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Measuring and Modeling of Plant Root Uptake of Organic Chemicals

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MEASURING AND MODELING OF PLANT ROOT UPTAKE
OF ORGANIC CHEMICALS

by

Erik Dettenmaier

A dissertation submitted in partial fulfillment
of the requirements for the degree
of
DOCTOR OF PHILOSOPHY
in
Civil and Environmental Engineering

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ABSTRACT

Measuring and Modeling of Plant Root Uptake of Organic Chemicals

by

Erik Dettenmaier, Doctor of Philosophy

Utah State University, 2008

Determining the root uptake of xenobiotic organic chemicals into plants is critical for assessing the human and ecological health risks associated with the consumption of plants growing in contaminated environments. Root uptake of xenobiotic organics occurs passively in conjunction with transpiration and the transport from root to shoot is ultimately controlled by passage through one or more lipid root membranes. The transpiration stream concentration factor (TSCF), the ratio between the concentration of a chemical in the xylem to that in the solution used by the roots, is used to describe the relative ability of an organic chemical to be passively transported from root to shoot. However, relatively few experimental TSCF values exist due to the cost and the lack of regulatory requirements for generating such data. Where literature data exist for chemicals having more than one TSCF, the variability is often large due to the lack of standardized methods and difficulty in accounting for metabolism and volatilization losses occurring during the uptake experiments. Because of the scarcity of experimental
values, estimated TSCFs are often used. Widely cited estimation approaches relating TSCF and the logarithm octanol/water partition coefficient (log $K_{OW}$) suggest that only compounds that are in the intermediate lipophilicity range (log $K_{OW} = 2$) will be taken up and translocated by plants. However, recent data for highly water soluble compounds such as 1,4-dioxane, MTBE, and sulfolane suggest that these estimation techniques should be critically reviewed. To re-evaluate the relationship between TSCF and log $K_{ow}$, TSCFs were measured for 25 organic chemicals ranging in log KOW from -0.8 to 5 using an improved pressure chamber technique. The technique provides an approach for efficiently generating consistent plant uptake data. By using this data, a new mass transfer model relating TSCF and log $K_{OW}$ was developed that indicates that neutral, polar organic compounds are most likely taken up by plant roots and translocated to shoot tissue. An extensive review of literature TSCF studies supports the updated model.

(201 pages)
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Erik Dettenmaier
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CHAPTER 1
INTRODUCTION

Determining chemical contaminant uptake by plant roots and subsequent translocation into above ground tissue is critical for assessing remediation options such as phytoremediation and monitored natural attenuation (MNA), and for assessing potential human and ecological health risks. The use of plants tissue concentrations to help identify soil and groundwater contamination is another possible benefit of understanding of plant uptake potential.

For most organic contaminants, plant uptake via the roots is passive, with chemicals moving into the plant along with the water used for transpiration. This implies that the longer the exposure and the greater the amount of water transpired the more contaminant that will be taken up by the plant. The concentration of contaminant in the above ground plant tissue may or may not actually increase over time depending on the rate of such processes as metabolism, volatilization, and growth dilution that all act to reduce a contaminates concentration in the plant.

Two descriptors, transpiration stream concentration factor (TSCF) and soil plant bioconcentration factor (BCF), have been used to quantitatively describe the relationship between plant tissue and exposure concentrations. The preferred descriptor often depends on the type of environment in which the plant uptake experiment was conducted. TSCF and BCF values are typically used as constants for a particular chemical but the validity of this assumption has not been rigorously investigated.
The Transpiration Stream Concentration Factor (TSCF) is a ratio of the contaminant concentration in the xylem sap to that in the root-zone hydroponic or soil solution, and has been widely used as a descriptor of chemical uptake by roots (1, 2). Chemicals actively taken up by plants have TSCF values greater than 1.0 (N, P, and K), while those that move into plants at the same rate as water have TSCF values of 1. Interactions with the lipid bi-layer in root membranes reduce the uptake of organic chemicals relative to water resulting in TSCF values of less than 1, even though the mechanism is passive.

TSCF values have typically been measured using one of two general approaches. In the first approach, plants are exposed to a constant root-zone concentration of the chemical of interest. A hydroponic environment is often used because exposure concentrations are more easily measured and controlled this way. Since direct collection of xylem, the transpiration water moving from root to shoot, is difficult for most intact plants, TSCFs are generally calculated from measured shoot concentrations normalized to the amount of water transpired during the exposure period (i.e., concentration in xylem sap is equal to the total mass of compound in shoots divided by the volume of water transpired during exposure). This assumes there is no loss of the chemical due to metabolism or volatilization once it reaches the shoot tissue, that the distribution of the chemical is uniform within the measured tissue, and that the plant is exposed to a constant root-zone concentration. The second approach involves placing the roots of a detopped plant (above ground tissue removed just below the lowest leaves) in a pressurized chamber containing a solution of a known concentration of the chemical of interest. The xylem is forced through the roots as the chamber is pressurized and is
collected and analyzed as it exits the cut stem. Because a plant in a pressure chamber is experiencing a compound for the first time, the concentration in the xylem begins to rise over time as water is forced through the plant. Once the xylem concentration has reached a steady state value, a TSCF is calculated from the ratio of the xylem to root exposure concentrations. In either case, the TSCF value is used as input to models for predicting contaminant movement and distribution into above ground tissue.

In soil exposure systems, whether performed in the laboratory or measured under field conditions, it is generally easier to measure the concentration of chemical in the soil surrounding the plant than the chemical concentration in the water taken up by the plant. Thus, uptake is typically described by a simple ratio of the chemical concentration in the above ground plant tissue divided by the concentration in the soil, yielding a BCF. The use of a BCF does not directly take into account the amount of water transpired, the size of the plant, or the length of exposure.

The relationship between TSCF and BCF has not been adequately evaluated although they should be closely related if both are determined for the same plant under the same exposure conditions and loss mechanisms are properly accounted for.

Relatively few experimental TSCF and BCF values exist, due in part to the high cost associated with their determination and the lack of regulatory requirements for such data. In addition, most experimental data have been generated for a small number of related chemicals. Where data exist for chemicals having more than one literature value, the variability can be quite large due to the variety of experimental methods (hydroponics, pressure chamber, soil) that have been used to measure the values. Key variables
include: the plant growth environment (soil or water), static or flow through exposure system, length of exposure, species of plant and age of plant. There are no accepted standard methods for conducting plant uptake studies.

Because of the scarcity of measured TSCF and BCFs, estimated values from quantitative structure activity relationships (QSAR) are widely used in risk assessment applications. The most widely used estimation techniques are based on correlating the uptake descriptor with the log octanol/water partition coefficient (log $K_{OW}$ or log $P$) of the chemical of interest. Lipophilicity was originally chosen as it is the physical property thought to be the most important factor related to media partitioning and membrane translocation of organic compounds (3). Gaussian curves relating TSCF to log $K_{OW}$ proposed by Briggs et al and others (2, 4), suggest an optimal lipophilicity for uptake and translocation and infer that compounds that are either highly polar (log $K_{OW} < 1$) or are highly lipophilic (log $K_{OW} > 4$) will not be significantly taken up by plants. In contrast, the widely cited log linear relationship between log BCF and log $K_{OW}$, reported in the literature review conducted by Travis and Arms (5) indicates that highly polar compounds have the highest propensity to bioconcentrate. In addition, the relatively high uptake observed in recent laboratory and field studies for non-ionizable, highly water-soluble organic compounds such as sulfolane, (6), 1,4 dioxane (7), and MTBE (8) suggest that the appropriateness of the bell-shaped TSCF vs log $K_{OW}$ curves should be reevaluated.

Despite the seemingly inconsistent outcomes, especially for compounds having low log $K_{OW}$ values, both estimated TSCF and BCF values are used in risk assessment depending
on the availability of data. If exposure solution data are known or can be estimated, a TSCF value is often used. If soil concentrations are available, an estimated BCF value can be used; however, there is no method for normalizing a BCF to the duration of a plant’s exposure to the contaminant.
Plant Root Uptake of Xenobiotic Organics

Plant uptake of chemicals via the roots can generally be divided into passive or active mechanisms. In passive transport, uptake is purely driven by entropic processes and requires no energy expenditure by the plant (9). Active transport mechanisms require that the plant expend energy. The most significant examples of active transport are proton pumps and redox gradients where specific substrates such as nitrate are moved across a membrane by way of specific protein channels linked to an energy source (9, 10).

Without evolutionary exposure to xenobiotic organic compounds, it is assumed that root uptake and subsequent translocation is passive. There is speculation that a class of plant hormone (auxin) mimicking compounds such as 2, 4-dichlorophenoxyacetic acid (2, 4-D) may exhibit minimal tendency to transport actively (11) however there is no theoretical basis and scant evidence for this premise.

Uptake Root Uptake Mechanisms

Uptake and transfer to and throughout a plant is driven by advective and diffusive mass transport, and exudation. The mass transport mechanism is a simple combination of moving with and spreading throughout the water the plant is accessing for transpiration. As soil water is drawn-up the plant by way of transpiration, dissolved chemicals that are associated with that soil water are also drawn up. In its simplest form the accumulated concentration can by described by Equation (1) (1, 12).
Exudation is a process by which a plant releases an organic compound that acts as a chelating agent. The chelating agent acts on a metal-based compound in the soil by complexing with a specific element in the soil, and in so doing, both cleaving the soil-nutrient bond and stabilizing the compound (10). This consequently increases the compound solubility and mobility in the soil matrix. An important exudation example is phytosiderophores in iron acquisition (9). Exudation, though not completely free of energy expenditures by the plant, is still more the less considered passive transport because the exudates themselves act as catalysts and are recycled in the system. Despite the important role exudation can play in metal and nutrient uptake, it is expected that it will have minimal impact, as there is no charge stabilization on neutral organic compounds.

**Root Membrane Permeation**

Ultimately, the uptake of all compounds by plants is controlled by their diffusion across a lipid bilayer membrane. In plants, these membranes regulate transport both on an individual cell basis, by way of plasma membranes between the cell wall and cytoplasm, and organism wide by way of a heavy wax strip which bands the endodermis known as the Casparian strip (10). When a substrate is laterally transported into a plant from the soil to the xylem, it can be transported by three separate pathways. In the first pathway, the solute must pass through the cell wall and plasma membrane, then into the cell cytosol. From the cytosol it is transported across the cell and out of the plasma
membrane and into an adjacent cell wall. The solute then crosses another plasma
membrane and finally into the next cell’s cytosol. In this way the solute is repeatedly
leapfrogging from inside one cell to the next through a chain of cells that connect to the
Casparian strip (Pathway 1, Figure 2-1) (10, 12, 13). The second form of transport is
analogous to cell wall surfing. The solute diffuses into a cell wall but before it passes
through the plasma membrane and into the cell it migrates into an adjoining cell wall.
The solute never passes through the membrane and into a cell until reaching the
Casparian strip. Upon reaching the casparian strip it either diffuses through the heavy
wax strip, is forced through a plasma membrane and into a cell or is inhibited from
entering the xylem and the rest of the plant (Pathway 2, Figure 2-1) (10, 12, 13). The
final form of transport occurs when a solute passes through the cell wall and into the
cytosol, where the solute then moves from cell to cell by way of interlinking
plasmodesmata (tunnels between cells) before passing through the endodermal cells
encompassed by the Casparian strip (Pathway 3, Figure 2-1) (10, 12, 13). In all cases,
transport is limited by the diffusion of a compound across at least one plasma membrane.

Transport across a plasma membrane can either occur by diffusion through the lipid
bilayer or through specialized transmembrane proteins spanning the width of the
membrane. To prevent cellular leakage, transmembrane proteins have evolved to be very
specific in their mode of action, thus limiting the passage of all but their target
compounds. The greatest example of a well-gated and selective transmembrane protein
class is the aquaporin. The aquaporin can passively transport at rates 20 times faster than
any other known protein (14). The aquaporin has evolved to transport water and most
aquaporins are known to do so exclusively. There are however, some specific animal versions which have recently been discovered to transport chemical and structural homologues of water such as urea, ethylene glycol, and glycerol (14). With most neutral organic compounds moving into plants passively while sharing few chemical and structural similarities with water, their uptake into plants is expected to be rate limited by diffusion across lipid bilayers and not through protein channels (Figure 2-2).

Diffusion gradient transport between two phases, or transport across a semi-permeable membrane can be described by a modified form of Fick’s Law (Equation 2).

\[
J = \frac{A}{t} \frac{dC}{dx}
\]  

(2)

Where:

\[
J = \text{Flux (mass length}^{-2} \text{ time}^{-1})
\]
\[
A = \text{Area (length}^2\text{)}
\]
\[
t = \text{Time (time)}
\]
\[
C = \text{Chemical concentration (mass length}^{-3}\text{)}
\]
\[
x = \text{distance (length)}
\]

As can be seen from Equation (2), a diffusion gradient will always flow from a high concentration, typically the soil, to a low concentration, the plant root. When a solute transports across the membrane the amount of energy required thermodynamically is zero. However, analogously to a chemical reaction, a minimum activation energy must be overcome before that transport will take place. The energy required to move a compound through the membrane is produced by the entropic forces related to the solute which are manifest in the form of a potential energy stored in a concentration gradient. Upon reaching, the other side of the membrane this energy is returned to the system as a localized reduction in entropy or increasing in concentration.
FIGURE 2-1. The three primary intercellular transport pathways in the root for water, nutrients and xenobiotics.

FIGURE 2-2. Schematic of a typical semi permeable plant membrane with integral membrane protein (blue oval) and diffusing molecules (circles & squares) from high concentration (left) to low concentration (right).
Compounds that are charged are likely not to be passively transported across a hydrophobic membrane due to high transport activation energy requirements. Therefore, these compounds must be transported synergistically by proton pumps in which a specifically designed protein, that utilizes the energy supplied from the ATP-ADP reaction, pumps protons across a membrane. The proton pump mechanism works by literally forcing hydrogen ions across a membrane producing both a concentration and a charge gradient. This charge gradient can then be utilized to drive cationic charged species down gradient through the membrane in order to regain charge balance. The concentration gradient can also be used for cotransport of anionic compounds, where a coupled movement of an anion and a cation across the membrane and down the gradient occurs (12).

Redox gradients are the other active mechanism used by plants for nutrient accumulation. Like the proton pumping mechanism, the plant must expend energy, usually as NADPH, to produce a gradient (9). What makes the redox pump different is that it pumps electrons instead of protons across the membrane and therefore creates the drive for anionic charged species to cross the membrane barrier.

**Plant Uptake Descriptors**

Regardless of which mechanism is involved, typically plant uptake is either quantitatively described using the bioconcentration factor (BCF) or the transpiration stream concentration factor (TSCF) descriptor.

**Bioconcentration Factors.** A plant BCF is analogous to the more widely used fish BCF (L/mg) and thus it is simply the ratio of the concentration in the plant tissue, to the
concentration in the soil that the plant is growing in. As mentioned previously, BCFs tend to come from plants grown in soil systems over long durations with most resulting from actual field measurements. BCFs are reported on either a dry or wet weight basis. Wet and dry BCFs are not symmetrical ratios and it is important to distinguish between the two, as the water content of soil and plant tissue differ greatly. The primary advantage of a BCF is its direct correlation to field conditions, often however, little is known about those conditions making their use in modeling difficult. Despite this difficulty, after a literature review of 24 peer review papers, Travis and Arms (5) proposed a plant uptake relationship for dry weight BCFs based on the octanol/water partition coefficient that results in a linear correlation on a log/log basis (Equation 3).

$$\log BCF = 1.588 - 0.578 \times \log K_{ow}$$ (3)

Where:
- BCF = Bioconcentration Factor (unitless)
- $K_{ow}$ = Octanol/water partition Coefficient (unitless)

The linear relationship proposed by Travis and Arms (5) suggests that hydrophilic compounds have the highest tendency to transport and bioconcentrate in vegetation under field conditions.

