THE WORM HAS TURNED: DEVELOPING STRATEGIES FOR ASSESSING THE RISKS OF ENDOCRINE DISRUPTING COMPOUNDS

A Dissertation
Presented to the Faculty of the Graduate School of Cornell University
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by
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Estrogen-like endocrine disrupting compounds (EEDCs) are found in a variety of products to which humans are exposed, such as plastics and personal hygiene products. These compounds are generally not removed by wastewater treatment systems, and can be found in streams, rivers, and land to which sewage sludge (biosolids) has been applied. Thus, EEDCs may pose a risk to human and ecosystem health.

This dissertation focuses on exposure analysis of EEDCs to soil organisms, with a further discussion of the communication of the risk of EEDCs. The dissertation details the development of a novel analytical method employing principles of solid-phase microextraction that can be used to quantify the potential exposure of soil organisms to four EEDCs that have been detected in land amended with biosolids. Conclusions are drawn regarding the ability of the method to quantify the bioavailability of the compounds under various circumstances that mimic field conditions, and the ability of the method to predict tissue concentrations of the compounds in the earthworm, *Eisenia fetida*.

The dissertation then shifts its focus to the issues surrounding the communication of the risks of EEDCs, using bisphenol A as the target compound for a case study in risk communication. Lessons from this case study are discussed.

The ubiquitous nature of EEDCs in personal products and in the environment, as well as the unique dose-response relationship of EEDCs, suggests that risk assessments for numerous EEDCs will be required in the near future. The research described in this dissertation, the development of a method that can be used to analyze exposure to the EEDCs of interest. In
addition, further understanding the communication of these risks will aid the overall risk management process.
BIOGRAPHICAL SKETCH

Katherine Jean Neafsey Engler was born in Stafford, Connecticut, and has been fascinated by dirt for as long as she can remember. She received a Bachelors of Science with Distinction in Research from Cornell University in 2008. During her undergraduate career, she performed research through the College of Agriculture and Life Science’s honors research program with Professor Ann Lemley on the use of the Fenton method, an advanced oxidation process, to degrade sulfonamides in water. She published a paper on this work in the *Journal of Agriculture and Food Chemistry* in 2010. She began her doctoral studies in Environmental Toxicology at Cornell University in 2008, and an article based on part of her dissertation research is currently in press in the *Journal of Environmental Toxicology and Chemistry*. During her graduate school years, she helped organize Expanding Your Horizons, an annual conference at Cornell designed to encourage middle school girls to pursue careers in math and science, and served on the Tompkins County Environmental Management Council. She has also enjoyed hiking around the Finger Lakes with her husband, Chris, and their two dogs, Hershey and Gizzy.
To my grandmother, who taught me to never stop learning and to enjoy every last drop of life.
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I am also grateful for the assistance of Dr. Shengpeng Sun and the Cornell Statistical Consulting Unit for their help with developing the array of experiments described in Chapter 2 and 3. I also wish to thank Elizabeth Henderson for her help in preparing the earthworm bioassay experiments in Chapter 2 and 3, and Professor Murray McBride for his help with the field soil collection described in Chapter 3.

I am tremendously grateful for the constant support of my committee chair, Professor Ann Lemley who, since my sophomore year in college, has always pushed me to be a better scientist but has also allowed me the freedom to pursue new research paths. I would also like to thank my committee members, Professor Murray McBride and Professor Jery Stedinger, for their helpful suggestions in developing my research topic and throughout the doctoral research process.

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CHAPTER 1

Introduction

Estrogen-like endocrine disrupting compounds (EEDCs) can be found in plastics, personal hygiene products, and in other products such as receipt paper. Humans can be exposed to these compounds through use of products containing EEDCs, either via ingestion or transdermal absorption [1, 2]. Humans can also be exposed to these compounds if they are present in drinking water, since EEDCs have been found to resist microbial degradation in waste water treatment systems [3]. Finally, due to their hydrophobic properties, EEDCs have been detected in sewage sludge, which is often added to agricultural fields as a fertilizer or to parks and play areas as a soil amendment [4-6]. While some research has been performed on the ability of crops to take up EEDCs, the impact this route of exposure has on human health is largely unknown [7].

With respect to the exposure of other organisms to EEDCs, the compounds have been detected in streams and rivers, often downstream from waste water treatment plants [3]. Exposure of aquatic organisms to EEDCS has been correlated with feminization, diminished reproductive success, and skewed population characteristics, but more research is needed to fully understand the entire spectrum of effects [8]. Due to these compounds’ presence in sewage sludge which is then often land-applied, soil represents a significant source of exposure for many organisms [4-6, 9, 10]. Soil organisms may also have the ability to bioaccumulate EEDCs, providing a potential magnification effect in higher trophic levels [4-6, 11].

This dissertation focuses on exposure analysis of EEDCs, part of the risk assessment process, with a further discussion on the communication of risk of EEDCs, part of the risk management process. There are several important steps in between the process of determining exposure to a
compound and the communication of the risks associated with such exposure. However, during the risk assessment process it is crucial to ensure that determination of the magnitude and route of exposure to the compound is accurate, just as it is important that the risks associated with the compound are accurately communicated to policy-makers and to the public during the risk management process.

With respect to exposure, the dissertation describes the development of a novel method of quantifying bioavailability, an important aspect of determining exposure for organisms in a soil environment. The bioavailability of a compound in soil controls what quantity of the compound that an organism could take up from the soil environment. It is, briefly, the amount of free compound available for uptake. While metabolism, excretion, and bioaccumulation may vary among organisms, the bioavailability of a compound in soil depends largely on the physical characteristics of the soil and the compound [12, 13].

Experiments employing earthworms have long been used to quantify the uptake/bioavailability of compounds such as EEDCs. These experiments are neither resource nor time effective and more efficient methods are needed. Therefore, this dissertation proposes a rapid method to determine the general bioavailability of four EEDCs commonly detected in soil. The proposed method is a passive sampling method, called thin-film solid-phase microextraction (TF-SPME). It draws on the principles of solid-phase microextraction (SPME) which typically employs glass fibers coated with a hydrophobic material, often polydimethylsiloxane (PDMS). The coated fibers are exposed to the soil of interest, and compounds freely dissolved in the soil pore-water partition into the hydrophobic material. The concentrations of the compounds in the soil and in the PDMS film are allowed to come to equilibrium, and the film is extracted for concentration analysis. In this way, SPME can be used to quantify the bioavailable portion of the compounds in
the soil. The proposed method improves on SPME in that it uses PDMS in thin-film form. The films have a higher surface to volume ratio than the coated fibers, resulting in shorter extraction times, and are also less expensive than traditional SPME fibers. Additionally, rather than using solvents to re-extract the compounds from the PDMS before quantifying their concentrations, as is often done in traditional SPME, the equilibrium concentrations of compounds in the TF-SPME films can be analyzed using thermal desorption and gas chromatography-mass spectrometry. In this way, TF-SPME can be considered a solvent-free method.

This dissertation describes the first step in the development of the TF-SPME method, the conduction of experiments to determine the amount of time required for each test compound to come to equilibrium in the films and the ideal temperature and time required for thermal desorption of each compound. The calculation of film-water partition coefficients for each compound and the use of the method to calculate the soil pore-water concentrations of each compound in artificial soil is described. Conclusions are also made regarding the ability of the developed method to be used as a proxy for earthworm uptake studies.

The dissertation then describes how the developed method was challenged with conditions that are likely to be encountered upon field-application. The method is used to calculate soil pore-water concentrations of each compound when the compounds are present in a mixture in artificial soil, when the compounds are present in a mixture in a high clay soil, and when the compounds are present in a mixture in a sandy loam soil. Finally, the results of these additional experiments are used to determine whether remediating contaminated soil with organic carbon could be an effective way to limit bioavailability, and thus exposure to soil organisms.

The dissertation then discusses risk communication, a part of the overall risk management process, which attempts to synthesize all of the available information on human and ecological
exposures to the compound, the toxicity of the compound, and the ways in which the exposure to or the effects of the compound can be mitigated. Bisphenol A is one of the EEDCs to which the novel bioavailability method is applied. Risk communication about bisphenol A, including research surrounding its biological effects, routes of exposure, and the reaction to the scientific reports of these effects by the public and the media, is examined.

The case study postulates that the reaction by the public and government agencies to the controversy surrounding BPA is a product of several established theories in risk communication: trust and credibility, public participation, and media effects. BPA has received a considerable amount of publicity, in both the scientific and general public realms, but it is not the only EEDC to which humans and other organisms are exposed. In fact, one might expect compounds similar to BPA, which are present in many products to which humans are exposed and which also elicit estrogen-like effects, to be scrutinized in the future by scientists and regulators to the same end as BPA. In this sense, it is prudent to examine the process and challenges involved in the process of communicating the risk of BPA, because the lessons learned from the case analysis could prove useful in future communication efforts about the risks of other EEDCs. Similarly, the EEDCs to which the novel bioavailability method is applied are only a subset of the known EEDCs present in the environment. However, it is important to have a method that can be used to assess the general bioavailability of EEDCs in soil, in order to inform future risk assessments regarding their environmental presence.
CHAPTER 2

Development of an in vitro Thin-Film Solid-Phase Microextraction Method to Determine the Bioavailability of Xenoestrogens in Soil

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ABSTRACT

Biosolids applied to agricultural fields, parks, and other areas represent significant sources of estrogen-like endocrine disrupting compound (EEDC) inputs to soil. It is important to determine the bioavailability of EEDCs in soil to inform risk assessment concerning their environmental presence, and Eisenia fetida (earthworms) are typically used in traditional in vivo bioavailability experiments. The development of an in vitro bioavailability method will decrease time, expense, and use of solvents in future analyses. A thin-film-solid phase microextraction (TF-SPME) method for determining the bioavailability of several EEDCs detected in biosolids was developed. It was found that the TF-SPME method could be used to calculate equilibrium pore-water concentrations of diethylhexyl phthalate, bisphenol A, benzophenone, and triclosan at environmentally relevant concentrations in artificial soil within 88 minutes. The potential and limitations of using TF-SPME generated pore water concentrations to predict E. fetida tissue concentrations are discussed.
INTRODUCTION

Estrogen-like endocrine disrupting compounds (EEDCs) include plasticizers, pesticides, and surfactants, as well as additives in personal hygiene products and cosmetics. Many of these xenoestrogens enter waste treatment plants via household and industrial waste streams and are not removed by conventional waste treatment processes. As a result, they are released in waste treatment plant effluent to streams and rivers, where they often resist degradation by microbes and ultraviolet light and persist in the environment [3]. A 2002 United States Geological Survey study, which aimed to quantify organic wastewater contaminants of concern in 139 streams near wastewater discharge areas in the United States, found eight xenoestrogens among the thirty most frequently detected contaminants in the tested streams [3], underscoring the ability of EEDCs to pass through the waste water treatment system unchanged into the environment. Due to their low water solubilities, low vapor pressures, and high octanol-water partitioning coefficients, many EEDCs partition into sediment and sewage sludge and have been detected in finished biosolids intended for land application [4-6, 9, 10]. Over 3 million metric tons of biosolids, sewage sludge that has been treated chemically or physically for further use, are applied to agricultural fields, parks, and other areas each year[9, 14]; these media may represent major sources of EEDC inputs to soil. Though they may differ in structure and chemical properties, xenoestrogens can bind to estrogen receptors found throughout the body and regulate gene expression, producing a variety of physiological effects [8].

While the toxicities of other compounds that are commonly released from waste treatment plants into the environment can be reduced by dilution of the effluent in rivers and streams, endocrine disruptors can elicit biological effects at very low concentrations, including those found in the
environment [15]. The exposure of aquatic vertebrates to xenoestrogens has been correlated with feminization, reduced reproductive success, and female-biased populations [8]. EEDC dose-response predictions are complex, as the relationship is only linear at very low EEDC concentrations [16]. In addition, there is also evidence that exposures can be cumulative, such that exposure to low doses of multiple endocrine disruptors can produce a response similar to that produced by exposure to a higher dose of a single endocrine disruptor [15]. The entire spectrum of the effects of EEDCs on aquatic and terrestrial organisms, including humans, has yet to be completely characterized.

While the exposure of aquatic organisms in various environments to EEDCs has been studied, data on the exposure of soil organisms are lacking. Several studies have focused on quantifying total concentrations or confirmed the presence of anthropogenic waste indicators, including several EEDCs, in earthworms in biosolids or sewage sludge [4-6, 11], and some have calculated bioaccumulation factors for these compounds. However, more data on the bioavailability of EEDCs to organisms in soil and water are necessary to fully assess risks associated with potential bioaccumulation and biomagnification.

