a suite of bioassay procedures. Briefly, immature rainbow trout, *Oncorhynchus mykiss,* were injected with equal portions of the extracts and the fish were examined for a variety of physiological parameters. In essence, the results of the bioassay procedures indicated that the SPMD extract from the most contaminated site was estrogenic while the SPMD extract from the less polluted site elicited no adverse effects. The consequences of long term exposure of fish, wildlife, and humans to the complex mixture of contaminants present in the main stem of the Missouri River are unknown, but could result in reproductive perturbations.

Research Results for the Second Reporting of the Project-

During the second reporting period of the project, SPMDs were again used to sequester hydrophobic contaminants from water, specifically in the region near Nogales, AZ, as part of a synoptic survey of ecological conditions, species diversity, habitat quality. As an assessment of the potential for contaminant impacts in the Santa Cruz River, SPMDs were deployed for 29 days. The Santa Cruz River is one of the major water sources in the extremely arid region of southern Arizona. In point of fact the Santa Cruz rises in the United States flows south into Mexico and turns north and flows back into the United States near Nogales, AZ. The United States State Department operates a sewage treatment plant at Nogales for the treatment of sewage from both sides of the United States-Mexico border. Because the population in growing rapidly and water resources are very limited, it is of paramount importance that the sewage effluent be treated in such a manner that in can potentially serve as a source for ground water recharge. This survey

was conducted as part of a larger project designed to determine the suitability of the sewage effluent for use in a constructed wetland, essentially a tertiary treatment.

Results and Discussion for Research Area 5-

The SPMDs were deployed at two sites, one set in the effluent weir of the International Wastewater Treatment Plant (IWWTP) and the second set in the Nogales wash (water entering the US from Mexico) and flowing into the Santa Cruz above the wastewater stream. The SPMDs were deployed for a period of 29 days. Following retrieval, the SPMDs were returned to the CERC and were processed and analyzed using procedures described in detail elsewhere (70). The analysis of these samples revealed the presence of OCs, PCBs, and PAHs in the effluent and in the waters of the Nogales wash. These data are presented in Tables 35, and 36.

Table 35. PAHs in SPMD Samples from Study Sites (μg/sample)

Component	IWWTP Effluent	Nogales Wash
Naphthalene	0.33	2.35
Acenaphthylene	<MDL	<MDL
Acenaphthene	1.52	1.00
Fluorene	<MDL	2.46
Phenanthrene	1.58	2.89
Anthracene	1.37	0.91
Fluoranthene	<MDL	3.50
Pyrene	<MDL	9.29
Benz[a]anthracene	0.38	0.37
Chrysene	1.16	<MDL
Benzo[b]fluroanthene	<MDL	<MQL
Benzo[a]pyrene	<MQL	<MQL
Indeno[1,2,3-cd]pyrene	<MQL	<MDL
Dibenz[a,h]anthracene	<MDL	<MDL
Benzo[g,h,I]perylene	<MDL	<MDL
Total PID Response as Pyrene	1700	340

Table 36. Organochlorine Compounds in SPMD Samples from Study Sites (ng/sample expressed to two significant figures)

Component	IWWTP Effluent	Nogales Wash
HCB (Hexachlorobenzene)	150	59
PCA (Pentachloroanisole)	74	<MDL
α-BHC (α-Benzenehexachloride)	<MDL	<MDL
Lindane	<MDL	<MDL
Heptachlor	15	18
β-BHC (β-Benzenehexachloride)	<MDL	<MDL
δ-BHC (δ-Benzenehexachloride)	<MDL	<MDL
Dacthal	<MDL	8.5
Oxychlordane	46	16
Heptachlor Epoxide	<MDL	<MDL
trans-Chlordane	80	11
trans-Nonachlor	120	33
cis-chlordane	37	<MDL
o,p'-DDE	<MQL	<MDL
p,p'-DDE	360	100
Dieldrin	49	1.2
o,p'-DDD	<MQL	<MQL
Endrin	<MQL	<MDL
cis-Nonachlor	42	<MDL
o,p'-DDT	<MQL	35
p,p'-DDD	180	<MQL
Endosulfan II	<MQL	<MQL
p,p'DDT	<MQL	<MDL
Endosulfan Sulfate	8.2	<MDL
Mirex	<MQL	<MDL
Methoxychlor	35	<MDL
Total PCBs	2100	2100

The PAH profiles in the samples were extremely complex and typical of alkylated PAHs. In addition to the individual priority pollutant PAHs given in Table 35, the entire PAH response was quantified by comparing the total area to the response of a standard of a ubiquitous PAH, pyrene. On this basis, the samples from the IWWTP effluent contained about five times the amount of a complex mixture of PAHs as did the Nogales Wash sample. In regards to the priority pollutant PAHs, both samples were similar with these residues being somewhat higher in the Nogales Wash sample. In regards to organochlorine contaminants, the IWWTP effluent sample contained more contaminants and higher levels of coincident contaminants than did the Nogales Wash sample.