**Transpiration Stream Concentration Factors.** The idea of a TSCF was first proposed by Sheet in 1961 (15) when he hypothesized that passive uptake of xenobiotics should be correlated to the amount of water transpired during exposure. The TSCF descriptor was officially codified by Shone and Wood (1) as a way of describing passive uptake of the herbicide simazine as a function of transpiration. As put forward by Shone and Wood (1), the TSCF is a simple ratio of the xylem concentration of a contaminant
divided by the hydroponic or soil solution concentration surrounding the immediate roots (Equation 4).

\[
TSCF = \frac{C_{\text{xylem}}}{C_{\text{sol}}}
\]  

(4)

Where:

- TSCF = Transpiration Stream Concentration Factor (unitless)
- \(C_{\text{xylem}}\) = Chemical concentration in the xylem (mass length\(^{-3}\))
- \(C_{\text{sol}}\) = Chemical concentration in the solution (mass length\(^{-3}\))

Because plant uptake of organic compounds is passive and the TSCF is a ratio of the chemical concentration in the xylem over the solution chemical concentration it is bound between 0 and 1.

Plant transpiration is a function of the growing conditions including factors such as leaf to air vapor pressure difference (absolute humidity), radiation intensity (sunlight), plant water status, radiation interception, health and the duration of exposure (10, 16). The TSCF, which includes transpiration, integrates plant and field conditions into the descriptor. This incorporation of several parameters has made the TSCF a favorite of modelers, and thus it is the most commonly used and measured plant uptake descriptor (17). Specifically, the TSCF is a way of normalizing the plant concentration to the root-zone concentration or soil water with respect to the amount of water transpired for individual compounds (Equation 5).

\[
TSCF = \frac{C_p \times M_{\text{Foliar}}}{T_w \times C_{\text{sol}}}
\]

(5)

Where:

- TSCF = Transpiration Stream Concentration Factor (unitless)
- \(C_p\) = Chemical concentration in the plant (mass mass\(^{-1}\))
- \(M_{\text{Foliar}}\) = Foliar Tissue Mass (mass\(^{-1}\))
- \(T_w\) = Volume of Water Transpired (length\(^3\))
$C_{\text{sol}} =$ Chemical concentration in the solution (mass length$^{-3}$)

A TSCF is based on the concentration of compound “available” to the plant root and the volume of water that is taken in by those roots. A TSCF for a given compound is assumed constant and independent of the method used to measure it (i.e., pressure chamber system, a hydroponics system or a soil system); however this has not been rigorously investigated. Theoretically, a laboratory determined TSCF could be used to predict the foliar concentration of a compound in a native soil system if the soil water concentration, transpiration rate, and potential losses (metabolism, volatilization) are known.

Because xylem sap is experimentally and analytically difficult to collect and measure in intact plants, it is often reverse calculated by multiplying the concentration of a contaminant in the plant by the mass of the plant compartment and then dividing this by the total water transpired during the exposure (Equation 6).

$$C_{\text{xylem}} = \frac{\sum C_i \cdot M_i}{T_w}$$  \hspace{1cm} (6)

Where:
- $C_{\text{xylem}} =$ Chemical concentration in the xylem (mass length$^{-3}$)
- $C_i =$ Chemical concentration in the $i$ tissue compartment (mass mass$^{-1}$)
- $M_i =$ Tissue Mass of $i$ compartment (mass$^{-1}$)
- $T_w =$ Volume of Water Transpired (length$^3$)

There are several empirical relationships that attempt to predict the TSCF for a compound based on its hydrophobicity. Perhaps the most widely used and simple of these regressions was developed by Briggs et al. (2) in which TSCF is related to $K_{OW}$ (Equation 7).
\[ TSCF = 0.784 \times e^{\frac{-(\log K_{OW} - 1.78)^2}{2.44}} \]  

(7)

Where:

\( TSCF = \) Transpiration Stream Concentration Factor (unitless)
\( K_{OW} = \) Octanol/water partition coefficient (unitless)

This relationship results in a bell shaped curve when plotted against log \( K_{OW} \) (Figure 2-3). As with the Travis and Arms relationship discussed earlier, the Briggs relationship represents one of the simplest plant quantitative structural activity relationships (QSAR).

The apex of the curve indicates that the maximum transport occurs at a log \( K_{OW} \) of approximately 1.8. This relationship was developed using barley grown in hydroponics dosed with 1 of 17 compounds from two chemical classes (oximes and phenylureas).

The study was conducted on 10-day-old plants over a period of 24-48 hours.

FIGURE 2-3. TSCF versus log \( K_{OW} \) relationship for nine O-methylcarbamoyloximes and nine substituted phenylureas in 10-day-old barely plant after 24-48 hours. Adapted from Briggs (2).
The Briggs relationship has been tested by others, most notably Hsu et al. using a pressure chamber on detopped soybeans (18), and Burken and Schnoor (4) growing hydroponic hybrid poplar tree whips. Both groups report a less conclusive bell shaped curve, and the optimal log $K_{OW}$ for maximum uptake is disputed by both. Incidentally, the Hsu group was attempting to validate the method of using a pressure chamber to measure TSCF. The Burken and Schnoor data, much like the Briggs data, were collected over a short period in very young plants. In a study by McCrady et al. (19) in which soybean stems were tested for translocation, the relationship between translocation and log $K_{OW}$ was linear and behaved like a reverse phase HPLC column (19) in which hydrophilic compounds transported to the greatest extent than hydrophobic compounds.

Studies in which a TSCF has been measured have typically been conducted on plants less than 14 days old over short exposure times of 24-48 hours. The use of small plants and short exposure periods are due to general difficulties in long-term plant cultivation. The use of such small, young plants may lead to experimental artifacts as the amount of water transpired over such a short time measured gravimetrically has typically resulted in only 500-1000 µL/day (1, 2, 20). Although the exact value is not known and is likely a function of physical and chemical properties of a particular compound, there is a minimum amount of water transpired before a representative TSCF can be determined. Furthermore the short exposure length, and the very young age of the plants creates problems when scaling values to field conditions where exposure times are 2 orders of magnitude longer and water transpiration rates are 3 orders of magnitude higher. With such small transpiration rates, factors such as exponential growth and evaporation,
combined with the difficulties in analytical methods of small systems, measurement precision and analytical concentration steps, can produce significant artifacts. Another concern of plant uptake in regards to the TSCF descriptor comes from the differing experimental methods used to carry out the measurement. TSCF values have traditionally been carried out using either “whole” plants grown hydroponically or detopped plants inserted into a pressure chamber. One study conducted by Hsu et al. (21) concludes that TSCF values resulting from a pressure chamber are similar to predicted TSCF measurements using the Briggs relationship developed for hydroponically grown plants. Other studies have also validated the method in regards to plant health (22) however no study has actually been carried out using identical species and compounds using both methods to truly validate this hypothesis.

In recent years, data for compounds such as 1,4-dioxane and sulfolane have led to the suggestion that the Briggs relationship for TSCF and log $K_{OW}$, is not a reliable predictor of plant uptake of chemicals from the class of polar neutral organics. Though in general agreement with the idea of an optimal log $K_{OW}$ for plant uptake, no other study concurs with the 1.8 value proposed by Briggs et al. (2, 21, 22). Little has been reported in the literature regarding reconciling this disagreement, with most models taking advantage of data sets from only one or two studies. When data from multiple studies are compiled, the idea of an ideal log $K_{OW}$ for plant uptake becomes much less tenable. A preliminary pass through the literature for TSCF values results in a more random picture than that reported by any individual study when plotted against log $K_{OW}$ (Figure 2-4). In particular, polar neutral organics such as sulfolane (6), MTBE (23) and 1,4-dioxane (7)
with low log $K_{OW}$s do not appear to agree well with the relationship proposed by Briggs et al. (2).

Quantitative structure activity relationships (QSARs) are analytical tools that describe the behavior of a compound based on its underlying molecular structure. The basis for this approach was first put forth by L. P. Hammet in 1935 when he reasoned “similar changes in structure produce similar changes in reactivity” (40). With the advent of advanced computing systems, the science of QSARs has progressed extensively. Despite the addition of many subtleties, nuances, and brute force methods brought by sophisticated computational methods, the underlying theory still pertains. The principles behind QSARs have proven to be so successful that they govern rational drug design and thus, much of the explosion in pharmaceuticals today. The QSAR approach is based on relating a biological process with one or more structural features. Without the use of a QSAR, the simplest method for predicting a compound’s biological activity would be to average the response given by a range of compounds and calculate the associated variance. A prediction of the unknown compound’s response would then likely be based on the calculated average response with the expected variability plus or minus the variance. A slightly more sophisticated method underlies the simplest of QSARs based on linear regression analysis, in which the biological response is linearly correlated to a property of the test compounds. With multiple discreet properties (hydrophobicity, size, hydrogen bond, etc.) related to the biological activity of a compound the basic QSAR can be expanded to the general (Equation 8),

$$Activity = c_0 + (c_1 \times P_1) + (c_2 \times P_2) \ldots (c_n \times P_n) \quad (8)$$

Where:

- Activity = Activity of concern (various)
- $c_n$ = Contribution coefficient (various)
P_i = Structural parameter (various)

Where P is the parameter or structural descriptor and C is a coefficient related to its contribution to the overall activity. More complicated QSARs are based on multivariate statistics such as principle component analysis (PCA), partial least squares (PLS), and complicated algorithms used in genetic function approximation (GFA) which use Darwinian evolution principles such as random mutations and crossover selection to “breed better models” (41). Some of the multivariate methods have even been combined in a way to mitigate their individual drawbacks such as the genetic partial least squares (G/PLS) method (42). In the creation of any QSAR, care must be taken to limit the analysis to parameters that are the most relevant descriptors. Adding too many parameters will lead to over fitting. In addition, many descriptors are inter-correlated and their use together can lead to violations of statistical assumptions. With over 1,660 computational descriptors of a compound currently available (43) the number of possible relationships from a given number of descriptors is 2^{n-1}, this would result in 10^{48} discreet combinations for just ten percent of the available descriptors. Therefore pertinent selection criteria are needed in selecting only those that provide chemical meaning to the activity while also abiding to the concept of parsimony. Consideration should also be made in selecting descriptors that are widely available or easily calculated such that the model is useful to as wide of an audience as possible.

QSARs are developed using a multitude of structural descriptors. These descriptors can range anywhere from the count of specific atoms, to the hydrophobicity of a compound as described by an octanol/water partition coefficient. The descriptors can fill
large volumes such as Hansch and Leo’s two volume set “Exploring QSAR” (44), however, with the continued advancement of computing software and the increasing array of known chemicals, most descriptors are now calculated directly from the energy minimized two and three dimensional structures in silico.

**Plant Uptake Models**

There have been several models proposed for the uptake of organic xenobiotics by plants over the years. These models include simple empirical regressions such as the Travis and Arms (5) relationship described above, equilibrium models requiring more input parameters such as the Chiou Partition Model (45), and the considerably more complicated mechanistic/dynamic models requiring the user to know a dozen or more variables such as the Trapp (46) four compartment model. In 2003 the Trapp (47) model was extended to include the uptake and subsequent translocation of contaminants to fruits, such as apples and pears, and was further improved in a paper published in 2007 (48).

The development of mechanistic models has been particularly important in furthering the understanding of the uptake process. These models have given insight not only into what could be important factors that govern plant uptake but have also helped guide researchers in their experimental designs. All these models, whether they are simple empirical relationships or extensive mechanistic models, rely on some type of uptake versus physical property relationship. The most common physical property chosen for plant uptake and translocation is the log K\textsubscript{OW} measurement (17). There are however a few models that incorporate other descriptors such as molecular weight (49), and in the case
of air deposition, the air water partition coefficient \( (K_{AW}) \) (50). Most models using a \( K_{OW} \) relationship, including the Trapp Fruit Model (47), have defaulted to using a modified form of the Briggs TSCF relationship rather than the Travis and Arms BCF relationship. As discussed previously, there are several advantages to the use of TSCF over BCF. The most prominent advantage to TSCF is that a TSCF measurement incorporates the amount of water transpired and thus it gives a measure of the uptake per unit time if transpiration for that time interval is known. A TSCF also gives modelers a value that can theoretically be scaled to any size plant using transpiration as the normalizing factor.

In many models, such as the Trapp Fruit Model, the Briggs relationship is often the most sensitive input parameter (47) affecting modeling results. This means that great care should be used in assessing the validity of the Briggs study. As mentioned previously, other studies have also seen a similar “humped” TSCF versus log \( K_{OW} \) relationship, however each study has called into question the location of the peak of the “hump.” The recent and most completely mechanistic model proposed by Trapp (48) no longer relies on the Briggs relationship and the result is that it predicts that hydrophilic compounds, rather than mid-range hydrophobic compounds, tend to transport most readily into plants. An inspection of Figure 2-4 yields no consensus on TSCF versus log \( K_{OW} \) trends except to show that polar, neutral compounds with log \( K_{OW} \) values below 1 are not well predicted by the “humped” relationship of Briggs et al. (2) and others and thus not well predicted by most models.

The focus of this study was to reevaluate the relationship between plant uptake as described by TSCF and chemical structure. The research attempts to determine if TSCF
is an appropriate parameter for predicting the translocation of xenobiotic organic chemicals from roots to shoots and to evaluate the suitability of currently used TSCF prediction methods based on log $K_{OW}$.

In Chapter 3, a plant exposure system that enables the determination of TSCF with a minimization of experimental artifacts associated with poor plant growth or losses associated with exposure durations will be presented. The exposure system, was adapted from work by others (21, 22) and includes some novel techniques that were employed to gain addition information about the uptake process.

In Chapter 4, the relationship between hydrophobicity, expressed as log $K_{OW}$ and root to shoot translocation expressed as TSCF will be thoroughly evaluated. Twenty-five neutral organic compounds ranging in hydrophobicity between -0.8 and 5 were determined using the methods laid out in Chapter 3. A determination of the applicability of current TSCF vs log $K_{OW}$ relationships is given stemming from a compilation of existing peer-reviewed plant uptake data.

In Chapter 5, an improved model is presented which explains the experimental data of Chapter 3 and 4. The model was validated using new collected and reported plant physiology data along with TSCF data presented in Chapter 4.
CHAPTER 3
VALIDATION AND IMPROVEMENTS TO MEASURING TSCF USING A PRESSURE CHAMBER

Abstract

The potential of xenobiotic organic compounds to be taken up by plant roots and transported to above ground tissue is an important consideration for phytoremediation projects and risk assessments alike. The transpiration stream concentration factor (TSCF), a ratio of the xylem concentration to the root-zone solution concentration, is the most commonly used parameter describing plant root uptake potential. There are two primary methods used to measure a TSCF. The first involves a mass balance, derived from intact plant experiments, while the second directly measures the TSCF of a detopped plant placed in a pressure chamber. Measuring a TSCF using intact plants can be problematic due to high cost, time requirements, and the difficulty in correcting for potential losses due to metabolism and phytovolatilization. The pressure chamber method for measuring TSCF has been successfully used to obviate these concerns. In this study adaptation of the basic method along with several novel techniques were developed for conducting plant uptake measurements using the pressure chamber. These adaptations and techniques amplify the robustness and scope, while increasing the overall validity of the method. Basic adaptations including, a root-zone solution sampling port, reducing excessive solution purging by using oxygen to pressurize the system, and the use of tritiated water as a conservative tracer. Measurements of several nutrient TSCFs and the use of mercuric chloride illustrating the effect of root membrane disruption and

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death on TSCF are presented. Finally, the results of pre-exposing plants before use in the chamber along with uptake kinetics are given. Incorporating these measurements and techniques into the basic pressure chamber method lends legitimacy to the measurements made and leads to an improved understanding of the uptake process.