Earthworms are the standard in vivo model to assess a compound’s bioavailability in soil. They take up compounds dissolved in soil pore water via diffusion both through the external skin as they move through the soil and through the internal gut wall as they ingest soil particles [17]. They are abundant in diverse soil types, and remain in constant contact with soil. The total amount of a compound taken up by an earthworm depends on the concentration of the compound in the soil pore water [18]. Their physiology has been extensively studied and thus they can be used in a variety of toxicity tests [17]. Furthermore, earthworms have low mixed function oxidase activity, indicating that rapid metabolism of organic compounds is unlikely [11].
While earthworms are a useful model for determining the bioavailability of compounds in soil, tests employing them can be lengthy, expensive, and require many solvents to extract and isolate the compounds of interest [17]. Replacement of traditional experiments employing earthworms with *in vitro* bioavailability methods would likely decrease analytical time, expense, and use of solvents in future research. The ability of the *in vitro* bioavailability method solid phase microextraction (SPME) to determine the interstitial pore water concentration of the chemical of interest, without affecting the equilibrium between the compounds adsorbed to soil particles and the compounds in the pore water, makes it ideal for measuring the bioavailable portion of a contaminated soil. In typical solid phase microextraction (SPME), glass fibers coated with a hydrophobic material, which serves as the extraction phase, are added to soil samples to passively sample the compounds in soil pore water in a manner similar to uptake by earthworms [19]. Successful sampling requires adherence to the principles of negligible-depletion SPME, which can be described by the equation $K_{oc}W_s+W_w>>K_fW_f$, where $K_{oc}$ is the compound’s organic carbon partition coefficient, $W_s$ is the weight of the organic carbon in the soil to be sampled (g), $W_w$ is the weight of the water in the sample (g), $K_f$ is the compound’s partition coefficient between the hydrophobic coating of the fiber and the water, and $W_f$ is the weight of hydrophobic coating on the fiber (g). In the absence of soil, the equation becomes $W_w>>K_fW_f$.

Observance of negligible-depletion SPME ensures that the equilibrium between the compound in the sorbed and aqueous phases is not perturbed during sampling [20]. Once the concentrations of the compound in the fiber coating and soil reach equilibrium, the fibers are removed and compounds are extracted, typically with a solvent, and concentrations are quantified [19]. SPME has been used to effectively predict the bioavailability of several persistent organic pollutants to earthworms in soil [19, 21].
A disadvantage of using traditional SPME is that it can take up to several weeks to measure the equilibrium concentrations of compounds [19, 21]. Therefore, a method that uses the same principles of SPME and retains the high sensitivity that SPME offers but decreases the sampling time is needed. Thin-film solid-phase microextraction (TF-SPME), which involves the use of a polydimethylsiloxane (PDMS) film as an extraction phase, is a new version of SPME that has been used recently to measure PAHs in water samples [22, 23]. PDMS is a common hydrophobic coating for SPME fibers, and it has been shown that the partitioning behavior of compounds into PDMS film is equivalent to that of compounds into the PDMS coating of SPME fibers [24]. However, the use of PDMS in film form requires less extraction time, due to the higher surface area to volume ratio of the PDMS extraction phase in a 1 cm$^2$ film versus in a typical 100µm fiber [23]. Further, each film costs ~0.03 United States dollars, which is much less expensive than a traditional SPME fiber, which costs ~144 United States dollars.

The overall goal of this research was to investigate the potential for a novel in vitro method to quantify bioavailable xenoestrogens in soil. The specific objectives were: i) to develop a method based on TF-SPME that can be used to calculate the interstitial soil pore water concentrations of bisphenol A, triclosan, diethylhexyl phthalate, and benzophenone, all of which have been detected in finished biosolids; ii) to determine the method conditions in artificial soil; iii) to calculate the interstitial pore water concentrations of the compounds using TF-SPME; and iv) to evaluate use of the pore water concentrations generated by TF-SPME to predict earthworm tissue concentrations of the compounds. The current research focused on developing a careful and detailed proof of concept, using artificial soil, with the final goal of testing the efficacy of the method in field soils and biosolids samples in future research.
MATERIALS AND METHODS

Chemicals and SPME films

Bisphenol A (2,2-Bis(4-hydroxyphenyl)propane, 4,4’-Isopropylidenediphenol) (>97%), bisphenol A diacetate (4,4’-isopropylidenediphenol diacetate) (98%), triclosan (Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenol) (>97%), benzophenone (diphenyl ketone) (>99%), diethylhexyl phthalate (bis(2-ethylhexyl) phthalate) (99%), benzyl benzoate (benzoic acid benzyl ester) (>99%), and acetic anhydride (>99%) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (HPLC grade), ethyl acetate (HPLC grade), methanol (HPLC grade), and water (HPLC grade) were purchased from Fisher Chemicals (Fair Lawn, NJ). Acetone was purchased from Macron Chemicals (Phillipsburg, NJ), granular sodium sulfate was purchased from VWR International (Radnor, PA), and potassium carbonate was purchased from Mallinckrodt Baker (Paris, KY). Silicone (polydimethylsiloxane) sheeting (127 µm thick) was purchased from Specialty Silicone Products (Ballston Spa, NY). The silicone sheeting was cut into 1 cm² pieces, which were cleaned by thermal desorption at 270°C for 14 hours under a constant flow of helium and stored in methanol until use. The standard deviation of the weights of a subset of the pieces was 10%. All reported film-water partition coefficients and interstitial pore water concentrations calculated using TF-SPME take this standard deviation into account. All glassware was silanized and rinsed in pure water and acetone and allowed to dry before use.

Artificial soil

Fine sand (50-70 mesh) and Kaolin clay were purchased from Sigma Aldrich (St. Louis, MO). Finely ground sphagnum peat was purchased from Fisher Scientific (Fair Lawn, NJ). Artificial
soil was prepared in accordance with the “Earthworm, Acute Toxicity Tests” section of the OECD Guideline for Testing of Chemicals and consisted of 10% peat, 20% kaolin clay, and 70% fine sand [17].

Thin-film SPME method

In order to determine the conditions for the thin-film SPME method in water and determine the water-film partition coefficients by TF-SPME, 5 mL HPLC grade water in a glass vial was spiked with an EEDC in methanol or acetone. In order to determine the conditions for the thin-film SPME method in soil and calculate the soil pore water concentrations of each EEDC using TF-SPME, 1g artificial soil in a glass vial was spiked with an EEDC in methanol or acetone and 5 mL HPLC grade water was added. One 1cm² PDMS film was added to each vial and each vial was stirred with a magnetic stir bar at 380 RPM. Since the acyl derivative of bisphenol A is more readily absorbed by PDMS and resolved in GC-MS than bisphenol A [25], 0.25 mL of K₂CO₃ and 0.25 mL of acetic anhydride were added to the water or water-soil solution prior to the addition of the PDMS film to derivatize bisphenol A in situ to bisphenol A diacetate. The derivatization efficiency was assumed to be 100%, since the gas chromatogram peak corresponding to bisphenol A was never detected in any of the TF-SPME experiments and the peak corresponding to bisphenol A diacetate was detected in all experiments [25]. Since only the bisphenol A dissolved in water is able to be derivatized, the concentration of bisphenol A diacetate taken up by PDMS was assumed to be equal to the concentration of bisphenol A in the pore water available for uptake. After each film was exposed to the soil or water sample for the allotted time, it was removed with forceps from the vial, rinsed with deionized water, blotted dry with a lint-free tissue, folded in half, and inserted in the port liner of the inlet of a gas-
chromatograph/mass spectrometer (GC-MS) for thermal desorption prior to concentration analysis. A 4 mm Agilent single-taper splitless deactivated injection port liner, packed with silanized glass wool, was used. The inlet of the GC-MS was kept at 220ºC during insertion, and was then heated to the optimum temperature for desorption. Desorption time was measured once the inlet reached this temperature. Initial experiments were performed to ensure that no measurable desorption occurred at this temperature for any of the compounds tested. While thermal desorption completely removed the compounds of interest from PDMS film, the films were not reused for subsequent experiments.

_Determination of thin-film SPME method conditions in water_

In order to determine the conditions that would produce the highest film-water partitioning coefficient for that compound, which indicates the most effective extraction of the compound, the TF-SPME method was first applied to water spiked with each EEDC individually. The film-water partitioning coefficient for a given compound can be described by the equation $K_{\text{film}} = \frac{C_{\text{film}}}{C_w}$, where $K_{\text{film}}$ is the film-water partitioning coefficient for the compound, $C_{\text{film}}$ is the concentration of the EEDC in the film ($\mu$mol/L PDMS), and $C_w$ is the concentration in the water ($\mu$mol/L water) [21]. Central composite design was used to develop the array of experiments required to establish the optimum PDMS extraction time, gas chromatograph (GC) inlet thermal desorption temperature, and thermal desorption time for each EEDC. The response factor was log $K_{\text{film}}$, with higher log $K_{\text{film}}$ values reflecting higher extraction efficiency. For each experiment, 5 mL HPLC grade water in a glass vial was spiked with an EEDC in methanol or acetone. Thirty TF-SPME extractions of each compound were performed, and the extraction and
Desorption times, desorption temperature, and concentration were varied at random for each extraction (TABLE 1).

Table 1. Experimental conditions used in the development of TF-SPME method conditions in water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water Concentration (µmol/L water)</th>
<th>Extraction Time (minutes)</th>
<th>Desorption Temperature (°C)</th>
<th>Desorption Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>0-0.637</td>
<td>0-40</td>
<td>230-310</td>
<td>1-21</td>
</tr>
<tr>
<td>BZP</td>
<td>0-0.439</td>
<td>0-120</td>
<td>220-340</td>
<td>1-33</td>
</tr>
<tr>
<td>DEHP</td>
<td>0-1.657</td>
<td>0-120</td>
<td>175-355</td>
<td>0-20</td>
</tr>
<tr>
<td>TRI</td>
<td>0-2.560</td>
<td>0-120</td>
<td>250-330</td>
<td>0-20</td>
</tr>
</tbody>
</table>

Design-Expert software was used to fit appropriate models to the relationships between the parameters tested and the response factor, and analyses of variance (ANOVA) were used to determine which parameters significantly ($p<0.05$) affected the response factor for each compound. The values for the significant factors that corresponded with the highest measured $\log K_{\text{film}}$ values were determined visually from the response surface analysis figures generated by Design-Expert (Appendix). The median values tested in the central composite design experiments were chosen as the values for each of the non-significant factors.

**Determination of thin-film SPME method conditions in soil**

Central composite design and response surface analysis were also used in the experiments to determine the conditions for the TF-SPME method in soil. For each experiment, 1 gram of artificial soil in a glass vial was spiked with an EEDC to produce environmentally relevant concentrations that encompass the median concentrations in biosolids found by Kinney et al. [10] and 5 mL HPLC grade water was added. It was assumed that the chosen GC desorption
temperature and desorption time would be unchanged by the addition of soil. Since the purpose of the method is to calculate the interstitial pore water concentrations when the compound is at equilibrium between the soil and the pore water, it was important to determine the time at which the distribution of the compound reaches equilibrium and begin the extraction by PDMS at that point. Therefore, the only factors tested for the soil experiments were the EEDC concentration added to the soil, the stirring time prior to the addition of PDMS to soil, and the PDMS extraction time. Soil concentrations encompassed those found in TABLE 2, stirring time ranged from 0.5-38 hours, and PDMS extraction times encompassed those found in TABLE 1.

Table 2. Soil concentrations used in TF-SPME experiments and earthworm bioassays, and water concentrations used in experiments to determine $K_{film}$ values for each compound.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Soil Concentrations Used in TF-SPME Experiments and Earthworm Bioassays ($\mu$mol/g soil)</th>
<th>Water Concentrations Used in $K_{film}$ Measurement Experiments ($\mu$mol/L water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>0.0009-0.0044</td>
<td>0.159-0.637</td>
</tr>
<tr>
<td>BZP</td>
<td>0.0026-0.0248</td>
<td>0.512-2.560</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.0017-0.0121</td>
<td>0.345-2.418</td>
</tr>
<tr>
<td>TRI</td>
<td>0.0003-0.0022</td>
<td>0.055-0.439</td>
</tr>
</tbody>
</table>

Twenty extractions were performed for each compound, and the EEDC concentration, stirring time prior to the addition of PDMS to soil, and the PDMS extraction time were varied at random for each compound. The log $K_{film}$ was again used as the response factor and the significant values for each factor were determined in the same way as in the determination of the conditions for TF-SPME in water. It was hypothesized that stirring times less than the time required for the compounds in the soil and water to reach equilibrium would result in higher log $K_{film}$ values than those measured once the compounds in the soil and water were at equilibrium. Therefore, it was decided that the values for stirring time prior to the addition of PDMS that corresponded to the
lowest log $K_{\text{film}}$ values would be chosen during the visual analysis of the response surface figures to ensure that the method measured equilibrium pore-water concentrations (Appendix).