The much larger total PID (photoionization detector) response observed in the SPMD sample extract from the IWWTP site may be attributable to a biological origin for these PID active components or perhaps to improper disposal of, for instance, waste oil in an

urban area. Another potential source of the complex mixture of PAHs is run-off from asphalt areas in and around Nogales.

The total concentration of individual OCs ranged from non-detectable to 360 ng/sample. The OC, p,p'-DDE was found at the greatest concentration in samples from both sites. Other OCs at relatively elevated levels (≥ 50 ng/sample) in the SPMD samples were HCB, PCA, *trans*-Chlordane, and p,p'-DDD. Interestingly, PCBs were present in the SPMD samples from both deployment sites at the same concentrations.

SPMD uptake kinetic data are required to accurately estimate ambient waterborne concentrations of environmental contaminants. Using models previously developed (47) and sampling rate data previously reported (3), the bioavailable waterborne concentrations of selected contaminants present in the water near the sediment-water interface at the sampling sites were estimated. An example of the overall estimation procedure is as follows. The analyte sampling rate (R_s) is determined from laboratory exposures conducted under similar conditions (i.e., water temperature, exposure duration, etc.) as the field study. However, unlike SPMDs exposed in the laboratory, the field deployed SPMDs were somewhat biofouled. Consequently, accounting for the potential impedance to uptake due to Aufwuchs layers (71), the environmental sampling rate (R_{sc}) is given by

$$R_{sc} = R_s F_i \tag{7}$$

where F_i is one minus the fractional reduction in uptake flux or sampling rate due to fouling impedance. The linear SPMD uptake of OCs with high K_{ow}s (octanol-water partition coefficients) was described by Huckins, et al. (47) as follows:

$$C_L = C_W k_o K_{MW} A t / V_L \tag{8}$$

substituting R_{sc} for $k_o K_{MW} A$ gives

$$C_L = C_W R_{sc} t / V_L \tag{9}$$

Where C_L is the concentration of the analyte in the lipid phase, C_W is the concentration of the analyte in the water, t is the exposure time in days, and V_L is the volume of the lipid. Rearranging the above equation gives

$$C_W = C_L V_L / R_{sc} t \tag{10}$$

Because the analytes present in the membrane were also recovered during the dialysis procedure, this equation can be rewritten as

$$C_W = C_{SPMD} M_{SPMD} / R_{sc} t \tag{11}$$

Where C_{SPMD} is the concentration of the individual analyte in the SPMD and M_{SPMD} is the mass of the SPMD. R_{sc} is defined as L/d on a per-gram of SPMD basis.

Because the SPMDs from both sampling sites were not extensively bifouled, an average fouling impedance of 20 % or $F_i = 0.80$ was employed. The estimated bioavailable waterborne concentrations of selected contaminants present at the sampling sites are presented in Table 37. These values were generated using an average R_{sc} for an ambient temperature of 26 °C.

Table 37. Estimated Water Concentrations of Selected Environmental Contaminants (concentrations in pg/L expressed to two significant figures)

Component	IWWTP Effluent	Nogales Wash
Total PAH-Like Compounds*	2.9×10^6	5.9×10^5
HCB	310	120
Heptachlor	2.5	41
Oxychlordane	110	40
trans-Chlordane	180	25
trans-Nonachlor	270	74
p,p'-DDE	890	250
Dieldrin	150	3.7
Total PCBs**	2100	2100

* Based on total PID response as pyrene

** Based on an uptake rate constant as the average of values for PCB congeners I-40 through I-119

While some variation exist between the sites, the estimated concentrations are elevated compared to more pristine aquatic systems. For example, the concentration of p,p'-DDE has been reported as 24 pg/L in Lake Huron (72) and as 73 pg/L in Lake Ontario (73). Kucklick, et al. (74), reported the following concentrations in Lake Baikal: p,p'-DDE (17 pg/L) and p,p'-DDD (17 pg/L). In addition, the PAH concentrations in SPMD samples from both sites are indicative of potentially significant hydrocarbon related contamination. The complexity of the PAH residue patterns do not permit a detailed comparison between the sites, however, it appears that the effluent of the IWWTP contains elevated levels of PAHs or PAH-like chemicals.