Introduction

Phytoremediation efforts and risk assessment determinations rely on the ability to determine the potential for xenobiotic organic compounds to be taken up by plant root systems and transported to foliar regions. The uptake potential is often measured using the TSCF (6, 11, 32, 51-53). A TSCF is the ratio of the concentration in the plant xylem or transpiration stream to the concentration in the solution the roots are using for transpiration (1). The usefulness of a TSCF is a result of the normalization of the amount of compound taken up by a plant to the total amount of water transpired during a plant’s exposure to that compound. Normalizing uptake to transpiration allows for linear scaling of total plant uptake to transpiration dependant factors such as exposure time, plant age, size, and climatic conditions (54). By knowing a compound’s TSCF, the total mass of compound in a plant shoot can be obtained by multiplying by the effective concentration of the exposure solution, the amount of water transpired during the exposure period, and then correcting for metabolism and volatilization losses (35).

The TSCF is measured in one of two basic ways (see Chapter 4). In the traditional approach, a TSCF is estimated using intact plants grown either in soil or under hydroponic conditions. Because collection of enough xylem sap for analysis is difficult for most living plants, TSCFs are generally determined from calculations using the measured shoot concentrations normalized to the amount of water transpired during the
exposure period, i.e., concentration in xylem sap is deduced as the total mass of the compound in shoots divided by the volume of water transpired (2, 4, 6, 7). These calculations require that metabolism or volatilization within the plant be corrected for, but this is often analytically difficult and there are no uniform methods for performing these corrections. Furthermore, a TSCF derived from intact plants is often time and money intensive. The second method requires the use of a pressure chamber. The pressure chamber specifically addresses many of the difficulties encountered in using intact plants. The pressure chamber method consist of sealing the roots of a detopped plant (i.e., the above ground tissues are removed just above the lowest leaves) in a pressurized chamber containing a solution of a known concentration of the chemical of interest (11, 21, 53). The solution is drawn up through the roots as the chamber is pressurized and the xylem is collected and analyzed as it exits the cut stem. Ideally, the pressure is carefully adjusted to provide a constant flow rate corresponding to the transpiration rate of an intact plant of similar size without damaging the roots (Chapter 4). A TSCF is calculated from the ratio of the steady state xylem concentration to the root exposure concentration (21, 53). The primary advantage of the pressure chamber method comes from its simplicity. The reduction in operational variables tends to increase the pressure chamber’s consistency and repeatability (Chapter 4). Nevertheless, some adaptations and measurement techniques for incorporation into the basic process are proposed in an effort to increase the validity, robustness, and scope of the basic method. These adaptations include the addition of a root-zone solution sampling port for measuring xylem and solution concentrations in parity, use of pure pressurize the system
oxygen to reduce purging, and the use of tritiated water as a conservative tracer. The use of mercuric chloride is used to demonstrate the viability of the method, the effect of root health on TSCF and elucidate the importance of the root membranes to the uptake process. Finally, the results of pre-exposing plants before use in the chamber along with uptake kinetics information are given.

**Materials and Methods**

The method of plant cultivation along with the fundamental techniques of measuring a TSCF in the pressure chamber are thoroughly described in several previously published works (11, 21, 53, 55). The basic method used in this study consists of detopping plants just below the first cotyledonary node (lowest leaves) and removing all of the shoot tissue except for a small section of stem. Following the prescription of Hsu et al. (21) for sealing the plant into the chamber using plastic sheeting wrapped around dental impression material, tended to develop leaks over time in the area between the stem and stem plate. Therefore, the method of sealing the plant into the chamber was modified to use short sections (5 cm) of butyl rubber (tomato) or rigid platinum cured silicone (soybean) tubing of various diameters fitted over the cut stem creating a stem gasket. For rigid stems like soybeans, the gasket-covered stem can then be slipped through a hole in an inverted rubber stopper. The inverted stopper is then inserted up through the chamber’s stem plate, thus sealing it into the pressure chamber. Because of the woody nature and uniform size of soybean stems, the use of an inverted rubber stopper controls for the pressure of the chamber. Under low pressure, the system seals based on the initial insertion pressure applied to the stopper. As the pressure in the chamber increases, the
inverted stopper is forced further into the stem plate, increasing the pressure around the stem in a gentle, uniform manner proportional to the pressure applied. For larger more malleable stems like those of tomato plants, a stainless steel hose clamp above and below a tight fitting stem plate works well. After the tubing covered cut stem is sealed in the stem plate, the roots are immediately immersed in a stainless steel vessel containing oxygen saturated nutrient solution spiked with a known concentration of a target compound (Figure 3-1). The stem plate is then secured to the vessel with a threaded collar and the tip of a disposable pipette is affixed over the cut stem and under the tubing. The chamber is pressurized (~150 kPa) using compressed oxygen resulting in a xylem flow rate of approximately 70% of the plant’s previous day average transpiration rate. The pressure difference between the roots and xylem typically used in the pressure chamber falls within the reported range of measurements for intact plant root and xylem differential pressures (56).

**Chamber Adaptations.** The pressure chamber apparatus Figure 3-1 consists of a stainless steel container with a threaded lid and stem plate. Modifications made to the pressure chamber device used in previous uptake studies (11, 21, 53) include a root-zone solution sampling port and stem plates with various diameter holes. Multiple stem plates with various size holes allow the fit of plant stems from a large age range as well as from different species without excess manipulation. The root-zone sampling port consists of a 1/4” (6.35 mm) stainless steel Swagelok™ bulkhead fitting attached to the lower end of the chamber with a teflon coated septum seated into the fitting by a ¼’ (6.35 mm) cap. The addition of a sampling port facilitates paired measurements of the xylem and solution
concentrations in real time. The ability to sample the root solution in parity with the xylem sap is particularly important for volatile and very hydrophobic compounds whose concentration might be expected to change over the experiment due to surface sorption and purging losses.

FIGURE 3-1. Pressure chamber used to measure transpiration stream concentration factors illustrating the tomato plant configuration of sealing at the stem plate.
Investigators of previous studies have pressurized their chambers with compressed air constantly bubbled through the solution (11, 21). This is done to both mix the chamber and to prevent the root-zone from becoming anoxic due to continued root metabolism. A significant drawback to bubbling compressed air into the solution is the potential loss of compound due to purging. The flow rate and thus the purge rate can be safely reduced by a factor of 5, from the typical 50 mL/min (21) to 5-10 mL/min, using pure oxygen rather than air without reducing the dissolved oxygen content. The reduced flow rate, in combination with the root-zone sampling port, results in solution concentrations that are better controlled due to less purging, and confirmed by measurement than in the original configuration.

**Estimating TSCF from exponential** $C_{xylem}/C_{solution}$ **data.** Typically, plants used in the pressure chamber are exposed to a compound for the first time upon insertion in the chamber. Because the plants have no previous exposure, the concentration in the xylem sap is essentially zero. As such, the ratio of the concentration in the xylem to that in the solution is infinitely small. As the xylem is pushed through the plant, the concentration in the xylem begins to rise exponentially (Figure 3-4). The determination of the steady state TSCF and the characteristic time $\tau$ values for pressure chamber measurements are calculated using the model described by Feinstein and Holt (57) for estimating the parameters of a time varying unstable exponential process. The model attempts to fit a generic exponential curve (Equation 1).

$$ y(t) = C_o - (C - y_o) e^{-\tau/t} $$

(1)

Where:

$C = $ Absolute amplitude (various)
\[ \tau = \text{Characteristic time (time}^{-1} \text{)} \]
\[ t = \text{Time (time)} \]
\[ y(t) = \text{signal as a function of } t \text{ (various)} \]

C and \( \tau \) from (1) can be related for any given time step by solving for C and rearranging into slope intercept form (Equation 2). Using Equation (2) results in a line of all possible C and \( \tau \) combinations for that given time step (t).

\[
C(t) = \hat{y} \tau(t) + y
\]  

(2)

\[
\hat{y} = \frac{dy}{dt}
\]  

(3)

Where:
\[ \hat{y} = \text{response change per time (time}^{-1} \text{)} \]

Under ideal conditions the resulting lines obtained from Equation (2) would all cross at the point C, \( \tau \). However, under most real data scenarios, the lines from Equation (2) create a curve of intersecting lines over an extended region. Feinstein and Holt use the Hough Transform to estimate both C and \( \tau \) from that curve in a consistent way by balancing both C and \( \tau \) motion.

The mathematical summary of the process adapted to pressure chamber data is shown in (4) where TSCF replaces C, \( y \) represents the measured values from the experiment and \( \hat{y} \) was calculated using (Equation 5).

\[
\begin{bmatrix}
\tau \\
\text{TSCF}
\end{bmatrix} = 
\begin{bmatrix}
- \sum_{i=1}^{N} \hat{y}_i & \sum_{i=1}^{N} 1 & \sum_{i=1}^{N} y_i \\
- \sum_{i=1}^{N} \hat{y}_i^2 & \sum_{i=1}^{N} \hat{y}_i & \sum_{i=1}^{N} \hat{y}_i y_i
\end{bmatrix}
\]  

(4)
Where:
\[ \tau = \text{Characteristic time (time}^{-1}\text{)} \]
\[ y = \text{Concentration in xylem/concentration in solution at time } t \text{ (unitless)} \]
\[ \text{TSCF} = \text{Transpiration Stream Concentration Factor (unitless)} \]

\[ \dot{y} = \frac{(y_t - y_{t-1})}{(t_t - t_{t-1})} \]  \hspace{1cm} (5)

Where:
\[ t = \text{Time (time)} \]
\[ y = \text{Concentration in xylem/concentration in solution at time } t \text{ (unitless)} \]
\[ \dot{y} = \text{Concentration in xylem/concentration in solution per time (time}^{-1}\text{)} \]

**Results**

**Xylem Volume Measurements.** After the TSCF is measured, plants can be sealed back into a now empty chamber and repressurized for xylem volume measurements. The xylem exuded is collected in a graduated cylinder until breakthrough of air is visually evident from bubbles formed in the exudate. Using this technique, a linear relationship between the volume of xylem exuded and wet weight root mass was found from a series of soybean plants as shown in Equation (9) and (Figure 3-2).

\[ V_{xylem} = 0.38 \times M_{root} - 1.46 \]  \hspace{1cm} (9)

Where:
\[ V_{xylem} = \text{Volume of expelled xylem sap (length}^3\text{)} \]
\[ M_{root} = \text{Root mass (mass)} \]

In Figure 3-2 the data are separated into two classes, impacted (herbicide and mercuric chloride) and non-impacted. The difference between the linear relationships of the impacted and non-impacted plants is useful for diagnosing problems that might occur during an experiment. When the membranes of the roots become damaged due to
manipulation, or are intentionally disrupted due to herbicide or mercuric chloride addition, the volume of xylem expelled by the roots increases compared to non-impacted roots (Figure 3-2). The slope of the linear regressions for both conditions is essentially equivalent at 0.38 and 0.36 while the intercept was -1.5 and 9.7 for the non-impacted roots impacted roots, respectively. The increased exuded volume seen in the impacted experiments is consistent with damaged membranes of cells allowing a portion of the cell contents to leak out under repressurization. Identifying this condition by measuring the exuded volume at the end of an experiment can serve to increase the validity of the data obtained.

![FIGURE 3-2. Linear regression of expressed xylem volume versus plant fresh root weight of non-impacted (Vxylem = 0.38 x Mroot -1.46, r² = 0.94), and herbicide (atrazine & 2,4-D) and mercuric chloride impacted (Vxylem = 0.36 x Mroot + 9.7, r² = 0.66), soybeans used in pressure chamber.](image-url)
**Nutrients.** Because plants grown long term by hydroponics generally have easy access to sufficient nutrients (16), uptake of most nutrients is expected to be passive resulting in a TSCF equal to 1. To investigate this hypothesis and determine their suitability as uptake tracers in water, TSCF measurements of seven plant nutrients were made in two non-exposed tomato plants. Nutrients were directly analyzed in the xylem and solution by ICP/MS. The plants in which the nutrient TSCF was measured were grown in hydroponic solution containing ample levels of all these nutrients for at least 3 weeks prior to testing. The measured TSCF of all seven nutrients were not statistically different from 1 (Figure 3-3). Measurements of nutrient TSCF appear to be a function of their solution concentration, with higher concentrations associated with lower variability.

**FIGURE 3-3.** Duplicate TSCFs for several nutrients in the exposure solution (error bars =SE).
**Tritiated Water/Conservative Tracer.** Adding tritiated water ($^3$H$_2$O) to the root solution has two general advantages. Primarily, $^3$H$_2$O acts as a conservative tracer useful for verifying the plant is behaving as expected. The shape of the $^3$H$_2$O uptake curve provides an indication of whether there was any root/stem bypass taking place during the experiment. Secondly, the kinetics of water uptake can be compared to an individual compound’s kinetics within the same plant simultaneously (Figure 3-4.)

**Mercuric Chloride Addition.** Mercuric chloride is used in root hydraulic conductance experiments to reduce the flow of water through roots by closing aquaporin channels which selectively allow water to pass (13). Although it closes the aquaporins and thus produces a reduction in flow, the longer-term effect is deleterious to root membranes, resulting in death and an increase in flow (Figure 3-4). The consequence of 0.5 mM mercuric chloride is quickly evident on root hydraulic conductance while at first having no effect on TSCF. After approximately 60 minutes, the root membranes begin to lose their integrity as evident from the increasing flow rate. The ultimate result is that the roots can no longer exclude the compound and the TSCF begins to approach 1 (Figure 3-4). The mercuric chloride addition demonstrates that plant roots under normal conditions, with membranes intact, have the ability to limit the uptake of organic compounds to some extent. The decline in $^3$H$_2$O in Figure 3-4 supports the idea that the exclusion ability is a consequence of the root membranes. When the membranes begin to leak, they cause a diluting effect of the tritiated water as the cell’s contents, which do not contain tritium, escape into the xylem. The data from the expelled xylem sap of Figure 3-2 further supports the membrane disruption hypothesis.
FIGURE 3-4. Mercuric chloride (0.5 mM) addition to root solution of $^{14}$C caffeine in pressure chamber after initial steady state, showing the lack of control in uptake as a result of root damage. Declining $^{3}$H$_2$O xylem/solution concentration ratios indicates a dilution effect from cell contents leaking due to membrane leakage.

**Effects of Select Herbicides.** Two herbicides, atrazine and 2,4-dichlorophenoxyacetic acid (2,4-D), were tested in the pressure chamber to determine their TSCF values. Similar to the mercuric chloride, the herbicides had an injurious effect on the roots. Both herbicides had TSCFs that transiently approached the passive maximum of 1. The herbicide had toxic effects that were visually evident by the browning of the roots, the increases in flow rate under steady pressure conditions (Figure 3-5), and excess expelled xylem sap (Figure 3-2). The herbicides 2,4-D was dosed in duplicate at both 10 and 0.02 mg/L nominal concentrations. The low dose concentration resulted in a longer initial lag and slower rise in the TSCF of the plants compared to the higher dose; however, the
ultimate effect was the same with steady state TSCFs approaching unity (data not shown). The effect of atrazine was similar with a concentration of 0.850 mg/L reaching a TSCF of 1 faster than the lowest concentration of 0.085 mg/L however; no initial lag was evident in the atrazine data (data not shown). The lag in the 2,4-D could be the result of slow or poor membrane translocation of the acid form, which predominated at the buffered pH (5.5) of the system. Similar in structure to plant auxins (hormones), 2,4-D might be less toxic at very low concentration, however, impact to root structure and development has been shown at concentrations comparable to those used in this study (58).

FIGURE 3-5. Herbicides Atrazine (closed symbols) and 2,4-D in (open symbols) pressure chamber showing adverse effect over time with increasing root hydraulic conductance (flow rate, upside down triangles) and TSCF (circles) approaching unity.
Variations on Pressure. Just prior to being used in the pressure chamber, the amount of water transpired by the plant was measured gravimetrically over a 24 hr period. The volume of water transpired was calculated by multiplying the mass lost by the density of water. The rate was then normalized to the 16-hr photoperiod of the greenhouse to approximate the transpiration rate. The pressure applied to the chamber was set to induce sap flow that was 70-100% of the plants estimated transpiration rate typically 100~200 kpa. The effect of pressure and therefore transpiration rate was investigated in two ways. In the first experiment, under the assumption that a flow rate effect on uptake would be most apparent at the extremes of flow conditions, after a steady state TSCF had been reached the pressure was removed, reducing the flow rate to zero for 60 minutes. Sixty minutes was chosen to maximize flow effects while minimizing possible metabolic effects. After 60 minutes, additional tritiated water was added to follow the repressurization kinetics. Xylem flow rates quickly returned to near previous levels under the same pressure. The removal and resumption of pressure over a short interval showed no significant effect on either the TSCF or the tritium kinetics (Figure 3-6).