**Earthworm bioassays**

Earthworms (*Eisenia fetida*) were purchased from Carolina Biological Supply (Burlington, NC). Earthworms were housed in a 2.5 gallon glass aquarium filled with Magic® worm bedding and fed Magic® worm food *ad libitum*, all purchased from Carolina Biological Supply (Burlington, NC). Three adult earthworms totaling about 1 gram were placed in glass petri dishes with 40 grams of EEDC-spiked soil with a 35% water content for 3 weeks. Each petri dish full of soil was spiked individually to eliminate batch mixing errors. Deionized water was added as needed to maintain water content. Assays were repeated in triplicate for each concentration of each compound tested. Earthworms were then removed from the soil, depurated on moist filter paper for 24 hours, weighed, and frozen until tissue analysis. To extract each EEDC from earthworm tissue, frozen earthworms were first homogenized with sodium sulfate using a mortar and pestle until a fine powder was produced. The powder was then transferred to a centrifuge tube and 20 mL of a 50:50 mixture of acetonitrile and ethyl acetate was added. The tube was shaken for 5 minutes, centrifuged for 5 minutes, and the supernatant was isolated and dried under a constant stream of nitrogen. The lipid content of the sample was then determined gravimetrically. The sample was reconstituted with methanol, vortexed, centrifuged, and the supernatant was transferred to a gas chromatograph (GC) vial until analysis. The extraction efficiency, the amount of compound extracted compared to a known amount added to the sample, was 100% for benzophenone, 87.6% for BPA, 92.1% for DEHP, and 82.8% for triclosan. Lipid-normalized concentrations of EEDCs in earthworm extracts, corrected for their individual extraction efficiencies, were used for analyses and comparison with the TF-SPME method.
Determination of time required for earthworm uptake of EEDCs in soil

The time required for each EEDC to come to equilibrium in earthworm tissue during the bioassays was also determined using central composite design and response surface analysis. EEDC concentration and exposure time were varied. Soil EEDC concentrations encompassed those found in TABLE 2 and exposure time ranged from 3 days to 4.5 weeks. The response factor was log $K_{\text{worm}}$, where $K_{\text{worm}} = C_{\text{worm}}/C_{\text{soil}}$. $C_{\text{worm}}$ is the concentration of each compound extracted from earthworm tissue ($\mu$mol/g lipid) and $C_{\text{soil}}$ is the concentration in the soil ($\mu$mol/g soil). A higher log $K_{\text{worm}}$ was considered indicative of higher assimilation of the compound into earthworm tissue, and the significance of exposure time and concentration was determined in the same way as the factors involved in the TF-SPME method. The values for exposure time, those that corresponded with the highest measured log $K_{\text{worm}}$ values, were determined visually from the response surface figures generated by Design-Expert (Appendix).

Determination of film-water partition coefficients

Experiments to determine the film-water partition coefficients ($K_{\text{film}}$ values) for each EEDC were performed in triplicate at each concentration of each compound tested, and the extraction time, desorption time, and desorption temperature used in each experiment was in accordance with the results of the experiments to determine the conditions for the TF-SPME method in water. The concentrations used to determine $K_{\text{film}}$ values can be found in TABLE 2. Based on the preliminary $K_{\text{film}}$ values for TRI measured during the experiments to determine method conditions in water, the sample volume was increased from 5 mL to 100 mL when measuring the experimental $K_{\text{film}}$ values in order to satisfy the requirements of negligible-depletion SPME. The
overall $K_{film}$ value for each compound was calculated by averaging the experimental values over the range of concentrations.

*Calculation of pore water concentrations of EEDCs using TF-SPME*

Experiments to measure the uptake of EEDCs in soil by TF-SPME were performed 10 times for each concentration of each compound. The soil concentrations chosen for each compound are shown in TABLE 2 and the stirring time, extraction time, desorption time, and desorption temperature used in each experiment was in accordance with the results of the experiments to determine the TF-SPME method conditions in soil. Equilibrium concentrations in PDMS were converted to interstitial pore water concentrations ($C_{pw}$) by the relationship in the equation $C_{pw} = C_{film}/K_{film}$, where $C_{film}$ is equal to the concentration in SPME film and $K_{film}$ is the measured film-water partitioning coefficient [21].

*Measurement of earthworm uptake of EEDCs in soil*

Bioassay experiments to measure the uptake of EEDCs in soil by earthworms were performed 3 times for each concentration of each compound. The chosen soil concentrations of EEDCs were the same as those used for the calculation of pore water concentrations of EEDCs (TABLE 2).

*Instrumental analysis*

Concentrations of EEDCs in PDMS and in earthworm extracts were analyzed on an Agilent 6890 gas chromatograph with an Agilent 5973 mass spectrometry detector. The samples were injected in splitless mode at a temperature of 270°C. An external standard (benzyl benzoate) was run after each TF-SPME and earthworm extract sample was analyzed. The oven temperature was programmed to hold a temperature of 50°C for 1 minute, increase to 280°C at 30°C/minute, and
then increase to 310°C at 15°C/minute. The temperature was held at 310°C for 0.5 minutes. Helium was used as a carrier gas, at 9.97 psi. The mass spectrometer was run using electron impact in selective ion mode (SIM), with a solvent delay of 2 minutes. The ions used for detection and quantification of each compound are shown in TABLE 3.

Table 3. Selected chemical properties and ions used for detection of the compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log K&lt;sub&gt;ow&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log K&lt;sub&gt;oc&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Detection Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>3.32</td>
<td>3.10</td>
<td>213&lt;sup&gt;c&lt;/sup&gt;, 228</td>
</tr>
<tr>
<td>BZP</td>
<td>3.18</td>
<td>2.63</td>
<td>105&lt;sup&gt;c&lt;/sup&gt;, 212</td>
</tr>
<tr>
<td>DEHP</td>
<td>7.60</td>
<td>4.94</td>
<td>167&lt;sup&gt;c&lt;/sup&gt;, 279</td>
</tr>
<tr>
<td>TRI</td>
<td>4.76</td>
<td>3.93</td>
<td>218&lt;sup&gt;c&lt;/sup&gt;, 290</td>
</tr>
</tbody>
</table>

<sup>a</sup>Log K<sub>ow</sub> values generated from United States Environmental Protection Agency’s EPI Suite KOWWIN program, version 1.67

<sup>b</sup>Log K<sub>oc</sub> values generated from United States Environmental Protection Agency’s EPI Suite KOCWIN program, version 2.0

<sup>c</sup>Ions used for quantification.

Data analysis

Central composite design and response surface methodology as part of the Design-Expert 7.0 software package (Stat-Ease, Inc., Minneapolis, MN) were used to design the experiments for and to analyze the results of the determination of method conditions in water and soil, as well as to determine the exposure time for the earthworm bioassays. Data plotting, curve fitting, and the calculation of slopes and their standard errors were performed using Sigma Plot 9.0 (Systat Software, Inc., Richmond, CA). Standard deviations were calculated using the arithmetic error propagation method for multiplying and dividing the standard deviations of variables, by the
equation $\frac{\sigma_x}{x} = \sqrt{\left(\frac{\sigma_a}{a}\right)^2 + \left(\frac{\sigma_b}{b}\right)^2 + \left(\frac{\sigma_c}{c}\right)^2}$ where $x$ is the result of multiplying or dividing the variables $a$, $b$, and $c$ [26].

### RESULTS AND DISCUSSION

*Determination of the method conditions in water and determination of film-water partitioning coefficients*

In determining the method conditions for TF-SPME method in water, extraction time was found to significantly affect ($p < 0.05$) the log $K_{\text{film}}$ values for BPA, DEHP, and TRI, whereas desorption temperature was found to significantly affect ($p < 0.05$) the log $K_{\text{film}}$ values for BZP. Since concentration did not significantly affect the log $K_{\text{film}}$ values, the method could be used to determine an average log $K_{\text{film}}$ over a range of concentrations. The extraction time, desorption temperature, and desorption time for each compound can be found in TABLE 4.

Table 4. TF-SPME experimental conditions and experimentally determined $K_{\text{film}}$ values and their standard deviations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extraction Time (minutes)</th>
<th>Desorption Temperature (°C)</th>
<th>Desorption Time (minutes)</th>
<th>Measured $K_{\text{film}}$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>20.</td>
<td>270</td>
<td>11</td>
<td>21.9 +/- 1.16</td>
</tr>
<tr>
<td>BZP</td>
<td>60</td>
<td>310</td>
<td>16</td>
<td>25.4 +/- 1.22</td>
</tr>
<tr>
<td>DEHP</td>
<td>60</td>
<td>290</td>
<td>15</td>
<td>Not measured</td>
</tr>
<tr>
<td>TRI</td>
<td>60</td>
<td>270</td>
<td>10</td>
<td>252 +/- 4.56</td>
</tr>
</tbody>
</table>

As has been found by others [27], the measured $K_{\text{film}}$ values for each compound were lower than their octanol-water partition coefficient ($K_{\text{ow}}$) values (TABLE 4). The $K_{\text{film}}$ value for DEHP could not be accurately calculated, since due to its high hydrophobicity, the volume of water
required during experimental $K_{\text{film}}$ measurements to ensure adherence to principles of negligible depletion would have needed to be over 100 L to overcome the high extraction phase volume used in the TF-SPME method. This issue may indicate a limitation of the method, in that $K_{\text{film}}$ values derived from the literature may need to be used for very hydrophobic compounds such as DEHP in order to satisfy the assumptions of negligible depletion. In order to calculate soil pore water concentrations for DEHP, a $K_{\text{film}}$ value of $128100 \pm 1100$ previously reported by Kotowska et al. [28] was used. DiFilippo et al. found that linear solvation energy relationships (LSER) can be used to predict the partitioning of compounds into PDMS [24]. These equations rely on specific physical and chemical characteristics of compounds. Although these characteristics may not be available for all compounds of interest, LSER equations may prove useful for estimating $K_{\text{film}}$ values rather than experimentally calculating the values if TF-SPME is used with additional compounds in future research.

*Determination of method conditions in soil*

The stirring time prior to the addition of PDMS was a significant factor ($p<0.05$) for all of the compounds tested; equilibrium between the soil and water phases was reached for BZP, BPA, and DEHP within 19 hours and within 22.75 hours for TRI. Vials were thus stirred for 24 hours prior to the addition of the PDMS film and the other experimental conditions determined in testing the method in water were followed for all soil experiments.

*Determination of time required for earthworm uptake of EEDCs in soil*

Exposure time was found to significantly affect the response factor for all compounds ($p<0.05$). It was determined that 3 weeks was a sufficient exposure time to allow all compounds to reach
equilibrium between soil and earthworm tissue. Therefore, 3 weeks was chosen as the exposure time for the experimental bioassays. This time is in agreement with equilibrium exposure times reported in the literature [29].

*Calculation of interstitial pore water concentrations*

Equilibrium concentrations in PDMS films exposed to soil spiked with the concentrations of each compound listed in TABLE 2 were measured. Concentrations in PDMS increased linearly with soil concentration. When PDMS concentrations were converted to interstitial pore water concentrations, all $r^2$ values were $>0.93$ using a linear model and the average relative standard deviation for all compounds was 10.2% (FIGURE 1).
Figure 1. Comparison of concentrations of A. BPA, B. BZP, C. DEHP, and D. TRI in spiked soil with soil pore water concentrations calculated using TF-SPME. Error bars show one standard deviation.

The non-zero y intercepts are likely due to the sensitivity of the gas chromatograph, and represent the point at which the compound cannot be measurably detected in the lower limits of the working curve used to calculate the concentrations. The good linear fit and low standard deviations for each compound indicate that the use of the TF-SPME method results in precise calculations of interstitial pore water concentrations.
**Ability of the TF-SPME Method to Predict Earthworm Tissue Concentrations**

The concentrations of all the compounds in earthworm tissue increased linearly with soil concentration. In order to assess the ability of the TF-SPME method to predict the uptake of the EEDCs by earthworms, the interstitial pore water concentrations calculated using TF-SPME were used to estimate earthworm tissue concentrations. The equation $C_{\text{worm, predicted}} = C_{\text{pw}} \cdot \text{BCF}$ relates the concentration of a compound in soil pore water to its concentration in earthworm tissue [18]. $C_{\text{worm, predicted}}$ is the predicted lipid-normalized concentration in the earthworm body and BCF is the estimated bioconcentration factor for the compound. There are many ways to calculate the BCF for a given compound. Most equations used in the calculation of a BCF rely on $K_{\text{ow}}$ values. Estimated BCF values for each EEDC were calculated using an earthworm-specific BCF equation: $\text{BCF} = (F_{\text{water}} + F_{\text{lipid}} K_{\text{ow}})/\rho_{\text{worm}}$, where $F_{\text{water}}$ and $F_{\text{lipid}}$ are the fractions of water and lipid in the worm, respectively, and $\rho_{\text{worm}}$ is the density of the worm on a wet-weight basis (kg/L) [18].

Using the calculated predicted earthworm tissue concentrations, prediction factors for each compound were determined by the slope of the relationship between the predicted and measured earthworm concentrations for each compound. Based on these prediction factors, it was determined that converting interstitial pore water concentrations (calculated using TF-SPME) into predicted earthworm tissue concentrations over-predicts the concentrations of BZP and TRI taken up by earthworms by factors of 7.9 (SE = 0.012) and 9.3 (SE = 0.012), respectively, and under-predicts the concentrations of BPA by 30.8 (SE = 3.2). Using the PDMS fiber partitioning coefficient for DEHP that was measured by Kotowska et al. to calculate the pore water concentrations, the prediction factor was 2.9 (SE = 0.022), indicating that TF-SPME can still be used to predict pore water concentrations of DEHP when an external $\log K_{\text{film}}$ value is used [28].
Due to the high sorption capacity of the artificial soil, all TF-SPME experiments performed in the presence of soil satisfied the assumption of negligible depletion, regardless of which log $K_{film}$ value was used.

Organic carbon-based equilibrium partitioning equations are currently used in many risk assessments of the bioaccumulation of organic compounds in soils. Organic carbon-based equilibrium partitioning equations, as well as TF-SPME, rely on the principle that earthworms mainly take up compounds via passive diffusion of the compound in soil pore water through the skin. Organic carbon-based equilibrium partitioning equations take the form $C_{worm} = C_{pw}BCF$, where $C_{pw} = C_s/(f_{oc}K_{oc})$, $C_s$ is the concentration in soil (mg/kg), $f_{oc}$ is the fraction of organic carbon in the soil, and $K_{oc}$ is the organic carbon partitioning coefficient [18]. In order to use organic carbon-based equilibrium partitioning equations, the $K_{oc}$ of the compound and the fraction of organic carbon in the soil to be tested must be known, which limits the applicability of these equations to use in novel soils, since organic carbon content would need to be experimentally determined.