The presence of elevated levels of persistent contaminants in these samples undoubtedly results from the former (or perhaps current) widespread use of these chemicals in the area. Most of these chemicals have been banned-some for nearly 20 years (14). The apparent longevity of these chlorinated contaminants raises questions about the quality of the water entering the Santa Cruz River and the groundwater and may result in a reduction in habitat quality. For instance, dieldrin, the DDT complex, and the chlordane components along with a much larger set of diverse environmental contaminants have been reported to cause endocrine-disruption for some organisms (75).

As an integral part of this research, extracts from contemporaneously deployed SPMDs were examined using a suite of bioindicator tests. This assessment of the toxicological relevance of the complex mixture of chemicals sequestered by the SPMDs was conducted by Dr. Susan Jones of CERC. Individual groups of chemicals often have multiple measurable effects in an animal. For example, many chemicals such as PAHs and PCBs, that are known for liver enzyme induction, may also affect the reproductive system by interacting with hormonal pathways. Some reproductive hormones have effects on seemingly unrelated neurotransmitter pathways. Thus, it is useful when addressing the effects of a single chemical to measure a number of physiological endpoints or biomarkers to have a more thorough understanding of the chemical's overall impact. This approach is even more critical when assessing the overall effects of complex mixtures of chemicals. In reality, organisms are not exposed to one chemical at a time, but rather to a number of chemicals that may result in aberrant physiological effects. Our approach to assessing the toxicological relevance of complex chemical mixtures is to utilize a suite of biomarkers that reflect exposure to specific classes of chemicals.

In the current research, rainbow trout were injected with enriched extracts from the SPMDs deployed at the two study sites. The following parameters were measured: 1) a traditional marker of some classes of organic pollutants, liver mixed function oxidase induction (EROD; 7-ethoxyresorufin O-deethylation) (76); 2) a traditional marker of neurotoxicity (acetylcholinesterase and muscarinic cholinergic receptor binding) (77), and 3) an endpoint representative of estrogenic exposure, production of vitellogenin (an egg-yolk precursor) in male or immature female fish where it is not normally found.

Exposure and Tissue Sampling-Three to four rainbow trout were placed in each of 7 tanks in 18°C well water in a flow-through system. Fish were fed once/day throughout the exposure. After 48 h acclimation, fish were injected with 100 µL of a 1:1 mixture of an enriched SPMD extract or appropriate control extracts in dimethylsulfoxide (DMSO) and corn oil. Controls included a freshly prepared SPMD, a size exclusion chromatography (SEC) blank, a reagent blank, a DMSO blank and estradiol (2 mg/kg) as a positive control. The same injections were repeated six days after the initial injection. Fish were sacrificed after a total of 11 days exposure to the extracts. The following tissues were immediately removed from each fish and frozen at –80 °C until assayed: plasma, liver, gills, and brain.

EROD Activity-A microsomal fraction of liver tissue was prepared by homogenizing 0.5 g of liver tissue in 50 mL of phosphate buffer, pH 8.0, followed by appropriate centrifugation techniques. The resulting preparation designated S9 was used in measuring the induction status of CYP1A1 enzymes in livers of fish by a modification of reported methods (76). Increased metabolism of ethoxyresorufin with fluorescent detection of resorufin would indicate the presence of enzyme inducing chemicals in the SPMD extracts. EROD activity was significantly increased in fish exposed to the IWWTP effluent sample extract compared to controls ($P<0.05$) and approached a significant increase for the fish exposed to the Nogales Wash sample extract. There were no significant differences in fish body or liver weights among treatments.