In a separate experiment, using methanol as the exposure compound, there was no measureable change in the steady state TSCF when the flow rate was reduced from 0.3 mL/min to 0.05 mL/min (Figure 3-7). As predicted by the passive uptake hypothesis, if there are no loss mechanisms such as metabolism, or phytovolatilization, a flow rate change that is within the expected transpiration rate fluctuations a plant would experience diurnally had little to no effect on the TSCF.
FIGURE 3-6. Effect of removing pressure for 60 minutes on the TSCF of caffeine and tritiated water in soybeans.

FIGURE 3-7. Effect of six-fold decrease in xylem flow rate on TSCF of methanol and tritiated water in soybean roots in a pressure chamber.
Kinetics. The kinetics of translocation was examined by treating the plant system analogously to a chromatography column. The plant was placed in the pressure chamber containing nutrient solution. At 30 minutes, the chamber was dosed to a concentration of 120 mg/L caffeine through the sampling port. During the experiment, the chamber was well mixed by both the typical oxygen bubbling through solution and by the addition of a magnetic stir bar placed in the bottom of the chamber. After 60 minutes exposure time to caffeine, the pressure was released, the solution was replaced with clean nutrient solution, and the roots were rinsed in distilled water. The plant was then restarted in the pressure chamber. The lag time between solution maximum concentration and maximum xylem concentration was approximately 90 minutes (Figure 3-8). The rate of change in the upward slope of the xylem concentration curve exceeds that of the downward slope. This suggests that the rate of sorption onto the root membranes is much quicker than the rate off the root membranes and into the xylem. It is also interesting to note that there was some loss from the well-rinsed roots back into solution, which occurred within 60 minutes of root reinsertion. The re-equilibration of the roots with the solution demonstrates the root is acting as reservoirs taking the compound up quickly and then slowly releasing it to the aqueous compartments. The total area under the desorption side of the curve combined with the repartitioning to the bulk solution demonstrates that the rate of sorption to the roots is much greater than the translocation out of the roots. The root-zone solution concentration reached a level of approximately 4.5 mg/L while the xylem concentration dropped to roughly 3.7 mg/L resulting in a final TSCF of 0.82, which is equivalent to the average reported TSCF for caffeine (Chapter 4).
Variations on Dosing. TSCFs are typically generated in the pressure chamber from plants whose first exposure to the chemical of interest comes when they are inserted into the chamber. The plants are suddenly introduced to the chemical and the TSCF is determined when the xylem sap concentration of the compound reaches steady state (11, 21, 53). Although this is the easiest method for determining TSCF, the TSCF can also be derived from plants that are pre-exposed to the compound of interest. It is however important that the pre-exposed plant transpire enough water for the compound to reach steady state within the plant. Figure 3-9 illustrates the kinetics obtained using the typical sudden introduction method along with pre-exposed plants under both low transpiration.
and high transpiration conditions. It appears that under high transpiration (full sunlight) the TSCF is equivalent to the steady state TSCF of the pressure chamber. However, when the experiment was carried out on a diminutively transpiring plant, which was placed in the chamber after 8 hours of darkness, the immediate TSCF (0.65) was somewhat lower than the measured value using the typical approach (0.83). All three treatments resulted in similar TSCFs within 200 minutes. Because the mass of compound being transported in a plant is proportional to the transpiration rate, the mass of compound being transported to the upper portions of the low transpiring plant is small and the metabolism rate of the roots may have been sufficient to slightly reduce the measured TSCF during the 8 hours of preceding darkness. The caffeine used in this experiment may be highly susceptible to metabolism in plants (59) and additional investigation with a compound not expect to be readily metabolized is warranted.

Figure 3-9 illustrates that under similar conditions a TSCF of an intact plant should be identical to a pressure chamber measured TSCF. When kinetic information is not expected to be important, pre-dosing plants prior to use in the pressure chamber is an appropriate method of measuring a TSCF provided the plant is kept under environmental conditions sufficient to produce transpiration rates equivalent to field scenarios or the pressure chamber is run long enough to induce steady state. The data also supports the traditional pressure chamber method of measuring TSCF. The data also suggest that for easily metabolized or volatilized compounds, erroneously low TSCF values may result from making measurements on intact or pre-dosed plants in the early morning when transpiration rates are low rather than mid to late afternoon when they are at their peak.
Based on previous studies (11, 21, 53), the pressure chamber is a viable method for measuring TSCF of xenobiotic compounds. It is generally quicker, more cost effective, and more robust than making measurements with intact plants, which often require significant and sometimes poorly understood corrections for metabolism and phytovolatilization. The pressure chamber is especially effective at providing kinetic information on the uptake process that may be useful in modeling the mechanisms of the process.

The technique of measuring xylem volume is useful for verifying plant health during the experiment while paired root-zone measurements and use of oxygen help better control the experimental variability, and pre-dosing, tritiated water, mercuric chloride and
herbicide data validate the use of the pressure chamber as a viable method for measuring TSCF. Using the techniques provided in this paper will increase the quality and validity of the data generated using the pressure chamber.
CHAPTER 4
CHEMICAL HYDROPHOBICITY AND PLANT ROOT UPTAKE

Abstract
The uptake of xenobiotic organic compounds by plant roots occurs passively in conjunction with transpiration, and the transport of these chemicals from root to shoot is ultimately controlled by the passage through one or more lipophilic root membranes. The transpiration stream concentration factor (TSCF), the ratio between a compound’s concentration in the xylem to that in the solution adjacent to the roots, is commonly used to describe the relative ability of an organic compound to be passively transported from root to shoot. Widely cited bell-shaped curves relating TSCF to the octanol/water partition coefficient (log $K_{OW}$) imply that significant root uptake and transfer into shoot tissues occurs only for compounds falling within an intermediate hydrophobicity range. Based on these curves, highly polar or very lipophilic compounds should not be readily transported from root to shoot. However, recent laboratory and field data for relatively water soluble compounds such as sulfolane, methyl tert-butyl ether (MTBE), and 1,4-dioxane suggest that these relationships are not universally applicable, especially for non-ionizable, highly polar, water soluble organics. To re-evaluate the relationship between root uptake and chemical hydrophobicity, TSCFs were measured for 25 organic chemicals ranging in log $K_{OW}$ from -0.8 to 5 using a pressure chamber technique. Using the TSCF values measured in this study, a new empirical relationship between TSCF (0 to 1) and log $K_{OW}$ (-0.8 to 5) is presented that indicates that non-ionizable, polar, highly

$^{1}$Authored by Erik Dettenmaier, William Doucette, Bruce Bugbee
water soluble organic compounds are most likely to be absorbed by plant roots and translocated to shoot tissue.

**Introduction**

Predicting the uptake of organic chemicals by roots and transport via the xylem to above ground tissues is critical for conducting risk assessments and determining the potential effectiveness of phytoremediation.

The uptake of xenobiotic organic chemicals by roots has long been observed to be passive, with the chemicals moving into the plant in proportion to the amount of water transpired (1, 15) which is in turn related to factors such as humidity, sunlight, and exposure duration (10, 16).

The transpiration stream concentration factor (TSCF) is the ratio of the contaminant concentration in the xylem sap to that in the root-zone hydroponic or soil solution, and has been widely used as a descriptor of chemical uptake by roots (1, 2). Chemicals actively taken up by plants have TSCF values greater than 1.0 (N, P, and K), while those that move into plants at the same rate as water have TSCF values of 1. Interactions with the lipid bi-layer in root membranes reduce the uptake of organic chemicals relative to water resulting in TSCF values of less than 1, even though the mechanism is passive.

Values of TSCF have typically been measured using one of two general approaches. In the first approach, intact living plants are exposed to a constant or measured root-zone concentration of the chemical of interest. A hydroponic environment is generally used for ease of measuring and controlling exposure concentrations. Since the direct collection of a sufficient amount of xylem sap for analysis is problematic for most living plants, TSCFs are generally determined from measured shoot concentrations normalized
to the amount of water transpired during the exposure period. Any loss of the chemical due to metabolism or volatilization within the plant should be corrected for, but this is often difficult to determine analytically and is not always performed.

To circumvent the problem of collecting insufficient xylem sap or the need to extract and analyze the plant tissue, the second approach for measuring TSCF involves sealing the roots of a detopped plant (i.e., the above ground tissues (shoots) are removed) in a pressurized chamber containing a solution of a known concentration of the chemical of interest (11, 21, 53). The solution is forced through the roots as the chamber is pressurized and the xylem is collected and analyzed as it exits the cut stem. Ideally, the pressure is carefully adjusted to provide a constant flow rate corresponding to the transpiration rate of an intact plant of similar size without damaging the roots (53). A TSCF is calculated from the ratio of the steady state xylem concentration to the root exposure concentration. Although transpiration is simulated by pushing water through the roots, the pressure chamber approach has several advantages over intact plant measurements. In addition to producing sufficient quantities of xylem sap, additional advantages include shorter experimental durations, direct measurement of xylem concentrations, and minimal losses due to volatilization and metabolism. Maintaining root health is critical for insuring normal membrane integrity and it is particularly important in hydroponic systems to maintain adequate oxygen and nutrient levels in the root-zone and avoid toxic chemical concentrations.

Overall, relatively few experimental TSCF values exist, due in part to the cost associated with their determination and the lack of regulatory requirements for such data. Where data exist for chemicals having more than one literature TSCF, the variability is
often relatively large due to the lack of standardized or generally accepted methods for TSCF determination.

Because of the scarcity of experimental values, estimated TSCFs are widely used in risk assessments. The most widely used estimation approaches are based on empirical relationships between TSCF and the logarithm octanol/water partition coefficient (log $K_{OW}$). These relationships suggest an optimal hydrophobicity for uptake and translocation and infer that compounds that are either highly polar (log $K_{OW} < 1$) or are highly lipophilic (log $K_{OW} > 4$) will not be significantly taken up by plants (2, 4, 21). However, reports of significant plant uptake of highly water soluble compounds with, low log $K_{OW}$ values such as 1,4-dioxane (7), MTBE (23), and sulfolane (6, 24) suggest that the general suitability of these estimation techniques should be re-evaluated, especially for these types of compounds. Polar, non-volatiles are also a class of organic chemical predicted to be the most likely transferred from soil to fruit in a recently published fruit tree model (48).

Thus, the main objective of this study was to evaluate the relationship between TSCF and log $K_{OW}$ using the pressure chamber technique and develop a more generally applicable relationship that encompasses both hydrophobic and hydrophilic neutral organic compounds. A comprehensive literature compilation of TSCF values was also used to evaluate the applicability of the resulting relationship and to examine the impact of experimental measurement methods on the variability of reported TSCF values.

Materials and Methods

Experimental Overview. Values of TSCF were determined for 25 organic chemicals (Table 4-1), using a pressure chamber technique. The chemicals were selected to cover,
or extend, the range of \( \log K_{OW} \) values used in previously reported TSCF-log \( K_{OW} \) relationships (from -0.8 to 5) from a list of compounds for which analytical methods had been previously developed in related projects. Analysis methods depended on the physical properties of the chemical. Volatile compounds were analyzed by headspace GC/MS, polar non-volatiles by HPLC, and \(^{14}\text{C}\)-labeled compounds by liquid scintillation counting (LSC).

Two plant species, soybean and tomato, were evaluated based on their previous use in related uptake studies and prior experience with their cultivation. Typically, only one chemical exposure concentration was evaluated. The concentration was chosen to be within the range of easily detected concentrations in the xylem. Only TSCF obtained in which no observable root toxicity, which was determined by visual discoloration of the normally white root tips; or abrupt changes in root hydraulic conductivity (measured as the flow rate exiting the stem), where used in the data analysis. Since the headspace GC/MS method allowed for the simultaneous determination of multiple volatile compounds, the TSCFs for these compounds were determined by exposing plants to mixtures of compounds. The TSCF of benzene measured as part of a mixture using headspace GC/MS and individually as \(^{14}\text{C}\) labeled compound using LSC, were statistically equivalent.

**Germination and Plant Culture.** Soybean (*Glycine max*) cv. Hoyt seeds were obtained from Utah State Crop Physiology Laboratory, Logan, UT. Tomato (*Lycopersicon lycopersicum*) cv. Red Robin seeds were obtained from Tomato Growers Supply Company, Fort Myers, FL. To initiate germination, seeds were inserted between damp paper towels and placed into transparent Plexiglas® containers with about 2.5 cm
of tap water maintained at the bottom. The containers were kept at 26 ± 1°C, while the seeds were kept moist by the capillary action of the paper towels. No significant microbial contamination of the seeds was observed. After 5-7 days (soybean) or 7-10 days (tomato), the rooted seedlings were transferred to a greenhouse hydroponic environment (16 hr photoperiod, 20/16 ± 1 °C day/night temperature) and grown to a uniform size for 4-6 weeks prior to use in the pressure chamber experiments.

**Pressure Chamber.** Immediately prior to the start of a pressure chamber experiment, test plants were removed from the hydroponic solution and cut just below the first cotyledonary node (lowest leaves) to remove all of the shoot tissue except for the stem base. A short length (5 cm) of butyl rubber (tomato) or rigid platinum cured silicone tubing (soybean) was then fit over the cut stem forming a gasket. In the case of tomatoes, the gasket was held in place with a hose clamp, and the gasket covered cut stem was inserted directly through the stem plate and the roots were immediately immersed in a stainless steel vessel containing oxygen saturated nutrient solution spiked with a known concentration of a compound (Figure 4-6, Supporting Information). The stem plate was then secured to the vessel with a threaded collar and the tip of a disposable pipette was affixed over the cut stem and under the gasket. Compressed oxygen was slowly (20 to 40 mL min⁻¹) bubbled into the nutrient solution to build pressure within the chamber to aerate and mix the root-zone. The pressure was gradually increased and the flow rate decreased (5 to 10 mL min⁻¹) until a xylem sap flow rate of approximately 70% of the intact plant maximum transpiration rate was reached (the pressure was usually about 150 kPa). The maximum transpiration rate was measure gravimetrically over the previous 24 hours and then normalized to the 16 hr photoperiod the plants were cultivated under.
For soybeans, the gasket-covered stem was first slipped through a hole in an inverted rubber stopper that was inserted up through the chamber’s stem plate, and sealed into the pressure chamber as previously described for the tomato plants. Because of the woody nature and uniform radius of soybean stems, the use of an inverted rubber stopper helped control the pressure exerted on the stem. As the pressure in the chamber increased, the inverted stopper was forced further into the stem plate, increasing the pressure around the stem in a gentle, uniform manner proportional to the pressure applied.

Samples of xylem sap exiting the cut stem and the root-zone exposure solution were collected every 10-30 minutes and analyzed for the compounds of interest. Xylem sap samples were collected using a fraction collector (ISCO, CYGNET, Lincoln, NE) and samples of the root-zone exposure solution were collected through a septum-sealed sampling port using a glass syringe. Depending on the physical-chemical properties of each compound (higher log $K_{OW}$ = longer exposure time), samples were collected for 5 to 50 hours, typically reaching steady state in less than 12 hours and not used after 24 hours.

**Analysis of Xylem Sap and Exposure Solutions.** The methods used to determine the concentrations of the test chemicals in xylem sap and exposure solutions depended on the physical chemical properties of the compounds (e.g. volatility, solubility, etc.).