Organic carbon-based equilibrium partitioning equations also over-predict earthworm concentrations of BZP and TRI by 3.24 (SE = 0.012) and 14.0 (SE = 0.006), respectively, and under-predict earthworm concentrations of BPA by 10.6 (SE = 1.09). The prediction factor for DEHP generated from organic carbon-based equilibrium partitioning equations was 833.36 (SE = 8.4x10^{-5}). This large over-prediction may be due to an increase in apparent water solubility of DEHP in the presence of dissolved organic matter [30]. While this higher solubility results in a lower measured $K_{oc}$, the soluble DEHP is bound to dissolved organic matter and is therefore unavailable for earthworm uptake [18]. As a result, the $K_{oc}$ used in partitioning equations for purposes of predicting earthworm uptake is an under-prediction of the true $K_{oc}$, and the predicted
amount of DEHP taken up by earthworms is an over-prediction. This issue underscores the importance of experimental measures of bioavailability such as TF-SPME rather than relying on organic carbon-based equilibrium partitioning equations to estimate bioavailability.

When converted to predicted earthworm tissue concentrations, neither the interstitial pore water concentrations generated from TF-SPME experiments nor those calculated using organic carbon-based equilibrium partitioning experiments closely predicted earthworm tissue concentrations. The biota of the earthworm gut produce enzymes that have been shown to break down organic matter in the gut [31]. As such, they may be able to dissociate organic carbon-bound compounds from ingested soil particles, increasing pore water concentrations of these compounds in the earthworm gut and therefore increasing the potential for uptake of these compounds into earthworm tissue. This may explain why both the organic carbon-based equilibrium partitioning equation and TF-SPME under-predicted earthworm concentrations of BPA.

On the other hand, both the TF-SPME method and the organic carbon-based equilibrium partitioning equations over-predicted the concentrations of DEHP, BZP and TRI in earthworms. Although limited information is available on the presence of Phase I enzymes in earthworms, it is possible that the earthworms or their flora are able to metabolize DEHP, BZP and TRI [32, 33]. Further research is needed to determine whether earthworms are capable of metabolizing organic compounds such as xenoestrogens.

Bioaccumulation factors (BAF) may more accurately describe how earthworms bioaccumulate a given compound than BCF values because BAF values are typically measured experimentally rather than simply calculated from a compound’s physical characteristics. A comparison of the BCF values derived for this study with bioaccumulation factors (BAF) for BZP and TRI that were measured in field-collected earthworms found in soils amended with biosolids as well as in
earthworms exposed to soils spiked with biosolids in the laboratory reveals that the measured BAF values are often lower than the calculated BCF values, and BAF values vary with the concentration of biosolids in soil [4, 5]. Thus, while BCF values do not capture the metabolism or dissociation of a compound from organic matter in the earthworm gut, BAF values vary with environmental conditions. Therefore, it may not be advisable to use either BCF or BAF values to convert soil pore water concentrations to earthworm tissue concentrations. This poses a challenge to recommending how to use interstitial pore water concentrations generated by TF-SPME to estimate earthworm tissue concentrations for a given compound in further use of the method.

However, given the high coefficients of determination for the linear fits between soil concentrations and interstitial pore water concentrations generated by TF-SPME (FIGURE 1), as well as the low relative standard deviations of the calculated pore water concentrations, TF-SPME may be best used with the goal of calculating soil pore water concentrations and thus general bioavailability of EEDCs and other compounds in soil. The use of TF-SPME to quantify bioavailability of such compounds would be preferable over non-experimental methods such as organic carbon-based partitioning equations due to the ability of TF-SPME to account for the sorption of compounds to dissolved organic carbon and the lack of need to measure the organic carbon content of a novel soil.

Conclusions

The developed novel TF-SPME method effectively and efficiently extracts and measures equilibrium concentrations of BPA, DEHP, TRI, and BZP in artificial soil pore water without the use of solvents and within a total of 88 minutes. While there are some challenges surrounding the
use of the method to predict equilibrium earthworm tissue concentrations of these compounds, TF-SPME can be used to generate a better estimate of bioavailability of a compound in soil than non-experimentally based equilibrium partitioning equations. This will aid in the development of risk estimates related to the presence of each of these compounds in the soil environment. Future research will test the TF-SPME method with mixtures of EEDCs, as well as with field soil of varying properties and samples with concentrations over several orders of magnitude to more accurately reflect how the method could be used to predict bioavailability to terrestrial organisms in the field.
CHAPTER 3

Application of an in vitro Thin-Film Solid-Phase Microextraction Method in Artificial and Field Soils to Determine the Bioavailability of Mixtures of Xenoestrogens

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ABSTRACT

A previously developed thin-film-solid phase microextraction (TF-SPME) method for determining the bioavailability of several EEDCs detected in biosolids was applied to mixtures of EEDCs in one artificial and two field soils. It was determined that TF-SPME could be used to calculate equilibrium pore-water concentrations of mixtures of diethylhexyl phthalate, bisphenol A, benzophenone, and triclosan at environmentally relevant concentrations in all of the tested soils. Pore-water concentrations were found to be dependent on the organic carbon content of the soil, and the possibility of using the relationships between organic carbon content and pore-water concentration to inform soil remediation efforts are discussed. Finally, the ability of the earthworm *E. fetida* to take up compounds in the artificial and field soils was investigated, and a dependency of earthworm tissue concentration of each compound on soil organic carbon was also found.
INTRODUCTION

Biosolids derived from treated sewage sludge may represent a major source of estrogen-like endocrine disrupting compounds (EEDCs), such as those found in household products and plastics. These compounds have been detected in both raw sewage sludge and soils to which sludge has been applied as a soil amendment or fertilizer [10]. There are very little data on the ecological risks that can be attributed to the presence of these compounds in the environment. Data on the bioavailability of these compounds to soil organisms is necessary for the calculation of these risks in soil, and new methods are needed that can be used to determine this bioavailability without necessitating the use of traditionally-used earthworm uptake experiments, due to the length and laborious nature of the experiments.

The development of a novel method using thin-film solid-phase microextraction (TF-SPME) to determine the bioavailability of several EEDCs was described in Chapter 1 [34]. The method involves the use of a polydimethylsiloxane (PDMS) film as an extraction phase. The use of the film requires less time than a fiber due to the higher surface area to volume ratio in the 1 cm² film versus in a typical 100µm SPME fiber [22]. It also costs ~3 US cents per film, much less expensive than a traditional SPME fiber, which costs ~144 US dollars. In TF-SPME, the film is exposed to soil containing the compounds, and after the compounds reach equilibrium levels in the film, they are extracted from the PDMS film via thermal desorption in a GC-MS and quantified. The method effectively extracted and determined equilibrium concentrations of the EEDCs bisphenol A (BPA), diethylhexyl phthalate (DEHP), triclosan (TRI), and benzophenone (BZP) in artificial soil pore-water without the use of solvents and within a total of 88 minutes [34]. The time required for the film to be exposed to the soil sample, the thermal desorption
temperature, and the time required for complete thermal desorption were determined; these
conditions are described in Chapter 1 [34]. However, in order to more accurately reflect how the
method could be used to predict bioavailability of these compounds in the field, the TF-SPME
method must be tested with mixtures of EEDCs, with concentrations of EEDCs spanning several
magnitudes, and with field soils of varying properties.

The goal of this research was to continue the development of the TF-SPME method by
challenging it with various conditions that reflect reasonable field characteristics. The specific
objectives were i) to calculate the interstitial pore-water concentrations of BPA, DEHP, TRI, and
BZP in artificial soil containing mixtures of these compounds using TF-SPME and determine
whether its ability to calculate interstitial pore-water concentrations of each compound was
significantly affected by the presence of the mixture of compounds; ii) to measure earthworm
tissue concentrations of each compound in artificial soil containing mixtures of the compounds
and determine whether the ability of earthworms to take up each compound was significantly
affected by the presence of the mixture of compounds; iii) to challenge the sensitivity of the
method by measuring interstitial pore-water concentrations of each compound over several
orders of magnitude in artificial soil spiked with mixtures of the compounds; iv) to calculate
interstitial pore-water concentrations of each compound using TF-SPME in two field soils spiked
with mixtures of the compounds, and v) to measure earthworm tissue concentrations of mixtures
of the compounds in two spiked field soils.
MATERIALS AND METHODS

Chemicals and SPME films

Bisphenol A (2,2-Bis(4-hydroxyphenyl)propane, 4,4′-Isopropylidenediphenol) (>97%), bisphenol A diacetate (4,4′-isopropylidenediphenol diacetate) (98%), triclosan (Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenol) (>97%), benzophenone (diphenyl ketone) (>99%), diethylhexyl phthalate (bis(2-ethylhexyl) phthalate) (99%), benzyl benzoate (benzoic acid benzyl ester) (>99%), and acetic anhydride (>99%) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (HPLC grade), ethyl acetate (HPLC grade), methanol (HPLC grade), and water (HPLC grade) were purchased from Fisher Chemicals (Fair Lawn, NJ). Acetone was purchased from Macron Chemicals (Phillipsburg, NJ), granular sodium sulfate was purchased from VWR International (Radnor, PA), and potassium carbonate was purchased from Mallinckrodt Baker (Paris, KY). Silicone (polydimethylsiloxane) sheeting (127 µm thick) was purchased from Specialty Silicone Products (Ballston Spa, NY). The silicone sheeting was cut into 1 cm² or 0.25 cm² pieces, which were cleaned by thermal desorption at 270°C for 14 hours under a constant flow of helium and stored in methanol until use. All glassware was silanized and rinsed in pure water and acetone and allowed to dry before use.

Artificial soil

Fine sand (50-70 mesh) and Kaolin clay were purchased from Sigma Aldrich (St. Louis, MO). Finely ground sphagnum peat was purchased from Fisher Scientific (Fair Lawn, NJ). Artificial soil was prepared in accordance with the “Earthworm, Acute Toxicity Tests” section of the
OECD Guideline for Testing of Chemicals and consisted of 10% peat, 20% kaolin clay, and 70% fine sand [17].

Field soils

Arkport soil and Hudson soil were collected from field sites on the Cornell University campus in Ithaca, New York. Both soils were dried and sieved to 1 mm before testing. The Arkport soil is a very fine sandy loam and the Hudson soil is a silty clay loam. The organic carbon contents of both soils were measured by the Cornell Nutrient Analysis Laboratory using the wet combustion method outlined in the Soil Survey Laboratory Methods Manual (National Soil Survey Center, National Resources Conservation Service, United States Department of Agriculture). The measured organic carbon contents of the Arkport and Hudson soils were 2.19% and 0.85%, respectively.

Thin-film SPME method

In order to determine the interstitial pore-water concentrations of each compound, the TF-SPME method was performed in a manner similar to that described in Chapter 1 [34]. Briefly, vials containing soil spiked with EEDCs and HPLC grade water were stirred at 380 RPM on a magnetic stir plate for 24 hours, the optimum time determined for the compounds between artificial soil and the pore-water [34]. One PDMS film was then added to each of the vials and each vial was stirred with a magnetic stir bar at 380 RPM. Previous research determined that the optimum PDMS exposure time for BZP, TRI, and DEHP was 60 minutes and was 20 minutes for BPA [34].
Prior to the addition of the PDMS film, bisphenol A was derivatized *in situ* to bisphenol A diacetate to improve uptake of the compound by PDMS by the addition of $K_2CO_3$ and acetic anhydride. Triclosan is also chemically susceptible to derivatization by the acetic anhydride since it contains a phenol group, but no analytical standards of triclosan acetate could be obtained.

Since both triclosan and bisphenol A were present in the soil for the experiments testing the effects of mixtures of compounds on TF-SPME, it was necessary to avoid the measurement of the derivatized triclosan. Therefore, initial experiments were performed in which PDMS was exposed to samples of artificial soil spiked with BPA, DEHP, TRI, and BZP for 60 minutes, allowing the DEHP, TRI, and BZP to come to equilibrium in the PDMS. The sample was not derivatized, and PDMS was then removed from the system after 60 minutes and the concentration of TRI in PDMS was measured. The results of these experiments were compared with those in which PDMS was exposed to samples containing BPA, DEHP, TRI, and BZP for 60 minutes, followed by the derivatization of the sample with acetic anhydride, and the exposure of PDMS to the sample for an additional 20 minutes to allow derivatized BPA to be taken up by the PDMS. The PDMS film was then removed from the system and the concentration of TRI in PDMS was measured. A *t*-test was performed to compare the PDMS concentrations of TRI in the experiments in which the sample was derivatized with those in which the sample was not derivatized. There was no significant difference ($p<0.05$) between the amount of TRI taken up by PDMS when it was exposed to the sample for 60 minutes and removed without derivatization, and the amount of TRI taken up by PDMS when it was exposed to the sample for 60 minutes, derivatized, and exposed for an additional 20 minutes before being removed. Therefore, for the mixture experiments, the PDMS film was first exposed to the sample for 60 minutes, allowing
for the uptake of BZP, TRI, and DEHP. The derivatizing agents were then added and the film was exposed to the sample for an additional 20 minutes to allow for the uptake of derivatized BPA.