Neurotoxicity Endpoints-Brain tissue was homogenized and fractions taken for acetylcholinesterase activity (AChE) and muscarinic cholinergic receptor (MChR), and β-adrenoceptor (βAR) binding *in vitro* assays. AChE was performed according to published procedures (78) in which the activity of the enzyme is measured by hydrolysis of acetylthiocholine and reaction of the thiocholine product with a colorimetric reagent. MChR binding was performed according to the method of Jones, et al. (77), in which a crude membrane preparation is incubated with a radioligand specific for MChR. βAR binding was done in a similar manner. Following incubation, samples were filtered through GF/B glass fiber filters, with the radioactivity being measured by liquid scintillation counting. There were no significant differences in brain MChR affinity (K_D) or number among the treatments. Additionally, brain cholinesterase activity levels were similar in all treatments. However, plasma cholinesterase was significantly depressed in the plasma of fish exposed to the IWWTP effluent compared to controls. There were no significant differences in brain β-adrenoceptor number or affinity among the treatments. However, gill receptor numbers in fish exposed to the sample extract from the Nogales Wash were increased compared to controls.

Vitellogenin Production-Vitellogenin was detected by SDS-PAGE followed by immuno-detection of vitellogenin by published Western blotting techniques (79). Briefly, proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes and incubated with polycolonal primary antibody specific for rainbow trout vitellogenin. A second antibody was added with associated colorimetric detection to visualize as little as 50 ng of vitellogenin. Plasma from all four estradiol injected fish exhibited a band of 160K corresponding to purified rainbow trout vitellogenin. In addition, all fish exposed to the sample extract from the IWWTP effluent and three of four fish exposed to the sample extract from Nogales Wash exhibited a corresponding band, indicating overall estrogenicity of the extracts.

Discussion:
The overall physiological effects of the enriched extracts from the IWWTP samples indicate liver enzyme induction, perturbation of two neurotransmitter systems, and overall estrogenic effects. EROD results indicate that the extracts from both the IWWTP and the Nogales Wash induced increased levels of enzyme activity compared to controls, although only the IWWTP sample extract was significantly higher. These results are consistent with the chemical analysis which indicated higher PAH levels, and other chemicals known to induce liver enzymes, in the sample from the IWWTP effluent compared to the sample from the Nogales Wash.

Plasma cholinesterase values were depressed in fish exposed to the sample extract from both sites compared to controls. A number of chemicals demonstrated to be present in the sample extracts, particularly the organochlorines are known to inhibit cholinesterase, so these results are not surprising. We were surprised, however, that a similar response did not occur with brain cholinesterase. It may be that brain cells are able to metabolize the compounds more readily or that the effect occurred earlier in the exposure period permitting recovery of the enzyme activity.

A wide variety of chemicals are known to cause perturbations in the endocrine systems of organisms (14). As presented above, the presence of vitellogenin in fish exposed to the sample extracts from both sites is indicative of potential adverse effects associated with long term exposure to these waters.

Whereas time and concentrations of individual chemical effects were not determined in this assessment, the totality of effects of exposure to enriched extracts from the deployment sites indicate that mixtures of environmentally derived chemicals can be assessed using a suite of bioindicator tests. Moreover, these bioassays can be employed to define the toxicological relevance of exposure to a complex mixture of chemicals without foreknowledge of the specific chemicals present in the mixture.

Sampling of Airborne Contaminants-

In an effort to demonstrate the applicability of the SPMD samplers as monitors of human exposure to airborne contaminants in enclosed areas, we initiated a joint project with the U.S. EPA to employ SPMDs as integrative samplers as a focused part of a much broader study of human exposure. The SPMDs were deployed in enclosed areas along the border between Arizona and Mexico. The main objective of this project was to determine the applicability of the integrative sampling approach of the SPMD technology to define the potential exposure of people living in the sampled areas to complex mixtures of chemicals. This project, while not funded as part of the current research, did provide data applicable to the development of a holistic exposure assessment approach of direct application to the interest of DOD. The full details of this research have been reported to EPA (80).

Briefly, the SPMDs were deployed for thirty days and the sample extracts were subsequently analyzed for residues of organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocsarbons (PAHs), and selected current use pesticides, diazinon, chlorpyrifos, ednosulfan, permethrin, and trifluralin. Residues of all analytes were determined to be present at varying concentrations in the samples from all deployment sites. Total levels of contaminant classes ranged from ng to μg quantities. In particular, the DDT complex and the current use pesticides were found at higher levels. The PAH profiles were very complex and appear to contain a broad array of alkylated aromatics hydrocarbons.