**Volatile Compound Analysis.** Samples (10 mL) of xylem sap and pressure chamber solutions were transferred to headspace vials containing enough sodium chloride (NaCl), to saturate the 10 mL sample. The NaCl was used to increase the headspace concentrations of the volatile organic compounds. The concentrations in the xylem sap and pressure chamber solution were determined indirectly from the concentrations of the compounds in the headspace. External standards, made by spiking known amounts of a
commercial standard (Supelco, Bellefonte, PA, USA) into NaCl saturated water, were used to define the relationship between the headspace and the water concentrations.

Headspace samples (2 mL) were introduced into a Hewlett-Packard® 6890 GC/5973 MS using a Tekmar 7000HT Headspace Analyzer/Autosampler. The autosampler platen/sample temperature was set to 80 °C, the sample equilibrium time was 20 minutes, and the transfer line and sample loop temperatures were 180 °C. Chromatographic conditions were as follows: DB-624, 30 m x 0.25 mm, 1.4 µm film thickness column (J&W Scientific, Folsom, CA); helium carrier gas at 0.7 mL/minute (3.52 psi); temperature program 40 °C for 2 minute to 230 °C at 10 °C/minute; and split ratio of 2:1.

**Sulfolane and Trichloroethanol.** A liquid-liquid extraction method was used for sulfolane and trichloroethanol. Aqueous samples (20 mL) were placed into 50 mL centrifuge tubes and were saturated with sodium chloride. The extraction solvents, methylene chloride (4 mL) for sulfolane and methyl-tert-butyl ether (7 mL) for trichloroethanol, were added and the tubes were shaken on an orbital shaker for 20 minutes at 180 oscillation/minute. After shaking, the tubes were centrifuged for 15 minute at 7500 rpm to separate the phases, and the solvent layer was transferred into a 25 mL sealed vial. The extraction process was repeated two additional times and the combined extracts were analyzed by gas chromatography using a temperature program of 50°C (2 minute hold) to 220 °C at 10°C/minute. Spike recoveries for sulfolane and trichloroethane were typically greater than 80%. Aqueous phase concentrations were not corrected for spike recoveries.

**Caffeine Analysis.** The concentration of caffeine in aqueous samples was directly measured using an Agilent Model 1100 High Performance Liquid Chromatograph
equipped with a Lichrospher RP 18 (250 mm x 4.6 mm, 5 µm particles) column
(Supelco, Bellefonte, PA), a diode array detector 1100 and autosampler. The elution
program was isocratic 70% water, 30% methanol with a flow rate of 1 mL/minute.

$^3$H$_2$O and $^{14}$C Analysis by Liquid Scintillation Counting. Liquid scintillation
counting (LSC) (Beckman LS 1701, Beckman Instruments, Inc., Fullerton, CA) was used
to directly determine the concentration of tritiated water ($^3$H$_2$O) and $^{14}$C labeled
compounds in aqueous samples, after adding appropriately 5 mL of Ready Gel®
scintillation cocktail to 2 mL of sample. Samples were counted to greater than 1%
precision. The exact sample volume was determined gravimetrically by multiplying the
measured mass by the density of water.

Calculation of TSCF. For each compound, the measured chemical concentration in
the xylem sap was divided by the concentration in the root-zone samples collected over
the same time interval and were plotted as a function of time in an approach analogous to
column breakthrough studies (Figure 4-1). The TSCF was approximated as the
calculated steady state value from the Hough Transform of the exponential fit approach
as described by Feinstein and Holt (57).

Compilation of TSCF Values from the Literature. TSCF values from 26 studies (1,
2, 4, 6, 7, 11, 15, 21, 23, 26, 28-30, 32-39, 53, 60-63) were compiled from the literature
and entered into a Microsoft Access® database developed specifically to record available
experimental information such as length of exposure, plant species, exposure
concentration, experimental method (e.g., soil/hydroponics/pressure chamber), in
addition to the TSCF value. The database is available from the authors upon request.
Results and Discussion

Pressure Chamber TSCF Measurements. The measured TSCF values for 25 compounds are summarized in Table 4-1 along with the corresponding log $K_{OW}$ values and the general methods used for analysis of the compounds. The TSCF values ranged from 0.01 to 0.98 while the log $K_{OW}$ values ranged from -0.77 to 5. The increased analytical sensitivity associated with the use of $^{14}$C-labeled compounds (10 of 26 compounds) enabled the direct measurement of chemical concentrations in the xylem fluid and root-zone samples along with tritiated water ($^3$H$_2$O). The $^3$H$_2$O was used to provide a direct comparison between water and chemical transport (Figure 4-1). As expected, the steady state TSCF value of $^3$H$_2$O was 1.0 in all experiments. The shape of the curves also suggests that the health and integrity of the root membranes was not impacted and no preferential flow paths were formed during root pressurization or sampling.

The effect of chemical hydrophobicity is illustrated in the TSCF versus time plot (Figure 4-1) for methanol (log $K_{OW} = -0.77$), benzene (log $K_{OW} = 2.13$), and pyrene (log $K_{OW} = 4.88$). The TSCF for methanol increased more rapidly and reached a higher steady state TSCF value (0.88) than benzene (0.4) and pyrene (0.04). The TSCF values obtained for the other highly polar compounds sulfolane, N-nitrosodimethylamine, 1,4-dioxane, caffeine, tert-butyl alcohol, and methyl tert-butyl ether, provide additional evidence that polar, neutral compounds appear to be rapidly taken up by roots and translocated to shoots. The inverse relationship between the rate of uptake of xenobiotics in a pressure chamber system and hydrophobicity has been previously observed (21, 53).
TABLE 4-1. Average measured TSCF, reported log $K_{OW}$ (reference) and method of analysis for compounds investigated (GC = Gas Chromatography, MS = Mass Spectrometry, ECD = Electro-Conductivity Detector, FID = Flame Ionization Detector, HPLC = High Performance Liquid Chromatography, LSC = Liquid Scintillation Counter, LLE = liquid liquid extraction, HS = headspace, N/A not applicable, (*measured using slow stir method and $^{14}$C-labeled compounds))

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS# or Formula</th>
<th>log $K_{OW}$</th>
<th>Average Measured TSCF</th>
<th># of Plants</th>
<th>SE</th>
<th>Analysis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water ($^{3}$H$_2$O)</td>
<td>7732-18-5</td>
<td>-1.38 (64)</td>
<td>1.00</td>
<td>26</td>
<td>0.01</td>
<td>LSC</td>
</tr>
<tr>
<td>Trichloroethanol</td>
<td>115-20-8</td>
<td>1.42 (64)</td>
<td>0.99</td>
<td>1</td>
<td>N/A</td>
<td>HS/GC/MS</td>
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<tr>
<td>1,4-Dioxane</td>
<td>123-91-1</td>
<td>-0.27 (64)</td>
<td>0.98</td>
<td>6</td>
<td>0.013</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>N-nitrosodimethylamine</td>
<td>62-75-9</td>
<td>-0.57 (64)</td>
<td>0.97</td>
<td>2</td>
<td>0.016</td>
<td>LSC</td>
</tr>
<tr>
<td>Methanol</td>
<td>67-56-1</td>
<td>-0.77 (64)</td>
<td>0.88</td>
<td>4</td>
<td>0.008</td>
<td>LSC</td>
</tr>
<tr>
<td>Sulfolane</td>
<td>126-33-0</td>
<td>-0.77 (64)</td>
<td>0.86</td>
<td>3</td>
<td>0.121</td>
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</tr>
<tr>
<td>Caffeine</td>
<td>58-08-2</td>
<td>-0.07 (65)</td>
<td>0.83</td>
<td>13</td>
<td>0.018</td>
<td>LLE/HPLC &amp; LSC</td>
</tr>
<tr>
<td>Methyl tert-butyl ether</td>
<td>1634-04-4</td>
<td>0.94 (64)</td>
<td>0.82</td>
<td>12</td>
<td>0.034</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>tert-butyl alcohol</td>
<td>75-65-0</td>
<td>0.35 (64)</td>
<td>0.80</td>
<td>4</td>
<td>0.024</td>
<td>LSC</td>
</tr>
<tr>
<td>Chloroform</td>
<td>67-66-3</td>
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<td>0.69</td>
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<tr>
<td>Toluene</td>
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<td>0.64</td>
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<td>0.073</td>
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</tr>
<tr>
<td>1,2-Dichloropropane</td>
<td>78-87-5</td>
<td>1.98 (66)</td>
<td>0.63</td>
<td>8</td>
<td>0.063</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>Compound</td>
<td>CAS# or Formula</td>
<td>log K&lt;sub&gt;OW&lt;/sub&gt;</td>
<td>Average Measured TSCF</td>
<td># of Plants</td>
<td>SE</td>
<td>Analysis method</td>
</tr>
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<td>------------------------------</td>
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</tr>
<tr>
<td>Benzene</td>
<td>71-43-2</td>
<td>2.13 (64)</td>
<td>0.59</td>
<td>7</td>
<td>0.075</td>
<td>HS/GC/MS &amp; LSC</td>
</tr>
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<td>1,2-Dichloroethylene</td>
<td>540-59-0</td>
<td>2.09 (64)</td>
<td>0.51</td>
<td>7</td>
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</tr>
<tr>
<td>Methylene Chloride</td>
<td>75-09-2</td>
<td>1.25 (64)</td>
<td>0.46</td>
<td>1</td>
<td>N/A</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>1,1,1-Trichloroethane</td>
<td>71-55-6</td>
<td>2.49 (64)</td>
<td>0.44</td>
<td>9</td>
<td>0.082</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>Carbon Tetrachloride</td>
<td>56-23-5</td>
<td>2.83 (64)</td>
<td>0.44</td>
<td>6</td>
<td>0.053</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>79-01-6</td>
<td>2.42 (64)</td>
<td>0.43</td>
<td>10</td>
<td>0.064</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>1,1,2,2-Tetrachloroethane</td>
<td>79-34-5</td>
<td>2.39 (64)</td>
<td>0.36</td>
<td>2</td>
<td>0.017</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>127-18-4</td>
<td>3.4 (64)</td>
<td>0.30</td>
<td>7</td>
<td>0.056</td>
<td>HS/GC/MS</td>
</tr>
<tr>
<td>Nonylphenol Tetraethoxylate</td>
<td>26027-38-3</td>
<td>3.1* (64)</td>
<td>0.21</td>
<td>2</td>
<td>0.003</td>
<td>LSC</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>84852-15-3</td>
<td>3.2* (64)</td>
<td>0.18</td>
<td>2</td>
<td>0.011</td>
<td>LSC</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>85-01-8</td>
<td>4.46 (64)</td>
<td>0.15</td>
<td>1</td>
<td>N/A</td>
<td>LSC</td>
</tr>
<tr>
<td>Nonylphenol Nonylethoxylate</td>
<td>26027-38-3</td>
<td>3.0* (64)</td>
<td>0.07</td>
<td>1</td>
<td>N/A</td>
<td>LSC</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>87-86-5</td>
<td>5.12 (64)</td>
<td>0.07</td>
<td>1</td>
<td>N/A</td>
<td>GC/ECD</td>
</tr>
<tr>
<td>Pyrene</td>
<td>129-00-0</td>
<td>4.88 (65)</td>
<td>0.04</td>
<td>2</td>
<td>0.015</td>
<td>LSC</td>
</tr>
</tbody>
</table>
FIGURE 4-1. TSCF values versus time for methanol and tritiated water run simultaneously. Results from separate experiments with benzene and pyrene shown for comparison. Solid lines represent Hough Transform exponential fitting of the respective data used to calculate steady state.

Unlike previous bell-shaped relationships (2, 4, 21), Figure 4-2 shows a nearly sigmoidal relationship between TSCF and \( \log K_{ow} \) with the TSCF data approaching the theoretical bounds of TSCF at 1 and 0, for hydrophilic and hydrophobic compounds, respectively. A sigmoidal fit of the experimental data (SigmaPlot version 8.0 nonlinear regression wizard) using a maximum TSCF of 1 produces Equation (14) \( (r^2 = 0.68, SE = 0.16) \).

\[
TSCF = \frac{11}{1 + 2.6^{\log K_{ow}}} 
\]

(14)

Where:
- TSCF = Transpiration Steam Concentration Factor (unitless)
- \( K_{ow} \) = Octanol/water coefficient (unitless)
A linear model (TSCF = -0.15 log $K_{OW} + 0.87$, $r^2 = 0.66$, SE= 0.16) gives similar statistics but could generate estimated TSCFs outside the theoretical bounds. The trend observed in Figure 4-2 is also consistent with the increasing number of observations in the literature reporting high root uptake for low log $K_{OW}$ compounds such as sulfolane, MTBE, TBA, and 1,4-dioxane (7, 11, 23, 30, 36, 38).

The difference between the relationship between TSCF and log $K_{OW}$ obtained in this study and those previously reported is not clear, but could be due to a variety of factors including: differences in methods used to generate TSCF (intact vs. pressure chamber), plant growth conditions (hydroponic vs. soil), plant species, plant age, duration, transpiration, losses due to metabolism and volatilization, etc. For example, the widely referenced relationship by Briggs et al. (2) reported TSCF values for 18 compounds that were obtained using 10 day old barley plants (root weight 0.1 g) that were exposed hydroponically to the chemicals for 24-48 hours. The small volume of transpired water during the Briggs experiment, (1 mL/day) makes it difficult to determine if the plants were healthy and actively growing during the very short exposure period.

The plants used in this study had root masses ranging between 20-110 g fresh wt and the volume of water pushed through the plants ranged from 300 to 3000 mL. While TSCFs are assumed independent of plant size and amount of water transpired, it is likely there is a minimum volume of water that needs to move through the plant before a representative TSCF can be determined as illustrated in the exponential rise of the xylem/solution concentration ratios (TSCFs) seen in the pressure chamber data (Figure 4-1).
Plant health and growth conditions are known to impact root membrane integrity and thus are likely to also effect plant root uptake of organics (10, 67-69). As illustrated in Figure 4-3, adding mercuric chloride at levels toxic to the roots during several TSCF measurements caused chemical concentrations in the xylem to quickly increase and reach a steady state value approaching that of the exposure solution (TSCF = 1). This apparent loss of root membrane integrity corresponded to a distinct change in root appearance, emphasizing the importance of root health in maintaining the normal regulation of plant root uptake.
**Literature TSCF Values and the Relationship with log $K_{OW}$.** In an attempt to better understand the potential reasons for the difference between the TSCF-log $K_{OW}$ relationship reported here and the previous bell-shaped curves, more than 150 refereed publications relating to root uptake of organic contaminants were reviewed. Seventeen papers contained reported TSCFs while nine additional publications provided enough information that TSCF values could be calculated (1, 2, 4, 6, 7, 11, 15, 21, 23, 26, 28-30, 32-39, 53, 60-63). This resulted in 132 total TSCF values for 93 individual compounds ranging in log $K_{OW}$ from -1.8 to 5.4. Due to differences in the level of experimental details provided, all TSCF values were assumed to be of equal quality and no values were rejected. Out of the 132 total TSCF values, 95 values came from studies where intact plants were used and 37 from pressure chamber studies. Intact plants were typically grown and exposed hydroponically but spiked soils were used in three separate experiments. Soybean, poplar, and barley were the most common species investigated. Corresponding log $K_{OW}$ values were obtained (in preferential order of their availability) from the individual studies themselves, Hansch and Leo (64), EPI Suite PhysProp Database (70), and estimated using EPI Suite KOWWIN (70) for compounds with no reported value.

Unlike previous reports from a single study (2, 4, 21) no apparent relationship (visually or statistically) between TSCF and log $K_{OW}$ was found using the combined dataset of 132 literature values (Figure 4-4). There did not appear to be any preferential scattering as a function of log $K_{OW}$ with a wide range (0 to 1) evenly spread across all log $K_{OW}$s.
FIGURE 4-3. Xylem/solution chemical concentration ratio uptake curves for caffeine with (open circle) and without (closed circle) mercuric chloride addition. The steady state TSCF values for caffeine were 0.83 and 1.0 for non-poisoned and poisoned experiments, respectively.