After each film was exposed to the soil for the allotted time, it was removed with forceps from the vial, rinsed with deionized water, blotted dry with a lint-free tissue, folded in half, and inserted into the port liner of the inlet of a gas-chromatograph/mass spectrometer (GC-MS) for thermal desorption prior to concentration analysis. A 4 mm Agilent single-taper splitless deactivated injection port liner, packed with silanized glass wool, was used. The inlet of the GC-MS was kept at 220ºC during insertion and was then heated to the optimum temperature for desorption. A desorption temperature of 310ºC was chosen. This temperature represents the chosen temperature for desorbing BZP as determined in the method development experiments described previously [34]. While previous research determined that desorption temperature significantly affected the water-film partitioning coefficients for BZP, it did not affect those for BPA, DEHP, or TRI [34]. Therefore, 310ºC was chosen as the desorption temperature for all four compounds since it is higher than the desorption temperatures determined for BPA, DEHP, and TRI, and is the optimum desorption temperature for BZP. Desorption time was measured once the inlet reached 310ºC. Although desorption time was not found in previous experiments to significantly affect the water-film partition coefficients ($p<0.05$), 16 minutes was chosen as the desorption time for these experiments since it represents the highest desorption time of any of the four compounds tested, and would thus allow for maximum desorption of the compounds [34].
**Earthworm bioassays**

Earthworms (*Eisenia fetida*) were purchased from Carolina Biological Supply (Burlington, NC). Earthworms were housed in a 2.5 gallon glass aquarium filled with Magic® worm bedding and fed Magic® worm food *ad libitum*, all purchased from Carolina Biological Supply (Burlington, NC). The procedure for earthworm bioassays was previously described [34]. Briefly, three adult earthworms totaling about 1 gram were placed in glass petri dishes with 40 grams of EEDC-spiked soil with a 35% water content for 3 weeks. Earthworms were then removed from the soil, depurated on moist filter paper for 24 hours, weighed, and frozen until tissue analysis, which is described in detail in Chapter 1 [34]. Lipid-normalized concentrations of EEDCs in earthworm extracts, corrected for their individual extraction efficiencies, were used for analyses.

**Calculation of interstitial pore-water concentrations of mixtures of compounds in artificial soil**

For the mixture experiments in artificial soil, a fractional factorial design was developed in SAS to randomly assign different concentrations of each compound to each experimental level. Environmentally relevant concentrations that encompass the median concentrations in biosolids found by Kinney et al. were chosen [10], and are the same concentrations tested in previous research described in Chapter 1 [34]. The tested concentrations of each compound represented the low, median, and high concentrations within the chosen concentration range. For BPA, concentrations of 0, 0.2, 0.6, and 1 µg/g soil were tested. For BZP, concentrations of 0, 0.05, 0.2, and 0.4 µg/g soil were tested. For DEHP, concentrations of 0, 1, 4, and 8 µg/g soil were tested, and for TRI, concentrations of 0, 0.5, 2, and 3.5 µg/g soil were tested. The fractional factorial design resulted in a set of 16 levels in which each concentration of each compound was randomly assigned to each level.
Experiments at each level in the experimental design were performed 10 times. In each experiment, one gram of artificial soil in a glass vial was spiked with EEDCs and 5 mL HPLC grade water was added. Equilibrium concentrations in PDMS were converted to interstitial pore-water concentrations \( (C_{pw}) \) as they were in the research described in Chapter 1 by the equation

\[
C_{pw} = \frac{C_{film}}{K_{film}},
\]

where \( C_{film} \) is equal to the concentration in SPME film and \( K_{film} \) is the measured film-water partitioning coefficient [21, 34].

**Calculation of interstitial pore-water concentrations for an extended concentration range of compounds in artificial soil**

Experiments were performed in artificial soil spiked with mixtures of compounds at concentrations that were one order of magnitude higher and one order of magnitude lower than the median concentrations tested in the mixture experiments described above. A fractional factorial design was developed in SAS to assign the concentrations of each compound to each experiment, resulting in 8 experimental levels. The concentrations tested were 0.063 and 6.3 \( \mu \)g BPA/g soil, 0.0213 and 2.13 \( \mu \)g BZP/g soil, 0.02 and 20 \( \mu \)g DEHP/g soil, and 0.033 and 33 \( \mu \)g TRI/g soil. Experiments at each level in the experimental design were performed 3 times, resulting in a total of 12 repetitions per concentration. In each experiment, one gram of artificial soil in a glass vial was spiked with EEDCs and 5 mL HPLC grade water was added. Equilibrium concentrations in PDMS were converted to interstitial pore-water concentrations by the equation described previously, \( C_{pw} = \frac{C_{film}}{K_{film}} \).
Measurement of earthworm tissue concentrations of mixtures of compounds in artificial soils

Earthworm bioassays were prepared as described above, and 40 g of artificial soil was used as the medium. The same experimental design was used in the calculation of interstitial pore-water concentrations of mixtures of compounds in artificial soil as was used for the measurement of earthworm tissue concentrations of the compounds. Each petri dish of artificial soil was separately spiked with EEDCs to avoid batch mixing errors. Bioassay experiments were performed 3 times for each level in the experimental design.

Calculation of interstitial pore-water concentrations of mixtures of compounds in field soils

For the experiments to determine the interstitial pore-water concentrations of mixtures of compounds in field soils, another fractional factorial design was developed in SAS to randomly assign different concentrations of each compound to each experiment. In order to reduce the number of experiments of mixtures of compounds in the two field soils, 0 µg/g soil was removed as a concentration level. Instead, control experiments were run with un-spiked field soils. The tested concentrations of each compound remained the same as in the mixture experiments in artificial soil.

The organic carbon content of both field soils was lower than that of the artificial soil. In order to still fulfill the requirements of negligible depletion for the uptake of DEHP, the amount of soil used for each field soil experiment was increased to 6 g, the water volume was increased to 30 mL, and the PDMS film was decreased in size to 0.25 cm². Experiments at each of the 9 experimental levels were performed 3 times for each field soil, resulting in a total of 9 experiments run per concentration of a given compound. Equilibrium concentrations of each
compound in PDMS were converted to interstitial pore-water concentrations by the equation described previously, \( C_{pw} = \frac{C_{film}}{K_{film}} \).

Measurement of earthworm tissue concentrations of mixtures of compounds in field soils

Earthworm bioassays were prepared as described above, and 40 g of field soil was used as the medium. The same experimental design used in the calculation of interstitial pore-water concentrations of mixtures of compounds in field soils was used for the measurement of earthworm tissue concentrations of the compounds. Each petri dish of field soil was separately spiked with EEDCs to avoid batch mixing errors. Bioassay experiments were performed 3 times for each level in the experimental design for each field soil.

Instrumental analysis

Concentrations of EEDCs in PDMS and in earthworm extracts were analyzed on an Agilent 6890 gas chromatograph with an Agilent 5973 mass spectrometry detector. The oven temperature program, injection mode, and ions used for quantification of each compound are the same as those described in Chapter 1 [34].

Data analysis

Data plotting and curve fitting, data analyses, and the calculation of slopes and their standard errors were performed using Sigma Plot 9.0 (Systat Software, Inc., Richmond, CA) and Excel (Microsoft, 2007). ANCOVA analyses were performed using XLSTAT 2013 (Addinsoft USA, New York, NY). Standard deviations were calculated using the arithmetic error propagation method for multiplying and dividing the standard deviations of variables, by the equation
\[
\frac{\sigma x}{x} = \sqrt{\left(\frac{\sigma a}{a}\right)^2 + \left(\frac{\sigma b}{b}\right)^2 + \left(\frac{\sigma c}{c}\right)^2}
\]
where \( x \) is the result of multiplying or dividing the variables \( a, b, \) and \( c \) [26].

RESULTS AND DISCUSSION

Calculation of interstitial pore-water concentrations of mixtures of compounds in artificial soil by TF-SPME

Previous research showed that the TF-SPME method can be used to determine the interstitial pore-water concentrations of BPA, BZP, TRI, and DEHP [34]. The current research seeks to test the TF-SPME method with mixtures of BPA, BZP, TRI, and DEHP in the soil to ensure that the method can be used to reliably measure the interstitial pore-water concentrations of any of the given compounds when other compounds are present.

The equilibrium concentrations of BPA, BZP, TRI, and DEHP in PDMS film exposed to soil containing mixtures of these compounds were measured. Interstitial pore-water concentrations of each of the compounds were calculated according to the equation described previously, \( C_{pw} = \frac{C_{film}}{K_{film}} \) and using the film-water partitioning coefficients previously calculated [21, 34]. Linear equations describing the relationship between soil concentrations of each EEDC and the corresponding pore-water concentrations determined by TF-SPME were generated (FIGURE 2).
Figure 2. Comparison of the relationships between soil concentration and soil pore-water concentrations (calculated by TF-SPME) in soil spiked with mixtures of the four compounds vs. in soil spiked with each compound individually. The four compounds shown are A. BPA, B. BZP, C. DEHP, and D. TRI. Error bars show one standard deviation.

To determine whether the presence of other compounds affected the ability of TF-SPME to generate interstitial pore-water concentrations of each of the compounds, an analysis of covariance (ANCOVA) was run for each compound assuming a 95% confidence level. The pore-water concentration of each compound was used as the dependent variable, and the soil concentration and type of experiment (single compound or mixture of compounds) were used as the quantitative and qualitative independent variables, respectively. Data from Chapter 1 were used for the single compound experimental data. As part of the ANCOVA analysis, a Type III
The sum of squares table was computed, which shows the impact of each variable (soil concentration or type of experiment) on the model to predict pore-water concentration. In other words, it can be used to determine whether the presence of the compound as the sole compound in the soil or as a mixture of compounds has an effect on pore-water concentration. The \( p \) values corresponding with the type of experiment in the Type III sum of squares table were >0.05 for DEHP, TRI, and BPA. The \( p \) value corresponding with the type of experiment for BZP was 0.023, which is significant under a 95\% confidence level. However, the correlation coefficient that was also calculated as part of the ANCOVA analysis, between type of experiment and pore-water concentration for BZP, was -0.053 versus a 0.968 correlation coefficient between soil concentration and pore-water (TABLE 5). Due to the low correlation coefficient describing the relationship between the type of experiment and pore-water concentration in the case of BZP, it was concluded that the type of experiment did not have a significant impact on the relationship between pore-water concentration and soil concentration.

Table 5. Results of analyses of covariance of soil concentrations and pore-water concentrations between TF-SPME experiments performed with individual compounds and with mixtures of compounds.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient of soil concentration vs. pore-water concentration</th>
<th>Correlation coefficient of type of experiment vs. pore-water concentration</th>
<th>Type III Sum of Squares Analysis: ( \text{Pr}&gt;F ) of soil concentration</th>
<th>Type III Sum of Squares Analysis: ( \text{Pr}&gt;F ) of type of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>0.973</td>
<td>0.022</td>
<td>&lt;0.0001</td>
<td>0.414</td>
</tr>
<tr>
<td>BZP</td>
<td>0.968</td>
<td>0.053</td>
<td>&lt;0.0001</td>
<td>0.023</td>
</tr>
<tr>
<td>TRI</td>
<td>0.967</td>
<td>0.026</td>
<td>&lt;0.0001</td>
<td>0.436</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.982</td>
<td>0.013</td>
<td>&lt;0.0001</td>
<td>0.833</td>
</tr>
</tbody>
</table>

Overall, the low correlation coefficients and the insignificant (>0.05) \( p \) values corresponding to the relationship between type of experiment and pore-water concentrations indicate that the
presence of compounds in the soil as individual compounds versus as mixtures does not have a significant impact on the relationship between soil concentration and the uptake of compounds by PDMS and subsequent conversion into pore-water concentrations. This is important, as it indicates that the TF-SPME method could be used to predict concentrations of the four compounds when mixtures of these compounds are present, as they often are in field scenarios, without the need for correction factors [4].

*Measurement of earthworm tissue concentrations of mixtures of compounds in artificial soil*

Previous research determined that there are several challenges surrounding the conversion of pore-water concentrations generated by the use of TF-SPME into predicted earthworm concentrations [34]. It was assumed that these challenges would be applicable to the current research. However, in order to add to the body of knowledge about how earthworms take up compounds in the soil, the manner in which earthworms take up compounds in the soil when the compounds are present individually versus in mixtures was assessed.

To investigate this, linear equations were developed that relate the earthworm tissue concentrations of each compound with concentrations of each EEDC in the soil when the compounds were present as mixtures in the soil (FIGURE 3).
Figure 3. Comparison of the relationships between soil concentration and earthworm tissue concentration in soil spiked with mixtures of the four compounds vs. in soil spiked with each compound individually. The four compounds shown are A. BPA, B. BZP, C. DEHP, and D. TRI. Error bars show one standard deviation.