By modifying the models used to estimate waterborne concentrations of hydrophobic organic chemicals, we estimated the airborne concentrations of selected organic chemicals present in the enclosed areas. An example of the overall estimation procedure is as follows. The analyte sampling rate (R_{SW}) is determined from laboratory exposures as described earlier. The models described earlier in this report can be modified to address airborne chemicals:

$$C_a = C_{SPMD}/(R_{sa}/M_{SPMD}) \qquad (12)$$

Where C_a is the concentration of the analyte in air and R_{sa} is the SPMD sampling rate in air. Although little SPMD sampling rate (calibration) data exist for vapor phase contaminants, there is a fairly extensive set of calibration data set for aqueous phase chemicals. Conversion of a chemical's sampling rate in water to its sampling rate in air is feasible because SPMD concentrations have been shown to be proportional to both levels in water and air. The following rationale can be used for the extrapolation;

Assumptions-

1) The ratio of the air-sampling rate constant (k_{ua}) divided by the water-sampling rate constant (k_{uw}), for similar compounds and conditions, is constant.

2) The air and water temperature are 26 °C.

3) Facial velocity of air and water is low, i.e., quiescent conditions during the exposures.

From existing laboratory data the k_{ua} and k_{uw} of congener 52 (IUPAC no. 2,2',5,5'-tetrachlorobiphenyl), under the conditions described above is 600 L/d· g and 1.3 L/d· g, respectively. Note that the dramatic difference is the volumes of the two matrices sampled is due to their difference in density, i.e., at sea level the density of water is 1220 times greater than air. The ratio of the SPMD-air and –water sampling rates ($S_{ra/w}$) iss given by

$$S_{ra/w} = k_{ua}/k_{ua} \qquad (13)$$

Using equation 13 and the sampling rate values given above for congener 52

$$S_{ra/w} = (600 \text{ L/d}\cdot \text{ g})/(1.3 \text{ L/d}\cdot \text{ g}) = 460 \qquad (14)$$

and $$R_{sa} = k_{ua} \cdot M_{SPMD}$$

Using approximate SPMD air sampling rates, the estimated airborne concentrations of selected chemicals present at representative sampling sites were calculated. Examples of these estimated concentrations are presented in Table 38.

Table 38. Estimated Airborne Concentrations of Selected Contaminants at Representative Sampling Sites (ng/m^3)

Analyte	Site 2	Site 43	Site 46
Total PAHs*	590	3200	960
Total PCBs	23	16	12
Chlordanes	1.4	3.7	1.5
Nonachlors	0.31	1.4	0.47
Endosulfans	1.7	1.1	0.99
Trifluralin	NA**	1.3	0.19
Diazinon	240	130	7.6
Chlorpyrifos	650	390	0.44
Permethrins	10	0.21	1.1
o,p'-DDT	33	0.50	0.95
o,p'-DDE	6.0	0.25	0.18
o,p'-DDD	1.2	NA	NA
p,p'-DDT	28	0.44	0.88
p,p'-DDE	4.0	0.29	0.40
p,p'-DDD	2.7	NA	NA

*As pyrene (i.e., total GC-PID response using the response factor of pyrene to quantify).
**NA = Not applicable.

The presence of persistent contaminants in the air of the deployment sites undoubtedly results from the former or present use of these chemicals in the area of the sampling sites. While the estimated ambient concentrations of the chemicals are in general below the NIOSH time weighted average (TWA) exposure limits based on a maximum 10 h work day and a 40 h work week, the values estimated from the SPMD data are representative of up to 24 h per day, 7 d per week. This extended exposure period and the complexity of the chemical mixtures present in these homes and the potential for synergistic effects require further investigation to assess possible health effects. However, the application of SPMDs as integrative samplers of airborne organic chemicals under field deployment conditions was successfully demonstrated.

SUMMARY

The research conducted towards development of an Area Monitor during the second reporting period of the project was generally centered in area 3, development of an integrative sampler for vapor phase Hg species, area 4, continued development of an integrative sampler for more hydrophilic chemicals, and area 5, evaluation of extracts from standard SPMDs deployed in the field using bioassay procedures. In research area 3, we field tested the prototype neutral Hg sampler in a ninety day outdoor exposure and in an indoor situation involving a Hg spill and mitigation efforts. In both cases, the PIMS performed remarkably well and provided a means to obtain data generally unavailable by other means.