In addition to the overall scatter in the data (Figure 4-4), for compounds having TSCF values reported in more than one publication, large variations were observed. Because intact plant measurements may include loses not associated with the pressure chamber, the difference in the experimental approach used (intact vs. pressure chamber) was assumed to be the most important factor in TSCF variability. However, differences in operational variables such as exposure duration, plant age, growth conditions, and species, could also contribute to the variability, especially for intact plant studies. To examine this possibility, the literature data were separated into two groups: TSCF values generated from intact plants and those generated using a pressure chamber method.

Since the pressure chamber technique has fewer operational variables, the approach would be expected to yield more consistent data than intact plant studies. In addition, soybeans of similar age and exposure period (6-48 hours) were used in the three literature
pressure chamber studies (11, 21, 53) further reducing potential sources of variability. As shown in Figure 4-5, the relationship between the literature pressure chamber TSCF values and log $K_{OW}$ appears to reach a maximum approaching 1 and a minimum approaching 0, very similar to the results reported in this study with neutral, polar organics having the highest potential to be taken up by plant roots.

It can also be observed that pressure chamber derived TSCF values are generally higher than those obtained from intact plant studies (Figure 4-5). Chemical losses due to volatilization and metabolism are likely greater during the longer duration and open exposure experiments typically used for intact plant TSCF determinations. If not
completely corrected for, such losses result in lower TSCF values. Low extraction efficiencies and non-uniform distribution of compound within shoot tissues could also contribute to low TSCF values, especially if subsamples are used to calculate the amount of chemical within the entire foliar tissue.

Overall, the TSCF values generated in this study and obtained from the literature suggest that bell-shaped curves between TSCF and log $K_{ow}$ greatly underestimate the potential plant uptake and transport into shoot tissue of highly polar, water soluble, neutral organics with low $K_{ow}$ values. The literature data also suggest that pressure chamber derived TSCF values tend to be greater than those derived from intact plant studies.

The pressure chamber approach provides a rapid and reproducible method for generating TSCF values that can be used in risk and phytoremediation assessments. The pressure chamber TSCF values may be considered a measurement of the maximum translocation potential. Examining the difference between pressure chamber and intact plant TSCF values may give an indication of the importance of loss mechanisms such as volatilization and metabolism. The lack of standardized methods or guidelines for TSCF measurement will continue to hamper the development of approaches or models that can be used to accurately predict plant uptake and tissue concentrations for risk assessment purposes. As a result of less operational variables, the pressure chamber is the most robust method for determining plant uptake potential. It results in the most consistent data and indicates that the relationship between plant uptake potential and log $K_{ow}$ is
sigmoidal with polar compounds most likely to be taken up and not bell-shaped as previously reported.

**Supporting Information**

Figure 4-6 Contains a diagram of the pressure chamber system used to measure Transpiration Stream Concentration Factors. The diagram specifically illustrates the configuration used for measurements in tomato plants.

![Pressure chamber in tomato plant configuration used to measure Transpiration Stream Concentration Factors](image)

**FIGURE 4-6.** Pressure chamber in tomato plant configuration used to measure Transpiration Stream Concentration Factors
**Determination of Steady State TSCF.** The steady state TSCF value was calculated using a Hough Transform approach as described by Feinstein and Holt and adapted for use with measured pressure chamber data (Equation 10). The derivative (\( \hat{y} \)) of the measured value (\( y, \text{mg L}^{-1}/\text{mg L}^{-1} \)) was approximated using Equation (11).

\[
\begin{bmatrix}
\tau \\
TSCF
\end{bmatrix} = \begin{bmatrix}
- \sum_{i=1}^{N} \hat{y}_i & \sum_{i=1}^{N} 1 \\
- \sum_{i=1}^{N} \hat{y}_i^2 & \sum_{i=1}^{N} \hat{y}_i \\
\end{bmatrix} \begin{bmatrix}
\sum_{i=1}^{N} y_i \\
\sum_{i=1}^{N} \hat{y}_i y_i
\end{bmatrix}
\]

\( \hat{y} = \frac{y_i - y_{i-1}}{t_i - t_{i-1}} \)  

Equation (11)
CHAPTER 5

PLANT ROOT UPTAKE OF ORGANIC COMPOUNDS:
A MODEL FOR PREDICTING TSCF

Abstract

Plant root uptake models range in complexity from simple partition models to complex mechanistic models. The majority of models rely on bell-shaped curves relating TSCF to octanol/water partition coefficient (log $K_{OW}$) implying an optimal lipophilicity is necessary for significant uptake and transport of compounds. Compounds that are either highly polar or are very lipophilic are not expected to be significantly translocated using these models. Recent experimental data suggests that the bell-shaped relationships the models are based on are not appropriate, especially for neutral, polar organics. Using experimental values generated in a recent study, a new model is developed based on mass transfer rates resulting in a new relationship between TSCF and Low $K_{OW}$. The relationship is a result of a new modeling approach, which has its basis in mass transfer rates of organics as a function of their log $K_{OW}$. The model appears to be the first for organic uptake that also includes the important physiological pathway of water uptake through aquaporins. The model assumes that root uptake of most xenobiotic organic compounds occurs passively in association with transpired water and the movement of these chemicals from root to shoot is ultimately controlled by the passage through one or more lipophilic root membranes. The model was used to investigate the species dependence of TSCF based on tomato and soybean measurements. The model suggests

$^{1}$Authored by Erik Dettenmaier, William Doucette, Bruce Bugbee
that one of the most widely used regressions for estimating plant uptake by roots in risk assessments is not appropriate, especially for the relatively polar, neutral organics.

Introduction

Predicting the root uptake and subsequent transport of organic contaminants to above ground tissues is important in conducting both phytoremediation studies and risk assessments. Root uptake of organic compounds is a passive process correlated to the amount of water transpired (15). Introduced by Shone and Wood (1), the Transpiration Stream Concentration Factor (TSCF) is defined as the ratio of the chemical concentration in the xylem to the concentration of the chemical in the water used by the plant for transpiration and is widely used in root uptake models. By relating uptake to transpiration, differences related to factors such as environmental conditions, plant size and exposure time can be accounted for. A simple estimate of the mass of chemical taken up by roots can be made by multiplying the TSCF by the volume of water transpired and the concentration of chemical in the water used by the plant.

Unfortunately, experimentally determined TSCF values are lacking so values estimated from empirical relationships with the logarithm octanol/water partition coefficients (log $K_{\text{OW}}$) are often used (2). These relationships suggest an optimal hydrophobicity for uptake and translocation and infer that compounds that are either highly polar (log $K_{\text{OW}} < 1$) or are highly lipophilic (log $K_{\text{OW}} > 4$) will not be significantly taken up by plants (2, 4, 21). However, recent TSCF data generated for 25 organic compounds using a pressure chamber technique and other reports of highly water soluble, low log $K_{\text{OW}}$ compounds being readily taken up by plants (7, 11, 30, 36, 38, 71, 72), suggest that the general suitability of these estimation techniques should be re-evaluated.
Using the newly reported pressure chamber dataset (Chapter 4), a new model of chemical root uptake potential, as measured by TSCF, is presented. This model also incorporates the important aquaporin water uptake pathway that can account for a significant fraction of water uptake (73-76) depending on the environmental conditions and may account for some of the variability observed in measured TSCF values. The model was also used to investigate the cause of the subtle differences between the chemical root uptake of tomatoes and soybeans seen in the dataset reported in Chapter 4. The model serves as a revised translocation module for more complete uptake models that incorporate phytovolatilization, deposition, and metabolism.

**Materials and Methods**

**TSCF Values.** The TSCF values used in model development were measured using a pressure chamber technique (Chapter 4). Briefly, 25 organic chemicals ranging in log $K_{OW}$ from -0.8 to 5 were used. Immediately prior to the start of a pressure chamber experiment, test plants (soybean and tomato) were removed from the hydroponic solution and cut just below the first cotyledonary node (lowest leaves) to remove all of the shoot tissue except for the stem base. A short length (5 cm) of butyl rubber (tomato) or rigid platinum cured silicone tubing (soybean) was then fit over the cut stem forming a gasket that was inserted directly through the stem plate finally the roots were immediately immersed in a stainless steel vessel containing oxygen saturated nutrient solution spiked with a known concentration of a compound. The stem plate was then secured to the vessel with a threaded collar and the tip of a disposable pipette was affixed over the cut stem and under the gasket. Compressed oxygen was slowly (20 to 40 mL min$^{-1}$) bubbled into the nutrient solution to build pressure within the chamber to aerate and mix the root-
zone. The pressure was gradually increased and the flow rate decreased (5 to 10 mL min\(^{-1}\)) until a xylem sap flow rate of approximately 70% of the intact plant maximum transpiration rate was reached as measured gravimetrically over the previous 24 hours. The pressure was usually about 150 kPa. Samples of xylem sap exiting the cut stem, and the root-zone exposure solution were collected every 10-30 minutes and analyzed for the compounds of interest. Xylem sap samples were collected using a fraction collector (ISCO, CYGNET, Lincoln, NE) and samples of the root-zone exposure solution were collected through a septum-sealed port using a glass syringe. The methods used to determine the concentrations of the test chemicals in xylem sap and exposure solutions depended on the properties of the compounds. Volatile compounds were analyzed by headspace GC/MS, polar non-volatiles by HPLC, and \(^{14}\)C-labeled compounds by liquid scintillation counting (LSC).

**Lipid and Water Fraction of Plant Roots.** Fresh tissue weights were recorded and the plant tissue was air-dried for 7 days. The tissue was then placed in a desiccator until a constant mass was obtained, usually 24-36 hours. The water content was determined from the difference of the fresh and dry weights. After drying, the tissue was finely ground in a coffee grinder and approximately two grams dry weight was placed in 25/80 mm cellulose thimbles. Thimbles were then placed in desiccators until a steady mass was obtained, again approximately 24 hours. After noting the dry weight, thimbles were placed in a Soxhlet Extractor and extracted for 24 hours with ethyl ether. At the end of the extraction, the remaining ethyl ether was evaporated and the Erlenmeyer flasks were placed in desiccators until a steady mass was obtained. Differences in post-experiment
and pre-experiment flask weights were used to determine the mass of lipid extracted. Spikes were performed by adding a known quantity of olive oil to selected thimbles.

**Model Development**

**Uptake and Transport Process.** During transpiration, water and organic chemical solutes move passively through the roots and into the xylem column (10, 77) by one or more pathways (Figure 5-1). In symplastic transport, water and solutes move through the plasma membranes and into the interior of individual root cells, while apoplastic transport occurs when water and solutes pass between the roots cell membranes until reaching the Casparian strip (10). In addition to the symplastic and apoplastic routes, aquaporins act as gated channels that allow water transport across the plasma membrane with minimal resistance (78). These channels are very selective, preventing nearly all other compounds from entering, and represent bypass routes for water and possibly a few other compounds that have very similar structural features to water (e.g., glycerol) (14, 79, 80). Overall transport is thought to be a combination of all pathways in which the properties of the compound being transported and the rate of plant transpiration determine the relative fraction of each (77). The passive root uptake of organic solutes is ultimately controlled by diffusion across one or more semi-permeable, lipid bilayer membranes.

**Mass Transfer Model.** Outside of empirical fits, current chemical root uptake models range from the simple partition-limited approach of Chiou et al. (45), to more complex mechanistic approaches involving diffusive and advective transport (e.g., Trapp 17, 48). However, none of the models account for water moving through aquaporins (Figure 5-2).
Conceptually, this model is based on three compartments as illustrated in Figure 5-2: the aqueous solution, the membrane encompassing the Epidermis, Cortex and Endodermis cells (Figure 5-1), and the xylem. Organic solutes are brought towards the roots with the water used for transpiration. The continuous advective transport of organic solutes to the roots helps maintain an elevated concentration of chemical in the solution near the roots and physically transports the chemical directly to the surface of the root lipid material. Since water passes through the root membranes faster than the organic solutes, the diffusion out of and not into the regulating membrane becomes the rate-limiting step for a compound entering the xylem stream. The model’s main postulate is that, the TSCF is a function of the partitioning rate of the compound out of a lipid membrane and into the xylem as illustrated in Figure 5-2.
As previously mentioned, the TSCF is a ratio of the chemical concentration in the xylem divided by the chemical concentration in the solution surrounding the root (1):

\[
TSCF = \frac{C_{xylem}}{C_{sol}}
\]  
\(15\)

Where:

- \(TSCF\) = Transpiration Steam Concentration Factor (unitless)
- \(C_{xylem}\) = concentration of chemical in xylem (mass length\(^{-3}\))
- \(C_{sol}\) = concentration of chemical in solution (mass length\(^{-3}\))

The chemical concentration in the xylem is equivalent to the mass of compound in the xylem stream divided by the volume of that xylem stream and can be calculated using Equation (16).

**FIGURE 5-2. Simplified conceptual illustration of model for the plant root uptake process**
\[
C_{\text{xylem}} = \frac{M_{\text{xylem}}}{V_{\text{xylem}}}
\] (16)

Where:
- \(C_{\text{xylem}}\) = concentration of chemical in xylem (mass length\(^{-3}\))
- \(M_{\text{xylem}}\) = Mass of chemical in xylem (mass\(^{-1}\))
- \(V_{\text{xylem}}\) = Volume of xylem (length\(^3\))

The xylem volume in Equation (16) can be calculated from the transpiration rate. The transpiration rate or flow rate of the xylem is equal to the volume of xylem per time, thus the xylem volume is simply the flow rate of xylem, multiplied by time as shown in Equation (17).

\[
V_{\text{xylem}} = Q_{\text{xylem}} \times t
\] (17)

Where:
- \(V_{\text{xylem}}\) = Volume of xylem (length\(^3\))
- \(Q_{\text{xylem}}\) = Flow rate of xylem (length\(^3\) time\(^{-1}\))
- \(t\) = Time (time)

The mass of compound in the xylem in Equation (16) can be calculated directly from the flux of the compound into and out of the membrane accounting for both the area the compound is transporting through, and the duration of that flux as shown in Equation (18).

\[
M_{\text{xylem}} = J \times A_{\text{flux}} \times t
\] (18)

Where:
- \(M_{\text{xylem}}\) = Mass of chemical in xylem (mass\(^{-1}\))
- \(J\) = Flux (chemical-mass length\(^{-2}\) time\(^{-1}\))
- \(A_{\text{flux}}\) = Area perpendicular to the flux (length\(^2\))
- \(t\) = Time (time)

Fick’s first law for calculating flux assumes simple diffusion in a gas phase however it can be adapted for calculating flux across a membrane (81). Fick’s first law for flux at
steady state conditions, assuming the direction is implied by the process, is calculated using Equation (19):

\[
J = D_m \times \frac{dC_m}{dx}
\]  

(19)

Where:
- \( J \) = Flux (mass length\(^{-2}\) time\(^{-1}\))
- \( D_m \) = Diffusivity of chemical through the membrane (length\(^2\) time\(^{-1}\))
- \( \frac{dC_m}{dx} \) = change in concentration with distance (mass length\(^{-4}\))

The flux into the root is modeled as a two-step process over three-phases in which the compound fluxes from bulk solution (phase 1) into the membrane (phase 2) then out of the membrane into the xylem (phase 3). The flux into mammalian epithelial membranes is commonly approximated using a modified form of Fick’s law in which the distribution coefficients between the bulk solution phase and the membrane phase is used to approximate the permeability of the membrane. Using the octanol/water distribution coefficient \( K_D \), for the distribution coefficient gives the flux into the membrane as:

\[
J_{in} = D_m \times K_D \times C_{sol} / h
\]  

(20)

Where:
- \( J_{in} \) = Flux into the membrane (mass length\(^{-2}\) time\(^{-1}\))
- \( D_m \) = Diffusivity of chemical into the membrane (length\(^2\) time\(^{-1}\))
- \( K_D \) = Octanol/water distribution coefficient (unitless)
- \( C_{sol} \) = Concentration of chemical in solution (mass length\(^{-3}\))
- \( h \) = Distance of diffusion (length)