To determine whether the presence of other compounds affected the uptake and bioaccumulation of compounds by earthworms, an analysis of covariance (ANCOVA) was run for each compound. The earthworm tissue concentration of each compound was used as the dependent variable, and the soil concentration and type of experiment (single compound or mixture of compounds) were used as the quantitative and qualitative independent variables, respectively. Data from Chapter 1 were used for the single compound experimental data. As part of the
ANCOVA analysis, a Type III sum of squares table was computed, which shows the impact of each variable (soil concentration or type of experiment) on the model to predict earthworm tissue concentrations. It can be used to determine whether the presence of the compound as the sole compound in the soil or as a mixture of compounds has an effect on earthworm tissue concentrations. The $p$ values corresponding with the type of experiment were $>0.05$ for DEHP and BPA. The $p$ value corresponding with the type of experiment for BZP was 0.03, which is significant under a 95% confidence level, but the correlation coefficient that was also calculated as part of the ANCOVA analysis, between type of experiment and earthworm tissue concentration for BZP, was 0.152 versus a 0.913 correlation coefficient between soil concentration and earthworm tissue concentration (TABLE 6). Therefore it was concluded that the type of experiment did not have a significant impact on the relationship between earthworm tissue concentration and soil concentration. These data indicate that earthworms take up and assimilate BPA, BZP, TRI, and DEHP when the compounds are present in mixtures in the same manner as when they are present individually in artificial soil.

The $p$ value associated with the type of experiment for TRI was $<0.0001$, indicating that the presence of compounds in the soil as mixtures may have an impact on the relationship between soil concentration and earthworm tissue concentration. However, the correlation coefficient between type of experiment and earthworm tissue concentration was 0.414, indicating a very weak correlation between the type of experiment and earthworm tissue concentration. Upon examination of Figure 3, it appears that the measured earthworm tissue concentrations are lower under mixture conditions. This may indicate that the presence of other compounds increased the metabolism of TRI by earthworms, possibly by the induction of a cytochrome involved in the
metabolism of TRI. Further research should be performed to determine by what mechanisms earthworms metabolize TRI and what factors influence its metabolism.

Table 6. Results of analyses of covariance of soil concentrations and pore-water concentrations between TF-SPME experiments performed with individual compounds and with mixtures of compounds.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient of soil concentration vs. earthworm tissue concentration</th>
<th>Correlation coefficient of type of experiment vs. earthworm tissue concentration</th>
<th>Type III Sum of Squares Analysis: Pr&gt;F of soil concentration parameter</th>
<th>Type III Sum of Squares Analysis: Pr&gt;F of type of experiment parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>0.961</td>
<td>0.020</td>
<td>&lt;0.0001</td>
<td>0.732</td>
</tr>
<tr>
<td>BZP</td>
<td>0.913</td>
<td>0.152</td>
<td>&lt;0.0001</td>
<td>0.030</td>
</tr>
<tr>
<td>TRI</td>
<td>0.824</td>
<td>0.410</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.954</td>
<td>0.080</td>
<td>&lt;0.0001</td>
<td>0.074</td>
</tr>
</tbody>
</table>

Calculation of interstitial pore-water concentrations of extended concentrations of compounds in artificial soil

TF-SPME was tested with concentrations one order of magnitude higher and one order of magnitude lower than those used in previous experiments in order to test the sensitivity of the method. To determine whether the relationship between soil concentration and pore-water concentration was affected by the extended concentration range, an analysis of covariance (ANCOVA) was run for each compound. The pore-water concentration of each compound was used as the dependent variable, and the soil concentration and type of concentration range (original or extended) were used as the quantitative and qualitative independent variables, respectively. Data from the mixture experiments were used for the original concentration range.
data. The \( p \) values corresponding with the type of experiment were >0.05 for all of the compounds (TABLE 7).

Table 7. Results of analyses of covariance of soil concentrations and pore-water concentrations between TF-SPME experiments performed with the original concentration and the extended concentration range.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient of soil concentration vs. pore-water concentration</th>
<th>Correlation coefficient of type of experiment vs. pore-water concentration</th>
<th>Type III Sum of Squares Analysis: Pr&gt;F of soil concentration parameter</th>
<th>Type III Sum of Squares Analysis: Pr&gt;F of type of experiment parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>0.897</td>
<td>0.357</td>
<td>&lt;0.0001</td>
<td>0.862</td>
</tr>
<tr>
<td>BZP</td>
<td>0.973</td>
<td>0.407</td>
<td>&lt;0.0001</td>
<td>0.836</td>
</tr>
<tr>
<td>TRI</td>
<td>0.991</td>
<td>0.406</td>
<td>&lt;0.0001</td>
<td>0.714</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.924</td>
<td>0.157</td>
<td>&lt;0.0001</td>
<td>0.198</td>
</tr>
</tbody>
</table>

This indicates that the range of compounds did not have a significant impact on the ability of TF-SPME to assess the bioavailability of compounds. These findings also indicate that TF-SPME can be used in a wide range of soils in the field, which may contain extremely low or high levels of the four compounds.

Calculation of interstitial pore-water concentrations of mixtures of compounds in field soils

The equilibrium concentrations of BPA, BZP, TRI, and DEHP in PDMS exposed to each field soil were measured using TF-SPME, and interstitial pore-water concentrations of each compound were calculated as described previously. No measurable levels of any of the compounds were detected in either of the un-spiked field soils.
For each field soil, the interstitial pore-water concentrations of each compound were plotted against the soil concentrations of each compound. Linear equations were generated to relate the pore-water concentrations with soil concentrations for each compound in each field soil. Pore-water concentrations generated by TF-SPME experiments increased linearly with soil concentrations in both field soils (FIGURE 4, FIGURE 5).

Figure 4. Comparison of concentrations of A. BPA, B. BZP, C. DEHP, and D. TRI in spiked Arkport soil with soil pore-water concentrations calculated using TF-SPME. Error bars show one standard deviation.
Figure 5. Comparison of concentrations of A. BPA, B. BZP, C. DEHP, and D. TRI in spiked Hudson soil with soil pore-water concentrations calculated using TF-SPME. Error bars show one standard deviation.

The average relative standard deviation was <17.5% for all compounds in both field soils. This indicates that TF-SPME can be used to reliably determine pore-water concentrations of BPA, BZP, TRI, and DEHP when those compounds are present in mixtures in field soils of differing properties, a scenario that more accurately reflects field conditions.

Organic carbon content has been shown to be a major predictor of the sorption of endocrine disrupting compounds to soil [35, 36]. The artificial soil used in the TF-SPME experiments has a
calculated organic carbon content of 5.8%; Hudson soil has a measured organic carbon content of 0.85%; and Arkport soil has a measured organic carbon content of 2.19%. Thus, it was hypothesized that the pore-water concentrations measured by TF-SPME would increase as the organic carbon content of the soil decreased, with Hudson soil having the highest pore-water concentrations and artificial soil having the lowest. For each compound, an exponential relationship was generated to relate the organic carbon content of the soil with the previously determined relationships between pore-water concentration and soil concentration (FIGURE 6).

Figure 6. Comparison of the relationships between soil organic carbon content and soil concentration-adjusted pore-water concentrations of BPA, BZP, TRI, and DEHP. A. shows the relationships on a linear scale and B. shows the relationships with a logarithmic scale on the vertical axis.

This exponential relationship represents the change in pore-water concentrations, adjusted for soil concentration, with each percentage change in soil organic carbon content. A general trend was noticed based on the slope of the relationships, in which BZP had the highest slope, followed by BPA, TRI, and DEHP. This trend indicates that the bioavailability of BZP in soil is
the most sensitive to changes in soil organic carbon content. The trend is also inversely related to that of the $K_{oc}$ values of the compounds. As can be seen in Table 2 of Chapter 1, DEHP has the highest $K_{oc}$ value, followed by TRI, BPA, and BZP [34]. These two findings reveal that as the $K_{oc}$ of a compound increases, its dependence on soil organic carbon content for changes in its bioavailability decreases. This is perhaps because compounds with high $K_{oc}$ values are so tightly bound to soils containing organic carbon that any incremental change in organic carbon content would not affect the equilibrium between bound and unbound compound as dramatically as one with less affinity for organic carbon.

Adding organic carbon to soil has been used as a remediation method for decreasing the bioavailability of several compounds [12, 13], and knowledge of the amount of additional organic carbon that would adequately decrease the bioavailability of a compound would be beneficial. The equations generated in FIGURE 6 could be used to identify target points for each compound at which the organic carbon content of the soil is high enough that an increase in soil concentration would result in an increase in pore-water concentration that has been deemed acceptable by regulators. For example, if one wanted to remediate the soil by adding organic carbon such that soil concentration-adjusted pore-water concentrations of all compounds are 50% of what they would be if the organic carbon content in the soil were 0% (i.e. 50% of maximum bioavailability), the soil would need to contain 2.5% organic carbon for BPA, 3.5% for BZP, 2.3% for TRI, and 0.7% for DEHP. Alternatively, if one wanted to remediate the soil such that the pore-water concentrations were 1% of maximum bioavailability, which would be a more radical reduction in bioavailability, the soil would need to contain 16.7% organic carbon for BPA, 23.5% for BZP, 15.2% for TRI, and 4.3% for DEHP. Field soils that have been amended with biosolids or manure have been found to have organic carbon contents of 1.2-2.7%
[4]; amending to achieve a much higher organic carbon percentage in order to decrease bioavailability may prove difficult. Therefore, the bioavailabilities of the four EEDCs tested are likely to exceed 1% of their maximum bioavailability levels in most field soils.

Measurement of earthworm tissue concentrations of mixtures of compounds in field soils

The pore-water concentration of a given compound in soil controls its uptake into earthworm tissue. Therefore, it was hypothesized that the organic carbon contents of Hudson and Arkport field soils would affect the earthworm tissue concentrations of each compound in the same manner as it affected the pore-water concentrations calculated by TF-SPME. Earthworm tissue concentrations of each compound were plotted against the corresponding soil concentration in each field soil. Similar to the pore-water concentrations measured by TF-SPME, earthworm tissue concentrations of each compound increased linearly with soil concentration. Linear equations were generated to relate earthworm tissue concentrations with soil concentration for each soil, and the slopes of each equation were compared with those generated for each compound in artificial soil. Exponential equations were fitted to the data, and as hypothesized, earthworm tissue concentrations of each compound increased as the soil’s organic carbon content decreased (FIGURE 7).
Figure 7. Comparison of the relationships between soil organic carbon content and soil concentration-adjusted earthworm tissue concentrations of BPA, BZP, TRI, and DEHP. **A.** shows the relationships on a linear scale and **B.** shows the relationships with a logarithmic scale on the vertical axis.

The relationship between soil organic carbon content and bioavailability, in this case represented by earthworm tissue concentration, followed the same trend among compounds as was observed in the TF-SPME experiments.

As with the exponential equations describing the interaction between soil concentration-adjusted pore-water concentration and organic carbon content (FIGURE 6), the exponential equations in FIGURE 7 could also be used to determine the organic carbon content that would result in acceptable earthworm tissue concentrations for each compound. In order to remediate the soil by adding organic matter to achieve a soil concentration-adjusted earthworm tissue concentration that is 50% of what it would be if the organic carbon content in the soil were 0%, the organic carbon content would need to be 4.1% for BPA, 0.88% for BZP, 1.3% for TRI, and 1.7% for
DEHP. However, although reduced, the resulting earthworm tissue concentrations may not be sufficiently low to effectively reduce risks to organisms in higher trophic levels. If a much lower soil concentration-adjusted earthworm tissue concentration is desired, such as one that is 1% of its maximum potential level, the organic carbon content would need to be 26.9% for BPA, 5.8% for BZP, 8.9% for TRI, and 11.0% for DEHP. As discussed previously, achieving these organic carbon contents in the process of soil remediation may be difficult in practice.

Conclusions
Upon additional testing of the developed TF-SPME method, the presence of the compounds singularly or as a mixture in artificial soils had no significant impact on the relationship between pore-water concentrations generated by TF-SPME and soil concentrations. The range of concentrations also had no significant impact on the relationship between soil concentration and pore-water concentration when TF-SPME was applied to soils containing concentrations of compounds one order of magnitude higher and lower than the median concentration found by Kinney et al. [10], indicating that TF-SPME can be used on soils containing a wide range of concentrations. Further, the pore-water concentrations generated by TF-SPME increased linearly with soil concentration in both sandy loam and silty clay field soils. This indicates that TF-SPME can also be used to predict general bioavailability of the four EEDCs in field soils when mixtures of the compounds are present. These observations are crucial to the further use of TF-SPME, as they support the efficacy of TF-SPME to predict bioavailability in field conditions.

In addition, the relationships between pore-water concentrations generated by TF-SPME and soil concentrations for each compound reflected the relationship between soil organic matter and sorption of compounds to soil, as has been described by others [35, 36]. The relationships
between the concentrations of each compound in earthworm tissue and soil concentration also depended on the organic carbon content of the soil in a similar fashion as those determined by the TF-SPME experiments. While the compounds tested represent a relatively wide range of chemical properties, further research should test the TF-SPME method with additional compounds before using the method on novel field soils. Finally, the calculated organic carbon contents that would result in an acceptable level of bioavailability, determined either by soil concentration-adjusted pore-water concentrations calculated from TF-SPME experiments or by soil concentration-adjusted earthworm tissue concentrations, may be higher than what can be achieved by amending field soils with organic matter. Therefore, it is likely that BPA, BZP, TRI, and DEHP would be bioavailable in most field conditions, and the environmental consequences of their presence in soil should be assessed.
CHAPTER 4

The Challenges of Risk Communication: A Case Study on the Xenoestrogen, Bisphenol A

INTRODUCTION

Bisphenol A (BPA) has received a tremendous amount of attention by the news media in the past 10 years, due to its ubiquitous presence in plastic products, unusual form of toxicity, and controversy surrounding its exact effects in the human body. This analysis attempts to introduce the case of BPA as a harbinger for a changing risk communication landscape.