Research continued in area 4 and a prototype integrative sampler for waterborne polar organic chemicals was developed. An optimum membrane, polyethersulfone, and a solid phase sequestration medium consisting of a polystyrene-divinyl benzene sorbent and a carbonaceous sorbent were characterized. This admixture of sorbents provides a sequestration phase applicable to a wide variety of chemicals. The prototype polar organic chemical integrative sampler (POCIS) was deployed in a constructed as a proof-of-concept field test. A wide variety of polar organic chemicals, including; antibiotics, analgesics, surfactants, pesticides, etc., were detected in the sample extracts. The POCIS forms the basis for assessing the presence and identity of a broad spectrum of polar organic chemicals potentially present in a wide variety of exposure situations.

We also examined the purified extracts of field deployed SPMDs using a suite of bioassay procedures. The deployment sites were located in the arid region near Nogales NM and represented an input into the Santa Cruz River, a major water source in this area. The overall physiological response indicated that the complex mixture of chemicals in the water at these two sites were toxicologically of concern, particularly in the broad area of endocrine disruption. The research conducted in this area of emphasis provides a basis for exposure assessment employing rapid bionindicator tests. This approach has many potential applications, including potential exposure to chemical warfare agents.

As part of a much broader U.S. EPA sponsored research project, SPMDs were used to integratively sample airborne organic chemicals in enclosed areas along the border between Arizona and Mexico. Numerous organic chemical residues were determined to be present in the SPMD sample extracts. Many of these residues are toxicologically significant at very low concentrations. While the health related effects associated with exposure to the complex mixture of chemicals found in the samples is uncertain, the application of the SPMD as an integrative sampler of airborne bioavailable organic chemicals was successfully demonstrated.

In summary, the development of multiple integrative samplers for chemicals in aqueous and atmospheric phases was successfully accomplished. In addition, the incorporation of bioindicator tests, i.e., enzyme induction and inhibition, neurotoxicity, etc., as an assessment approach for the toxicological assessment of exposure to complex mixtures of chemicals was demonstrated. By employing the integrative samplers, i.e., the SPMDs for hydrophobic chemicals, the POCIS for polar organic chemicals, the PIMS for neutral mercury species, and the SLMD for ionic metals, a holistic assessment approach for defining organism exposure to chemicals in air and water has been designed and developed. Further, incorporation of bioindicator test to delineate the toxicological relevance of exposure to environmental contaminants or other chemical stressors, provides the basis for a broadly based hazard/risk assessment procedure currently unavailable. The research described in this report has resulted in the development and proof-of-concept validation of technology forming the basis of Area Monitors for use by the DOD in situations requiring exposure assessment. The samplers described above can be readily combined into a single module. Further refinement of the technology, including miniaturization and remote sensing approaches are possible.

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REPORTABLE OUTCOMES

Publications:

1) Petty, J.D., B.C. Poulton, C.S. Charbonneau, J.N. Huckins, S.B. Jones, J.T. Cameron, and H.F. Prest. 1998. *Determination of bioavailable contaminants in the lower Missouri River following the flood of 1993.* Environ. Sci. Technol., 32:837-842. Publication directly applicable to research project, however, not funded by this MIPR.

2) Petty, J.D., S. B. Jones, J.N. Huckins, W.L. Cranor, J.T. Parris, T.B. McTague, and T.P. Boyle. 2000. *An approach for assessment of water quality using semipermeable membrane devices (SPMDs) and bioindicator tests.* Chemosphere. In Press. Publication directly applicable to research project, however, not funded by this MIPR.

Presentations:

1) *Observations on Declining SPMD Sampling Rates for High K_{OW} Compounds.* 1998. J.N. Huckins, J.D. Petty, R.W. Gale, K. Booij, H.F. Prest, and. R.C.Clark, 19th Annual National Meeting, Society of Environmental Toxicology and Chemistry, 15-19 November, 1998, Charlotte, NC.

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Degrees Obtained:

1) W.G. Brumbaugh, Ph.D. in Environmental Analytical Chemistry, University of Missouri-Columbia, Columbia, MO, 1997. Thesis Adviser, Dr. J.D. Petty. Thesis Title: *Development of an Integrative Sampler for Bioavailable Metals.*

2) D.A. Alvarez, Ph.D. in Environmental Analytical Chemistry, University of Missouri-Columbia, Columbia, MO, 1999. Thesis Adviser, Dr. J.D. Petty. Thesis Title: *Development of an Integrative Sampling Device for Hydrophilic Organic Contaminants in Aquatic Environments.*

Research Staff Supported:

1. Walter Cranor, Chemist

2. Randal Clark, Technician

3. Timothy McTague, Technician

4. David Alvarez, Doctoral Student