Like the flux into the membrane the flux out is partition driven, so that the flux out of the lipid membrane and into the xylem, another aqueous compartment, would be inversely proportional to \( K_D \).
\[ J_{\text{out}} = \frac{D_m \times C_{\text{sol}}}{K_D \times h} \]  

(21)

Where:

- \( J_{\text{out}} \) = Flux out of the membrane (chemical-mass length\(^{-2}\) time\(^{-1}\))
- \( D_m \) = Diffusivity of chemical into the membrane (length\(^{2}\) time\(^{-1}\))
- \( K_D \) = Octanol/water distribution coefficient (unitless)
- \( h \) = Distance of diffusion (length)
- \( C_{\text{sol}} \) = Concentration of chemical in solution (mass length\(^{-3}\))

Under a constant stir scenario, assuming a membrane thickness of 125 μm or less, flux by diffusion into the membrane can be achieved in less than 3 minutes (82). For plants undergoing transpiration, the solution is continuously drawn into the root, further decreasing the into membrane equilibration time while increasing the out of membrane equilibration time. These differences in time to steady state, means that the flux into the membrane is larger than the flux out of the membrane.

\[ J_{\text{in}} > J_{\text{out}} \]  

(22)

Where:

- \( J_{\text{in}} \) = Flux into the membrane (mass length\(^{-2}\) time\(^{-1}\))
- \( J_{\text{out}} \) = Flux out of the membrane (mass length\(^{-2}\) time\(^{-1}\))

The flux of a compound across the membrane is therefore limited by the flux out of the membrane and \( J_{\text{total}} \) can be approximated use the equation for \( J_{\text{out}} \).

\[ J_{\text{total}} = \frac{D_m \times C_{\text{memb}}}{K_D \times h} \]  

(23)

Where:

- \( J_{\text{total}} \) = Total flux through the membrane (mass length\(^{-2}\) time\(^{-1}\))
- \( D_m \) = Diffusivity of chemical through the membrane (length\(^{2}\) time\(^{-1}\))
- \( K_D \) = Octanol/water distribution coefficient (unitless)
- \( h \) = Membrane thickness (length)
- \( C_{\text{memb}} \) = Concentration of chemical in the membrane (mass length\(^{-3}\))
If the mass transport in is much greater than the mass transport out and thus effectively at steady state in relation to $J_{\text{out}}$ Equation (22), the concentration in the membrane can be calculated from the solution concentration and $K_D$

$$C_{\text{memb}} = C_{\text{sol}} \times K_D$$  \hspace{1cm} (24)

Where:
- $C_{\text{memb}} = \text{Concentration of chemical in the membrane (mass length}^{-3})$
- $C_{\text{sol}} = \text{Concentration of the chemical in solution (mass length}^{-3})$
- $K_D = \text{Octanol/water distribution coefficient (unitless)}$

For neutral organics, the distribution coefficient, $K_D$, can be replaced by the partition coefficient, $K_{OW}$.

$$C_{\text{memb}} = C_{\text{sol}} \times 10^{\log K_{OW}}$$  \hspace{1cm} (25)

Where:
- $C_{\text{memb}} = \text{Concentration of chemical in the membrane (mass length}^{-3})$
- $C_{\text{sol}} = \text{Concentration of the chemical in solution (mass length}^{-3})$
- $K_{OW} = \text{Octanol/water partition coefficient (unitless)}$

Values for $K_{OW}$ are typically reported on a log basis and although $10^{\log K_{OW}}$ is simply $K_{OW}$, they are displayed explicitly, for convenience and, to eliminate potential confusion.

$$J_{\text{total}} = \frac{D_m \times C_{\text{sol}} \times 10^{\log K_{OW}}}{10^{\log K_{OW}} \times h}$$  \hspace{1cm} (26)

Where:
- $J_{\text{total}} = \text{Flux through the membrane (mass length}^{-2} \text{ time}^{-1})$
- $D_m = \text{Diffusivity of chemical through the membrane (length}^2 \text{ time}^{-1})$
- $K_{OW} = \text{Octanol/water partition coefficient (unitless)}$
- $h = \text{Membrane thickness (length)}$
- $C_{\text{sol}} = \text{Concentration of chemical in solution (mass length}^{-3})$
The diffusivity of the compound in the xylem divided by the diffusion thickness is the partitioning rate or velocity (83), and represents a mass transfer coefficient that has units of conductance. The flux of compound out of the membrane and into the xylem stream becomes (83):

\[ J_{\text{total}} = P_m \times C_{\text{sol}} \] (27)

Where:
- \( J_{\text{total}} \) = Flux through the membrane (mass length\(^{-2}\) time\(^{-1}\))
- \( P_m \) = Permeability of the membrane to the compound (length time\(^{-1}\))
- \( C_{\text{sol}} \) = Concentration of chemical in solution (mass length\(^{-3}\))

Because the uptake of most organic compounds is a passive process (1, 2, 15, 21, 45) the compound partitioning rate must be fractionally bounded between 0 and 1 times the permeation rate of water through the membrane. This is accomplished by postulating that the chemical permeation rate of the root membrane is relative to the permeation rate of water and the fugacity ratio of the xylem and membrane phases.

\[ P_m = \frac{Z_{\text{xylem}}}{Z_{\text{Membrane}}} \times P_w \] (28)

Where:
- \( P_m \) = Permeability of the membrane to the compound (length time\(^{-1}\))
- \( Z_{\text{xylem}} \) = Xylem fugacity of chemical (mass time\(^{-2}\) length\(^{-1}\))
- \( Z_{\text{Membrane}} \) = Membrane fugacity of chemical (mass time\(^{-2}\) length\(^{-1}\))
- \( P_w \) = Permeability of the membrane to water (length time\(^{-1}\))

The fugacity ratio is a function of the amount of lipid-like material, aqueous material, and the compound’s solubility in each (84), and can be approximated by \( K_D \) or \( K_{OW} \) in the case of neutral organics.

\[ \frac{Z_{\text{membrane}}}{Z_{\text{xylem}}} = \theta_{\text{water}} + \alpha \times 10^{\log K_{OW}} \] (29)

Where:
- \( Z_{\text{Membrane}} \) = Membrane fugacity of chemical (mass time\(^{-2}\) length\(^{-1}\))
\[ Z_{\text{xylem}} = \text{Xylem fugacity of chemical (mass time}^{-2} \text{ length}^{-1}) \]
\[ \theta_{\text{water}} = \text{Wet weight mass fraction of water in root (unitless)} \]
\[ \alpha = \text{Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)} \]
\[ K_{\text{OW}} = \text{Octanol/water partition coefficient (unitless)} \]

The rate of a compound’s translocation from the bulk solution to the xylem as a function of hydrophobicity has long been reported \((21, 53)\) as being inversely related to \(K_{\text{OW}}\).

\[
P_m = \frac{1}{\theta_{\text{water}} + \alpha \times 10^{\log K_{\text{ow}}}} \times P_w \tag{30}
\]

Where:
\(P_m = \text{Permeability of the membrane to the compound (length time}^{-1})\)
\(\theta_{\text{water}} = \text{Wet weight mass fraction of water in root (unitless)}\)
\(\alpha = \text{Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)}\)
\(K_{\text{OW}} = \text{Octanol/water partition coefficient (unitless)}\)
\(P_w = \text{Permeability of the membrane to water (length time}^{-1})\)

In Equation (30):

\[
\alpha = \theta_{\text{lipid}} \times \beta_{\text{lipid}} + \theta_{\text{Carb}} \times \beta_{\text{Carb}} + \theta_{\text{Protein}} \times \beta_{\text{Protein}} \tag{31}
\]

Where:
\(\alpha = \text{Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)}\)
\(\theta_{\text{lipid}} = \text{Wet weight mass fraction of lipid in root (unitless)}\)
\(\beta_{\text{lipid}} = \text{Affinity of chemical to lipid material in root (unitless)}\)
\(\theta_{\text{Carb}} = \text{Wet weight mass fraction of carbohydrate in root (unitless)}\)
\(\beta_{\text{Carb}} = \text{Affinity of chemical to carbohydrate material in root (unitless)}\)
\(\theta_{\text{Protein}} = \text{Wet weight mass fraction of protein in root (unitless)}\)
\(\beta_{\text{Protein}} = \text{Affinity of chemical to protein material in root (unitless)}\)

The membrane permeation rate of water represents only the water that passes through the membrane using the same route as the compound. This pathway is traditionally viewed as comprising the symplastic and apoplastic pathways. However, the total amount of water passing through the root at any given time also includes water that passes through special channels that span plant membranes known as aquaporins. These channels are very selective preventing nearly all other compounds from entering, and
represent bypass routes for water only (14, 79, 80). The total water permeation rate equals the membrane route and the fraction of total water making use of the aquaporin channels:

\[ P_{\text{total}} = P_w + \gamma \times P_{\text{total}} \]  \hspace{1cm} (32)

Where:
- \( P_{\text{total}} \) = Permeability of the membrane to water including aquaporin bypass (length time\(^{-1}\))
- \( P_w \) = Permeability of membrane to water (length time\(^{-1}\))
- \( \gamma \) = Aquaporin bypass fraction (unitless)

The total flow into the root must be equal to the flow out of the root and thus the xylem stream can be represented as the flux area, the same as the compound flux area, multiplied by the total flux or velocity of water:

\[ Q_{\text{xylem}} = A_{\text{flux}} \times P_{\text{total}} \]  \hspace{1cm} (33)

Where:
- \( Q_{\text{xylem}} \) = Flow rate of xylem (length\(^3\) time\(^{-1}\))
- \( A_{\text{flux}} \) = Area perpendicular to the flux (length\(^2\))
- \( P_{\text{total}} \) = Permeability of the membrane to water including aquaporin bypass (length time\(^{-1}\))

Making the above substitutions:

\[ TSCF = \frac{1}{\theta_{\text{water}} + \alpha \times 10^{\log_{10} K_{OW} (P_{\text{total}} - \gamma \times P_{\text{total}}) \times C_{\text{sol}} \times A_{\text{flux}} \times t}} \] \hspace{1cm} (34)

Where:
- TSCF = Transpiration Steam Concentration Factor (unitless)
- \( \theta_{\text{water}} \) = Wet weight mass fraction of water in root (unitless)
- \( \alpha \) = Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)
- \( K_{OW} \) = Octanol/water partition coefficient (unitless)
- \( P_{\text{total}} \) = Permeability of the membrane to water including aquaporin bypass (length time\(^{-1}\))
- \( \gamma \) = Aquaporin bypass fraction (unitless)
- \( C_{\text{sol}} \) = concentration of chemical in solution (chemical-mass length\(^{-3}\))
- \( A_{\text{flux}} \) = Area perpendicular to the flux (length\(^2\))
- \( t \) = Time (time)
Simplifying this equation results in a TSCF for neutral organics that is dependent on the fractional water content of the plant root and the fractional aquaporin pathway; while being inversely proportional to the octanol/water partition coefficient, and plant material affinity:

\[
TSCF = \frac{1 - \gamma}{\theta_{\text{water}} + \alpha \times 10^{\log K_{OW}}} \tag{35}
\]

Where:
- \( TSCF \) = Transpiration Steam Concentration Factor (unitless)
- \( \theta_{\text{water}} \) = Wet weight mass fraction of water in root (unitless)
- \( \alpha \) = Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)
- \( K_{OW} \) = Octanol/water partition coefficient (unitless)
- \( \gamma \) = Aquaporin bypass fraction (unitless)

**Results and Discussion**

**Calculated Species Differences.** The dataset used to validate the model is provided in Chapter 4. This dataset consists of TSCF values generated using a pressure chamber for 25 common organic compounds of concern and contains data for both soybean and tomato plants. The basic model, Equation (35), contains two general parameters, aquaporin bypass fraction and plant material affinity, which could reasonably be expected to be influenced by species differences. Investigating the possibility of these differences consisted of determining if there was any difference in the fit of these parameters by species:

\[
TSCF = \frac{1 - \gamma'}{\theta_{\text{water}} + \alpha' \times 10^{\log K_{OW}}} \tag{36}
\]

Where:

\[
\gamma' = \gamma_0 + \omega \times z \tag{37}
\]
\[ \alpha' = \alpha_0 + \varphi \times z \]  

TSCF = Transpiration Steam Concentration Factor (unitless)
\[ \theta_{\text{water}} = \text{Wet weight mass fraction of water in root (unitless)} \]
\[ K_{\text{OW}} = \text{Octanol/water partition coefficient (unitless)} \]
\[ \gamma_0 = \text{Aquaporin bypass fraction associated with soybean and tomato (unitless)} \]
\[ \alpha_o = \text{Affinity of chemical to and wet weight mass fraction of root lipid material to soybean and tomato (unitless)} \]
\[ \omega = \text{Aquaporin bypass fraction associated with only soybeans (unitless)} \]
\[ \varphi = \text{Affinity of chemical to and wet weight mass fraction of root lipid material associated with only soybeans (unitless)} \]
\[ z = \text{species dependant factor, equal to 0 for tomato, 1 for soybean (unitless)} \]

Using the nonlinear least squares method, a 95% confidence interval for \( \omega \) is between -0.163 and 0.047 and so it does not significantly differ from zero. This result implies that for this dataset there was no significant difference between tomato and soybean concerning aquaporin by-pass fraction under the exposure and flow rate scenario of the pressure chamber. The 95% confidence interval for \( \varphi \) was between -0.006 and -0.001 and was significantly different from zero (\( p<0.001 \)), indicating that there is a difference in either the amount or affinity to root material represented by \( (\alpha) \) between the two species (Table 5-1).

**Measured Species Differences.** In the TSCF model, only \( \theta_{\text{water}} \) and approximate \( \theta_{\text{lipid}} \) of \( \alpha \) were measured for this experiment. For \( \theta_{\text{water}} \), there was essentially no difference in measured values, 0.903 and 0.900, for the roots of either tomato or soybeans species respectively. There was, however, a statistically significant (\( p<0.05 \)) difference in the wet weight fractional lipid content as measured by Soxhlet extraction (Table 5-1). Spike recoveries of \( \theta_{\text{lipid}} \) for tomato were (96.6\% ± 8.7\%: 95\% CI) and (103\% ± 3\%: 95\% CI) for soybean.
TABLE 5-1. Results of species different nonlinear least squares fitting of pressure chamber data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tomato</th>
<th>Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha'$</td>
<td>0.0072</td>
<td>0.0072</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>-0.00564</td>
<td>-0.00564</td>
</tr>
<tr>
<td>$z$</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$\alpha_0$</td>
<td>0.0072</td>
<td>0.0016</td>
</tr>
<tr>
<td>$^{*}\theta_{\text{lipid}}$</td>
<td>0.00062</td>
<td>0.00047</td>
</tr>
<tr>
<td>$\beta_{\text{lipid}}$</td>
<td>12</td>
<td>3.4</td>
</tr>
<tr>
<td>$^{*}\theta_{\text{water}}$</td>
<td>0.903</td>
<td>0.900</td>
</tr>
</tbody>
</table>

*Measured Value

Using a simplified form of Equation (31) assuming no significant species differences in protein or carbohydrate fraction, $\theta_{\text{lipid}}$ accounts for some but not all of the species differences inferred by the statistically fitted parameters of the model. The resulting values for $\beta_{\text{lipid}}$ would need to be 12 for tomato and 3.4 for soybean. Strictly from a plant lipid perspective, a difference in ($\beta$) of three times suggests that the tomato root lipid has an affinity for the compounds that is three times that of soybean and 12 times that of octanol. Nevertheless, it is probably indicative that the assumption that protein and carbohydrate fractions are equal between the two species is inappropriate, and/or that the compound has some affinity for material such as low molecular lipids, that were lost, incompletely extracted, or not extracted at all, using the method outlined for $\theta_{\text{lipid}}$ above. The differences seen in the two species would likely be account with more information.
about the structural and composition differences in roots, such as carbohydrate or protein
fraction and/or affinity. Ultimately, the calculated differences for the two species
combined is only important for midrange hydrophobicity compounds. The maximum
calculated difference in TSCF between the two species is 0.3 at a log $K_{\text{OW}}$ of 2.42. At
either end of the hydrophobicity spectrum, those differences effectively disappear (Figure
5-3).