The Food and Drug Administration (FDA) can regulate the use of BPA when it is used in packaging or products that contact food and, therefore, can be considered a food additive [37]. The Environmental Protection Agency (EPA) can also regulate the use of BPA, but only when it is used in non-food applications where it is likely to come into contact with the environment, such as in water pipe linings or as a coating for receipt paper [38]. However, simply because the FDA and the EPA have the right to regulate the use of BPA in certain applications, or ban its use outright, does not imply that either governmental agency will take any actions at all.

A 1992 United Nations Conference on Environment and Development affirmed that “In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation [39].” This statement has been used as the definition of the precautionary principle since then, and has been formally adopted by the governments of Canada and the European Union to guide decisions about which compounds to ban [40, 41]. The various
agencies of the United States’ government have not officially embraced this principle, and the decision process is typically based on years of review of published scientific material, initiation of independent scientific studies, testimonies, and attempts to capture the full extent of the risk of the compound to human and ecological health. Due to the extensive nature of this process, an agency’s decision to take a regulatory action is not rapid, and its action may be challenged by lobbying groups and corporations in lawsuits and congressional hearings. In fact, one of the major lobbying groups in the United States, the U.S. Chamber of Commerce, officially “support a science-based approach to risk management, where risk is assessed based on scientifically sound and technically rigorous standards; oppose the domestic and international adoption of the precautionary principle as a basis for regulatory decision making, and to educate consumers, businesses, and federal policymakers about the implications of the precautionary principle [42].”

The actions taken by United States state and local governments and corporations in response to the controversy surrounding the use of BPA, however, are contrary to the usual regulatory process. This analysis will investigate the hypothesis that this dichotomy is a product of the public’s perceived trust and credibility of the government, scientists, and corporations; the influence of the media in the communication of BPA’s risks; and the ability of the public to actively participate in the risk communication process.

**BACKGROUND INFORMATION**

*Origins and Use*

Bisphenol A is commonly used as a building block in shatterproof plastics and epoxy resins. The compound has received significant attention from scientists and public health groups in recent
years due to the discovery of its ability to act like endogenous estrogen in the human body. Its estrogenicity was first discovered in the 1930s during the push to develop synthetic estrogens [43]. However, it was never used for therapeutic purposes due to the subsequent synthesis of the much more estrogenically potent diethylstilbestrol [44]. In the early 1950s, BPA was instead used in the creation of epoxy resins [45]. In 1957, it was found that BPA could be polymerized to form polycarbonate plastic, and has since been used in that capacity in shatterproof water bottles, toys, electronics, food containers, and water pipe linings, and has also been used as a coating for receipt paper [45].

Routes of Exposure and Metabolism

In 1993, Krishnan et al. found that BPA was able to leach out of polycarbonate materials under intense heat conditions [46]. Subsequent studies have found that BPA leaches from can linings and hard plastic bottles to which humans are exposed [47-49]. Thus, the main route of exposure of BPA to humans is thought to be via ingestion of the leached compound in beverages and foodstuffs [50]. Dermal absorption has been recently identified as a new route of exposure, after it was found that BPA can be absorbed through skin exposed to receipts made from thermal paper coated with BPA, which allows the receipts to be printed with the use of heat rather than ink [2]. Once in the bloodstream, BPA is conjugated by liver enzymes to make it more water-soluble and able to be rapidly excreted by the kidneys. The half-life for BPA in humans is 6 hours, and BPA is excreted in the urine [51]. However, before its excretion, it is believed to be able to bind to estrogen receptors throughout the body [52].
Endocrine-Disrupting Properties

Bisphenol A can be classified as an estrogen-like endocrine disrupting compound (EEDC), one which is able to bind to estrogen receptors and elicit the production of mRNA and proteins in the same manner as endogenous estrogen [52]. The potency of each EEDC can be determined by quantifying how well the EEDC binds to estrogen receptors, which is typically compared to how well endogenous estrogen (estradiol) binds to the receptors. Typical EEDCs have a much lower potency than endogenous estrogen, and BPA is 10,000-100,000 times less potent than estrogen [53]. However, it is important to note that endogenous estrogen levels in the human body are tightly regulated and respond to various feedback systems. The available blood concentrations of estradiol in adult, non-menopausal women range from 0.5-33 pmol/L in a given menstrual cycle [54]. BPA has been found in blood at levels of 0.2-20 ng/mL serum [55]. Therefore, although BPA is much less potent than endogenous estrogen, its high concentrations may allow for a fair amount of estrogen receptor binding. Further, proteins in the blood bind to estradiol, controlling its bioavailability. BPA does not bind as well to these proteins, making it more available for binding to estrogen receptors [56].

Effects of BPA: Experimental Data

The effects of exposure to BPA have not been completely elucidated, and considerable controversy exists concerning the magnitude of these effects [57]. Much of the current scientific knowledge concerning its effects has come from laboratory studies involving mice or rats. These animals are more manageable subjects than humans (for time, monetary, and ethical reasons), and are considered to be an acceptable proxy for humans. There are numerous studies detailing the effects of exposure to BPA in mice and rats, some of which have found associations between
exposure to BPA and developmental effects, changes in mammary gland tissue, and errors during meiosis [58-60]. Data from human studies are more difficult to obtain, and most human studies involve surveys of concentrations of BPA in blood or urine [50, 61]. Several epidemiological studies have been published in recent years and have linked exposure to BPA with problems in boys’ reproductive development and girls’ behavioral development [62, 63]. There is a purported link between exposure to BPA and an increased risk for breast cancer; this link is based mainly on the mechanisms of breast cancer development and has not been supported with epidemiological data [64, 65].

Challenges Facing Risk Assessment

BPA is not a traditional toxin in the sense that it produces subtle, chronic effects and does not follow a linear dose-response curve. Its U-shaped dose-response curve indicates that low doses of BPA may elicit stronger effects than high doses [53]. Thus, it is difficult to design laboratory studies that are able to capture the range of effects of BPA at various doses, and it is more difficult to extrapolate the doses used in mice and rat studies to humans. As many of the effects are related to childhood development or are chronic and only later manifest as cancer, it is also difficult to correlate observed effects with exposure to BPA using epidemiological studies. Further, the effects of BPA may also be different depending on the life stage and gender of the exposed person [62, 63]. Finally, BPA is not the only estrogenic compound to which humans are exposed. Estrogenic compounds can interact as mixtures in a manner known as compound addition [66]. In this sense, small doses of multiple compounds can interact to form a large estrogenic dose. All of these factors challenge the development of appropriate experiments to determine the range of BPA’s effects.
Further, the complex mechanisms of action of BPA make it difficult for risk assessors to understand and characterize the risks, for risk communicators to communicate the risks, and for lay people to understand the risks involved with exposure to BPA and how best to minimize those risks. Risk assessments of BPA have focused on BPA’s overt toxicity rather than its hormonal effects [67]. This is due in part to the lack of sensitive tests to precisely detect and understand effects in the wide range of doses to which humans are exposed. Such tests are needed to determine the lowest observed adverse effect levels (LOAEL) and no observable adverse effect levels (NOAEL) required for regulation of BPA use [68]. While these metrics may not capture the entirety of BPA’s complex mode of action, the creation of these levels is a first step in the regulation process.

**Public Perceptions and Media Coverage**

While BPA’s estrogenic properties have been discussed in scientific papers for several decades, Patricia Hunt is one of the first scientists to cross the boundary between strict academic science and activism. In 1998, Hunt was a principal investigator at a laboratory at Case Western Reserve investigating the reproductive system in mice. When Hunt and colleagues discovered that their data were askew, they found that it was likely caused by the leaching of BPA from the plastic water bottles and cages to which mice were exposed [69]. Since that incident, Hunt, along with Frederick vom Saal, a professor at University of Missouri-Columbia, have published several papers discussing the estrogenicity of BPA and the effects of exposure to BPA in mice [53, 60, 63]. Their work, along with the work of others, has been picked up by various media outlets, including the New York Times, Scientific American, Science Magazine, and blogs.
CASE ANALYSIS

The public’s reactions to the scientific evidence of BPA’s estrogenicity, to the lobbying efforts by concerned individuals against government agencies and corporations, and to the pushback by the government and by lobbies for chemical corporations can be described by three theories in the field of risk communication. First, that trust and credibility play a significant role throughout the risk communication process and reflect trust in government agencies, scientists, physicians, and corporations. Second, that the extent of media coverage of the risks may play a role, particularly through the lens of print and web media. Finally, that the extent to which public participation in corporate and legislative lobbying efforts modulates risk perceptions is deserving of closer examination.

Trust and Credibility

One of the main issues surrounding the public’s perception of the risks of BPA is that while businesses and the FDA have maintained that BPA is safe as used [37], members of the scientific community have published papers suggesting otherwise [58-60, 62, 63]. Therefore, the public’s perception of the risks depends greatly on whether the public trusts businesses and government agencies, or they trust academic scientists on matters of safety and health. According to Trumbo and McComas, the trust that the public bestows on these groups is most likely not interpersonal trust, also known as credibility, but rather social trust, which encompasses the “complex social processes by which people make choices and assign management responsibilities to individuals, groups, or organizations” [70]. Unlike interpersonal trust, social trust does not need to rely on the credentials of a given business, agency, or scientist,
or the soundness of the scientific research performed. The public would likely find it difficult to assess the credibility of each of the scientists and regulators responsible for each research project on BPA, and would likely rely on the more general process of assigning trust to organizations. In this way, social trust is based on perceptions of trustworthiness, prior history with the groups, and in many cases, the ability of the groups to effectively communicate with them.

Brewer and Ley found that levels of trust in businesses and trust in the FDA were negatively associated with support for a ban on the use of BPA [71]. Confidence in scientists was positively associated with support for a ban, which Brewer and Ley hypothesized was a product of the inclusion of information in news reports that the concern surrounding BPA was due to scientific studies. In an additional study, Brewer and Ley found that when individuals were confronted with the statement that current scientific evidence is inadequate to determine whether BPA is harmful to humans, support for a ban on BPA decreased [72]. Support for a ban increased when individuals were exposed to the opposite statement. This may indicate that while trust in scientists can be correlated with support for a ban, when those scientists fail to provide absolute evidence of BPA’s harm, the public’s response is to assume that failure to provide evidence implies lack of harm.

Considerable uncertainty exists surrounding the exact effects of exposure to BPA, and Maxim and Mansier discuss the implications of the communication of this uncertainty [73]. Contrary to the findings of Brewer and Ley, Maxim and Mansier argue that many scientists incorrectly assume that communicating uncertainty negatively affects the public’s trust in science, specifically in endocrine disruptor toxicology. Their findings suggest that the public accepts the role of uncertainty in academic scientific efforts, and the communication of this uncertainty has no impact on the credibility of academic scientists. However, the communication of scientific
uncertainty does have a negative impact on the public’s perception of the credibility of industry scientists, who are already perceived as having low credibility due to their potential for having conflicts of interest.

The contrary findings of Maxim and Mansier and Brewer and Ley may indicate a disconnect between the public’s perception of an organization’s credibility and the policy implications that result from the organization’s findings. This is a concept discussed by Collins, who asserts that scientists have a responsibility to actively engage in the public debate of scientific findings, particularly on the topic of endocrine disrupting compounds [74]. This includes interpreting the findings for a wide audience and suggesting appropriate remedies, including policy actions.

**Media Coverage of BPA**

The second of the three theories considers media coverage. As discussed previously, BPA has received significant media attention since 2006. A Lexis Nexis search by Brewer and Ley revealed that there was only one story about BPA during evening news broadcasts in 2007, but 14 stories the following year [71]. Similarly, the *New York Times* only ran one story in 2006 and 2 in 2007, but 29 in 2008 [71]. The jump in number of stories correlates well with a Google Trend web search analysis of searches for “BPA” or “Bisphenol A” between 2003 and present day. The highest peak in web searches occurred in 2008, when Nalgene decided to remove BPA from its bottles, and searches for information on the compound since then have spiked as a result of reports of municipal bans on BPA, reports on new research concerning the effects of BPA, and reports of actions taken by the United States government. These web searches can be considered reactions to news stories, implying that the public’s interest in and desire to be more educated on the topic.
Brewer and Ley found that newspaper use predicted whether an individual was familiar with BPA and had ideas of how to reduce exposure to the compound. Television use did not, likely due to the comparably lower coverage of BPA stories in evening news versus print news [71]. Increased newspaper coverage of BPA between 2007 and 2008, combined with increased web searches for additional information on the compound, may be responsible for the public movements to ban the use of BPA in certain products that began in 2007.

Public Participation in BPA Risk Communication

The third theory relates to public participation in the risk communication of BPA. From 2007 to early 2008, three major outdoor retailers pulled BPA-containing polycarbonate plastic bottles from its shelves. In two of the cases, the retailers replaced bottles made by Nalgene Outdoor Products, the largest producer of polycarbonate plastics for laboratory, home, and outdoor use, with BPA-free products that Nalgene had developed. After Health Canada’s decision in 2008 to classify BPA as toxic and ban the use of BPA in all baby products, Nalgene Outdoor Products announced that it would stop using BPA in any of its plastics. While Nalgene maintained that its products were safe, it stated that its customers preferred not to use plastics containing BPA. Nalgene’s action also impelled major retailers in Canada, such as Wal-Mart, to remove BPA-containing plastic products used for food from their stores [75]. Canada supports the use of the precautionary principle to guide decision making [40]. Therefore, Health Canada’s action to ban the use of BPA in children’s products, as well as the actions of retailers in Canada, is not out of the ordinary.

The United States does not officially follow the precautionary principle, however, which makes the U.S. public’s reaction to BPA striking. Despite a report by the U.S. National Toxicology
Program in 2008, which indicated that the program had “some concern” about BPA’s effects on the development of the brain and prostate gland, as well as behavioral effects, in fetuses and children, the FDA decided not to ban BPA at that time. In 2009, a bill titled the Ban Poisonous Additives Act of 2009 was introduced in Congress, but was not enacted, nor was it enacted in 2011 when it was reintroduced [76]. In 2010, the FDA reported on concern about BPA’s health effects in children. It recommended “reasonable steps to reduce human exposure to BPA” but did not recommend the imposition of a ban on its use [37]. These actions fall in line with the federal government’s stance on the precautionary principle and their reading of the existing research on BPA’s effects.

Perhaps in response to the inactivity of the federal government on decisions regarding BPA, citizens encouraged state and local governments and corporations to ban the use of BPA, despite the lack of complete scientific evidence. In 2009, one year after Health Canada’s decision to ban BPA in baby products and FDA’s decision not to, the six largest producers of baby products decided that they would no longer sell baby bottles made with BPA in the United States after a request by the attorneys general of Connecticut and New Jersey to cease the sale of these products [77]. Also in 2009, Suffolk County, New York, became the first county in the United States to ban the sale of BPA-containing baby products. Minnesota and Chicago followed suit soon after, and Connecticut became the first state to ban the sale of baby food and infant formula containers containing BPA and also banned any reusable food or beverage container that contains BPA. By 2011, twenty-six states had introduced bills to ban BPA, though only eleven states successfully banned BPA in baby products as of 2012. Several groups, including the President’s Cancer Panel and the American Medical Association, urged a ban of BPA-containing baby products, with the President’s Cancer Panel stating that "because of the long latency period
of many cancers, the available evidence argues for a precautionary approach to these diverse chemicals, which include (...) bisphenol A” in its 2008-2009 annual report [78, 79]. All of the support for these bans took place despite the fact that scientific consensus on the exact effects and magnitude of those effects of BPA at various doses had not yet been achieved. The bans were so effective at influencing the public’s view of the safety of BPA-containing products that even the American Chemistry Council, the main lobbying organization that represents major chemical companies, including those that manufacture BPA, appealed to the FDA to ask for a ban on BPA-containing baby bottles in order to counteract the decline in consumer confidence in all BPA-containing products. The FDA banned the use of BPA in baby bottles and cups for toddlers in 2012, but stated that its decision was not based on safety concerns. Instead, it simply followed the growing trend in industry to avoid using BPA in baby products [80]. In response to the discovery of BPA in thermal receipt paper and the ability of BPA to be transdermally absorbed, bills banning the use of BPA in paper have been proposed and successfully passed in state legislatures, and the EPA has issued a draft report assessing the alternatives to BPA in thermal paper [38, 81, 82]. The corporate and governmental response to the controversy surrounding BPA has been a combination of corporate responsibility actions, public participation, and consumer purchasing power. Many of the bans enacted in states were a result of grassroots movements by citizens [83, 84]. Despite the federal government’s stance on the precautionary principle and their need to review extensive literature on a compound’s effects before making an official decision on a compound, the public generally relies on “intuitive toxicology” to guide their views on a compound [85]. Neil et al. have found that the public’s perception of a risk posed by a chemical is less dependent on dose and exposure than are perceptions of toxicologists [85]. Similarly, the
public finds any risk posed by chemical residues present in food to be unacceptable. These views, combined with an increased ability to generate grassroots movement, possibly due to the rise of social media and the accessibility of information on the internet, have made the shunning of BPA-containing products or legislative bans of BPA-containing baby products without scientific consensus possible. While the economics of banning BPA is beyond the scope of this discussion, it is also important to note that since many corporations had already moved toward replacing BPA with BPA-alternatives in many of their baby products as discussed previously, the monetary costs to states to ban BPA in baby products were likely low.

Arvai et al. have found that risk-policy decisions are more palatable by the public when the public is told that public input was sought during the decision-making process [86]. Perhaps in response to this finding, the FDA, several lobbying groups, and the state of California have emphasized the importance of public participation and public comments to proposed actions taken to minimize the risks of BPA to the public [37, 87-89].

LESSONS LEARNED

Bisphenol A is not the only endocrine disrupting compound to which the public is exposed, and BPA is not unique with respect to the lack of scientific information about its effects. Therefore, lessons should be taken from the case of BPA in order to effectively manage the risks and public communication of those risks of similar endocrine disrupting compounds. Credibility plays an important role in the acceptance of risk messages, and it would be prudent for government agencies to realize that the public’s trust of their messages depends on how forthcoming the messages are at addressing uncertainty in science and who performed the scientific studies [71].
As discussed above, the media plays an important role in providing citizens with information about BPA as well as increasing perceived efficacy in reducing exposure to the compound. With the advent of the internet, there are now multiple channels of information through which citizens can become informed. The spikes in Google searches between 2003 and the present day that seem to correlate with the BPA news stories, showing indicate that the public is willing and able to further educate themselves on an issue using the internet. Brewer and Ley only tested the connection between newspaper use and television use and familiarity with BPA. However, risk assessors should realize that the public is exposed not only to theoretically impartial newspaper and television coverage of events surrounding BPA, but also more unregulated channels of information about the compound, such as blogs and news sites with targeted audiences. Communication on BPA risks need to address the science and risks and also the potential misconceptions surrounding BPA to which people are exposed. Further, when assessing risk messages, the public considers how much public input has been solicited or will be solicited, and whether or not the government is willing to make a decision without all of the scientific facts in place [86]. Federal government agencies have been historically unwilling to promote a risk message before conclusive scientific evidence of harm has been demonstrated, due to their non-adherence to the precautionary principle. However, in the case of BPA, public impatience for the processes required to prove harm or lack of harm led to public demands of the removal of BPA from products through statewide bans and corporate responsibility-related actions by companies. It would be wise for government agencies to take note of the public’s reaction to BPA, as the reaction directly conflicts with the government’s negative stance on the precautionary principle. With greater empowerment by the public through grassroots movements and the influence of
consumer purchasing power on company behaviors, the disregard for the government’s rejection of the precautionary principle in practice may become more common. Government agencies should be aware that this public empowerment, combined with the concept of “intuitive toxicology,” may result in more calls by the public to ban or limit the use of certain compounds. Companies should also be aware that the public’s ability to incite change may influence how they will need to introduce products to the public and communicate risks. The American Bar Association has already taken note of the changing role of the public in risk communication, specifically in the realm of liability for product safety [90].

Finally, scientists, the public, and government agencies should be aware of the call to scientists to more actively participate in the formation of risk messages stemming from scientific research [74]. While this new persuasive risk communication may be beneficial to those attempting to understand the full ramifications of a scientific discovery, care must be taken, as the trust and credibility that the public bestows on scientists should not be taken lightly. Scientists are generally trusted more than any other source of scientific communication [91], but as Slovic states, the management of risks is typically done “within an adversarial legal system that pits expert vs. expert, contradicting each other’s risk assessments and further destroying the public trust” [92]. Scientists, the public, government agencies, and corporations must all be aware of the changing landscape of risk communication that the case of BPA signifies, and may have to adjust their ways of performing scientific studies, risk assessments, communicating risks, and taking action to conform to these changes.
A novel method involving thin-film solid phase microextraction was developed and used to quantify the bioavailability of several estrogen-like endocrine disrupting compounds. The method measured equilibrium concentrations of BPA, DEHP, TRI, and BZP in artificial soil pore-water without the use of solvents and within a total of 88 minutes. The method can be used to assess the general bioavailability of the compounds in soil. The method has potential for predicting equilibrium earthworm tissue concentrations of the compounds, but would require the use of chemical-specific bioaccumulation factors due to the suspected metabolism of the compounds by the earthworms or their flora. Further research on the metabolism of compounds by earthworms should be performed. Further, when tested with mixtures of compounds in artificial soil, the TF-SPME method was still able to quantify the bioavailability of the compounds. The method was also applied to the four compounds as mixtures in artificial soils in a concentration range that spanned several orders of magnitude, and again was able to be used quantify the bioavailability of the compounds. Finally, in order to more accurately reflect field conditions, the method was applied to mixtures of the four compounds in field soils of differing soil properties and was able to be used to quantify the bioavailability of each compound. Further, analysis of the resulting bioavailability estimates of each compound in the field soils revealed that the practice of remediating field soils with organic carbon in order to decrease bioavailability may not be an effective strategy, as impractically high amounts of organic carbon would need to be added to decrease the bioavailability to a near negligible level. Instead, on a holistic level, it may be wise
to decrease the concentration of EEDCs in biosolids through degradation of the compounds during the waste water treatment process, or to eliminate the practice of applying biosolids to land in order to decrease the potential for future uptake of the compounds by soil organisms. Further research should be performed in which the method is applied to soils containing additional EEDCs to the ones tested in this research. However, overall the method shows promise for use to assess the bioavailability of EEDCs and aid in the development of risk estimates related to their presence in the soil environment in field conditions.

Once the risks of the exposure of soil organisms, humans, or other organisms to EEDCs have been quantified and risk assessments are made, it is crucial that the communication of those risks to the public is clear and effective. Several lessons can be learned from the case of bisphenol A that can be used in future risk management and public communication of risks of other EEDCs. The credibility of the source of risk messages, the potential for public input, and the role of various types of media as an information source all play important parts in the effectiveness of risk communication efforts. Given the public’s intense response to the controversy surrounding bisphenol A, it is likely that a similar situation will soon occur with other EEDCs to which the public is currently exposed. Therefore, it is important to understand the successes and failures of the strategies of the communication of risk of bisphenol A, as this understanding will undoubtedly prove useful in the development of risk communication strategies for additional EEDCs. A working knowledge of effective risk communication strategies, combined with a method that can be used to quantify bioavailability, will be crucial for navigating the current and future risk landscape.
APPENDIX

Determination of TF-SPME method conditions in water

BPA Response surface figure of extraction time

BZP Response surface figure of desorption temperature

Design-Expert® Software

Kf

63.316
0

X1 = B: Extraction Time
X2 = D: Desorption Temp

Actual Factors:
A: Conc. = 0.20
C: Desorption Time = 16.1
TRI Response surface figure for extraction time

Design-Expert?Software

\[ \log K_f \]

3.10698
0.339311

X1 = A: Conc
X2 = B: Ex. Time

Actual Factors
C: Des. Temp = 265.00
D: Des. Time = 10.00

---

DEHP Response surface figure for extraction time

Design-Expert Software

\[ \log K_f \]

1.2189
0.0

X1 = A: DEHP Conc.
X2 = B: Extraction time

Actual Factors
C: Desorption temp. = 32
D: Desorption time = 15.1

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73
**Determination of TF-SPME method conditions in soil**

BPA Response surface figure of pre-extraction stirring time

![BPA Response surface figure of pre-extraction stirring time](image)

X1 = B: Stirring time  
X2 = C: Extraction time

Actual Factor  
A: BPA Concentration = 0

BZP Response surface figure of pre-extraction stirring time

![BZP Response surface figure of pre-extraction stirring time](image)

X1 = B: Stirring time  
X2 = C: Extraction time

Actual Factor  
A: BZP Concentration = 0
TRI Response surface figure of pre-extraction stirring time

Design-Expert?Software
Kf
1.53825
0.0602802
X1 = A: Soil Concentration
X2 = B: Stir Time
Actual Factor
C: Extraction Time = 68.1

DEHP Response surface figure of pre-stirring extraction time

Design-Expert?Software
PDMS conc
2.90015
0.436931
X1 = B: Stirring time pre-addition of PDMS
X2 = C: PDMS extraction time
Actual Factor
A: DEHP Conc. = 4.00

B: Stirring time pre-addition of PDMS
Determination of time required for uptake of compounds by earthworms

BPA Response surface figure of exposure time

Design-Expert® Software

Lipid Kf

171.38
117.68

X1 = B: Exposure time
X2 = A: Soil Concentration

BZP Response surface figure of exposure time

Design-Expert® Software

log Kf

1.84409
1.16554

X1 = A: Soil Concentration
X2 = B: Exposure time
TRI Response surface figure of exposure time

Design-Expert Software

log Kf

1.2742

0.582961

X1 = A: Soil concentration
X2 = B: Exposure time

DEHP Response surface figure of exposure time

Design-Expert Software

log Kf

1.128

-0.309159

X1 = A: Soil concentration
X2 = B: Exposure time
REFERENCES


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81. Maisel A. 2013. An act to amend the environmental conservation law, in relation to prohibiting the sale of thermal receipt paper containing bisphenol A. A1654.


83. Wright PB. Maine groups press for BPA ban after chemical found in baby and toddler food. *The Maine Public Broadcasting Network*.


88. American Association of Family and Consumer Sciences. Human Exposure to Bisphenol A.

89. California Office of Environmental Health Hazard Assessment. 2013. Proposition 65: Amendment to Section 25805 Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity - BPA. .

90. Taggart J. The CPSIA and social media make product issues public. *Litigation News*. 84