Root uptake difference related to species have been previously reported for organic
compounds (26). Care should be taken in interpreting the overall significance of the
difference inferred by this dataset without proper understanding of the meaning of the
difference and its result on the data. The location of the maximum species difference
coexists with the maximum slope of the relationship. Small differences in log $K_{\text{OW}}$ will
result in large differences in TSCF throughout this range. The difference in species,
though interesting, warrants further investigation, ideally with additional species
consisting of as wide a range as possible of root lipid material.

**Model Residuals.** The residuals of the model were calculated using the nonlinear least
squared function in the open source statistical software package R v2.6.2 and were
analyzed for three plant dependant factors and one physical chemical property of the
compounds. The plant dependant factor for which residuals were plotted, were plant
species (Figure 5-4); plant age (Figure 5-5); and plant size represented by root mass
(Figure 5-6), and the physical property that was analyzed was the log $K_{\text{OW}}$ of the
chemical.
FIGURE 5-3. Interspecies comparison of the model fit of pressure chamber data for 25 neutral organics ranging in log $K_{OW}$ from -0.8 to 5 from Chapter 4.

FIGURE 5-4. Model residuals using the nonlinear least squared modeling in the statistical package R v2.6.2 by species indicating good species predictivity.
FIGURE 5-5. Model residual by plant age using nonlinear least squared modeling in the statistical package R v2.6.2 indicating little age dependability to TSCF as predicted by the model.

FIGURE 5-6. Nonlinear least squares model residual by root weight (a good measure of overall plant size) using R v2.6.2 indicating little size dependability to TSCF as predicted by the model.
FIGURE 5-7. Model residual for log $K_{OW}$ using nonlinear least squared modeling in the statistical package R v2.6.2 indicating the model accounts for the dependability of TSCF and hydrophobicity.

FIGURE 5-8. General residuals of model for fitted values using nonlinear least squared modeling in the statistical package R v2.6.2.
The residuals indicate that the model does a good job fitting the data. The model makes the basic assumption that TSCF is independent of plant age, size and the even distribution of the residuals concerning size (Figure 5-6) and age (Figure 5-5) supports this premise. The model also predicts that there is a species difference between soybean and tomato plants. The model does a good job of predicting this difference as evident from the residuals (Figure 5-4). Overall, the residuals indicate good agreement between the data and the model with an even distribution associated with random error (Figure 5-8).

**Model Fitting of Data.** A generic solution of plant uptake potential regardless of species was developed for generic purposes in which there is little or no data for all the plant parameters for the species being considered to populate the general model. The tomato and soybean data both with a measured value of 0.90 for $\theta_{\text{water}}$ (Table 5-1) were used in Equation (35) to generate the generic solution. The result is a relationship between TSCF and $\log K_{\text{OW}}$ that is dependent on the aquaporin fraction which is likely to be dependent on environmental conditions (76, 85) and the octanol/plant lipid affinity factor ($\alpha$).

$$TSCF = \frac{1 - \gamma}{0.9 + \alpha \times 10^{\log K_{\text{OW}}}}$$

(39)

Where:
- TSCF = Transpiration Steam Concentration Factor (unitless)
- $\gamma$ = Aquaporin bypass fraction (unitless)
- $\alpha$ = Affinity of chemical to and wet weight mass fraction of root lipid material (unitless)
- $K_{\text{OW}}$ = Octanol/water partition coefficient (unitless)

The estimated value by nonlinear least squares fitting for the aquaporin bypass fraction is 0.25. Considering there is an environmental growth conditional dependency of
aquaporins (13, 85), it is comparable to reported root hydraulic conductance reduction of 32-87% (73-76) with aquaporin inhibition. Using the same nonlinear least squares fitting technique, the value derived for the affinity of a chemical and its wet weight mass fraction of root lipid material is 0.0024.

Assuming that the root lipid mass fraction difference measured between soybeans and tomatoes is representative of a wide range of plants then, a general root uptake potential equation becomes

\[
TSCF = \frac{312}{375 + 10^{\log{K_{OW}}}}
\]

(40)

Where:

- \( TSCF \) = Transpiration Steam Concentration Factor (unitless)
- \( K_{OW} \) = Octanol/water partition coefficient (unitless)

Equation (40) is similar to the purely empirical fit equation reported in Chapter 4 and results in a similar sigmoidal uptake potential relationship (Figure 5-9). Considering the large difference is lipid contents of tomato and soybean, the largest calculated variation between the generic and general TSCF models would likely be less than 0.21, and would occur at a \( \log{K_{OW}} \) 2.42.

A distinct advantage of this model in comparison to those based on the Briggs relationship, is its ability to account for water uptake by roots. Although not technically an organic compound, water presents a unique problem for these models due to its polar nature. Water has a range of reported \( \log{K_{OW}} \) of -1.15 (86) to -1.38 (64). A negative \( \log{K_{OW}} \) results in a TSCF of essentially zero using the Briggs relationship. Without a special mechanism for water, bell-shaped curves suggest that hydrophilic compounds, of which water is one, are not taken up by plant roots. Aquaporins can serve as a special
mechanism for water uptake to a certain extent (73, 74). Nevertheless, they cannot account for all the water uptake of roots. The new mass transfer model accounts for both the aquaporin fraction and the water fraction passing through the traditional membrane pathways. Using the fitted parameters suggested by the soybean and tomato data, and adding the aquaporin bypass fraction of 0.25, the model predicts the TSCF of water to be 1.08. This result is in remarkable agreement with the theoretical water TSCF of 1.0 and the measured tritiated water value of 1.00 ± 0.01.

FIGURE 5-9. A general TSCF versus log $K_{OW}$ relationship for all the compounds tested. The data are overlaid by the TSCF model Equation (40) with parameters fitted using nonlinear regression techniques available in R v2.6.2.
The data and model indicate that polar compounds do have a high potential to be taken up by plant roots unlike previously reported relationships (2, 4, 21). The model predicts uptake potential, and thus cannot fully predict final tissue concentrations. The potential for a compound to be taken up by plants is important when considering the appropriate use of phytoremediation techniques that involve plant metabolism, sequestration, and/or phytovolatilization. The uptake potential should also prove useful in conjunction with other models that account for soil concentrations and total water transpired over the exposure period, and can estimate losses due to metabolism and phytovolatilization over the same period, such as the Trapp model (48). Ultimately, this model serves in determining the extent of a compound’s root uptake potential as given by the TSCF, which is useful for phytoremediation assessment. When the model is incorporated into complete fate models, it should prove valuable in predicting final plant tissue concentrations for use in risk assessment analysis.
Summary

Engineering Significance

Given circumstances will determine whether the potential for compounds to be taken up and translocated by plants is either beneficial or a cause of concern. In the case of risk assessment and food chain contamination, the uptake and translocation of xenobiotics to edible portions of the plant pose a potential health risk, and therefore elevated uptake levels are considered troublesome. In the case of phytoremediation (remediation of an impacted site by living plants) a high tendency for uptake is often a prerequisite for the use of the technology and regarded as advantageous. For example, compounds with structural features that make them particularly well suited for plant uptake may potentially favor phytoremediation processes, but could cause apprehension concerning food chain contamination. Likewise, contaminants that are not easily taken up by plants have very little potential to be remediated under plant metabolism, sequestration, or phytovolatilization mechanisms but would also not likely cause concern in risk determinations. Similar to phytoremediation, a cost effective method of source identification for contaminated soils and groundwater may be possible through analysis of plant tissue concentrations for compounds with a high potential to be taken up by plants.

The research contained within this dissertation is designed to elucidate the understanding of uptake and translocation of chemicals to the edible portions of a plant and how this uptake and translocation relates to the compound’s structure and physical-
chemical properties. This research not only provides new data about plant uptake, but also compiles the data from past studies. Although the data from previous studies were found to be highly variable concerning methods, when understood properly they support the data generated in this study and the new model of plant uptake potential into roots. The root uptake potential model cannot be used independently to predict plant tissue concentration for use in risk assessment but it can be used as a screening tool. When incorporated with more encompassing plant uptake models like the Trapp model, this new model can be used to predict individual tissue concentrations important to risk assessment. Ultimately, the relationships developed because of this research should facilitate government regulators in conducting risk assessment studies of an impacted site and choosing remediation strategies.

This study and its review of the literature illustrate the importance of collecting data in a consistent and useful manner. Without standardized data, relationships will not be widely applicable. Unfortunately, because of a lack of pertinent data, relationships are sometimes used for predictions outside their applicability domain. The study not only helped widen that domain, but the detailed methods will also allow others to do so in a uniform manner.

**Conclusion**

Uptake and translocation of xenobiotic compounds is an important and growing concern for both the public and governmental regulators. The potential for a compound to bioaccumulate in plants directly relates to food chain contamination, risk assessment, phytoremediation potential and source identification. The TSCF is the primary descriptor of plant root uptake and the most often cited predictive relationship for the TSCF is the
Briggs (2) log $K_{ow}$ correlation. The Briggs relationship is not applicable for neutral, polar compounds based on recent literature reports and there is a large degree of variability within the literature, likely a result of non-standard experimental methods. The pressure chamber method of determining TSCF appears to be the most consistent. Its consistency is likely a result of having the least number of operational parameters. The pressure chamber method is also the quickest and cheapest method of determining the TSCF. Additional information as well as data that are more reliable can be generated using the basic pressure chamber method combined with minor modifications and supplementary techniques such as root-zone sampling, use of tracers, exuded xylem sap measurements and use of oxygen for pressurization. Using those techniques, TSCFs for 25 common organic contaminants over a wide range of hydrophobicities were measured. These data lead to a new model of plant root uptake potential, which unlike the Briggs relationship, predicts that more hydrophilic compounds have the highest potential for root uptake and translocation. Because the data encompass a wider range of chemical classes than previous models, government regulators can predict the uptake potential of a compound from its widely available log $K_{ow}$. Tissue concentration predictions used in risk assessments can be made from a compound’s plant uptake potential in combination with a model that includes phytovolatilization and metabolization within the plant, such as the Trapp (48) model. This greatly simplifies the process of risk assessment related to plant uptake and subsequent food chain contamination. By elucidating the root to shoot pathway of exposure and the potential of xenobiotic uptake, the relationship developed provides tools to aid in the protection of the public from unnecessary chemical exposure.
Likewise, this basic research is expected to be invaluable in the decision making process related to remediation of contaminated sites using phytoremediation.

Fundamentally, this literature review, study, and subsequent model illustrate the pitfalls and difficulties associated with measuring plant uptake while offering solutions and producing a clearer understanding of the guiding mechanisms. In particular, the contained model’s predictive capabilities are useful for determining the existence of phytoremediation potential or food chain risk from organic soil contaminants.
REFERENCES


(43) VCCLAB Virtual Computational Chemistry Laboratory. http://www.vcclab.org


APPENDICES
Appendix A: Data Analysis and Statistical Methods

**TSCF Relationship with other molecular descriptors.** Identification of possible physical properties and molecular indices related to the TSCF was conducted using basic correlation techniques. Correlations between TSCF and measured log $K_{OW}$, Molecular Weight, Solubility, Chem3D Ultra descriptors (Molar Refractivity, Connolly Accessible Area, Connolly Molecular Area, Connolly Solvent-Excluded Volume, Polar Surface Area) (87) and all 1666 E-Dragon molecular descriptors from VCCLAB (43, 88) were investigated. For three dimensional descriptors input structures were minimized in Chem3D Ultra using the General Atomic and Molecular Structure System (GAMESS) energy minimization routine (89, 90). Primary correlation screening was conducted by selecting the 20 most correlated factors from each of the following correlation tests, Pearson’s, Hoeffding’s D, Kendall’s $\tau$, and Spearman’s $\rho$. The resulting 80 physical properties and molecular descriptors were refined by removing all replicate descriptors. Further refinement was accomplished by removing inter-correlated descriptors (Pearson coefficient $> 0.8$) leaving only the most significant factors. For example, the most significant descriptor based on the octanol partitioning coefficient was the experimentally measured log $K_{OW}$, all inter-correlated descriptors such as the calculated values of the octanol partitioning coefficient MLOGP, and ALOGP with a Pearson coefficient $> 0.8$ to the experimentally measured value, were removed from the dataset. The result was 10 molecular descriptors with log $K_{OW}$ being the most significantly correlated by all methods.
TABLE A-1. Most Correlated Molecular Descriptors with TSCF (see Appendix B: for definitions)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson's r</th>
<th>Spearman's ρ</th>
<th>Hoeffding's D</th>
<th>Kendall's τ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log $K_{ow}$</td>
<td>-0.808</td>
<td>-0.831</td>
<td>0.308</td>
<td>-0.647</td>
</tr>
<tr>
<td>REIG</td>
<td>0.630</td>
<td>0.670</td>
<td>0.146</td>
<td>0.482</td>
</tr>
<tr>
<td>Mor18m</td>
<td>0.610</td>
<td>0.575</td>
<td>0.118</td>
<td>0.416</td>
</tr>
<tr>
<td>Mor22u</td>
<td>-0.571</td>
<td>-0.577</td>
<td>0.111</td>
<td>-0.409</td>
</tr>
<tr>
<td>Mor32m</td>
<td>0.565</td>
<td>0.652</td>
<td>0.150</td>
<td>0.478</td>
</tr>
<tr>
<td>MATS2v</td>
<td>-0.543</td>
<td>-0.586</td>
<td>0.099</td>
<td>-0.456</td>
</tr>
<tr>
<td>Mv</td>
<td>-0.542</td>
<td>-0.590</td>
<td>0.123</td>
<td>-0.413</td>
</tr>
<tr>
<td>Jhetv</td>
<td>-0.521</td>
<td>-0.591</td>
<td>0.162</td>
<td>-0.451</td>
</tr>
<tr>
<td>R2u</td>
<td>0.515</td>
<td>0.566</td>
<td>0.160</td>
<td>0.440</td>
</tr>
<tr>
<td>Mor06v</td>
<td>-0.367</td>
<td>-0.506</td>
<td>0.148</td>
<td>-0.409</td>
</tr>
</tbody>
</table>

The inter-correlation between log $K_{ow}$ and all the other ranked descriptors and was great enough to account for all of their correlation with TSCF.

R script used to determine species differences by nonlinear least squares analysis.

eData<-read.table("C:\RScripts\Data.txt",sep="\t",header=TRUE)
nData=subset(eData,Special=="No")
test=as.numeric(nData$Species=="Soybean")
testData=cbind(nData,test)
library (nlstools)

## are gamma and beta different for tomato and soybean?
m=TSCF ~ (1 - (gamma+alpha*test))/(.9 + (beta+psi*test)*10^(LogKow))
test.nls=nls(m,data=nData,start=list(gamma=.25,beta=.003,alpha=.01,psi=.0001))
overview(test.nls)

## is just gamma different for tomato and soybean?
m=TSCF ~ (1 - (gamma+alpha*test))/(.9 + (beta)*10^(LogKow))
test.nls=nls(m,data=nData,start=list(gamma=.25,beta=.003,alpha=.01))
overview(test.nls)

## is just beta different for tomato and soybean
m=TSCF ~ (1 - gamma)/(.9 + (beta+psi*test)*10^(LogKow))
test.nls=nls(m, data=nData, start=list(gamma=.25, beta=.003, psi=.0001))
overview(test.nls)

## Plot Residuals
test.resid=nlsResiduals(test.nls)
plot(test.resid)
testf=test.resid$sresi1[,1]
testr=test.resid$sresi1[,2]
testo=order(testf)
testb=cbind(testf[testo], testr[testo])
yData=cbind(testData, test.resid$sresi1)
attach(yData)
x11()
plot(LogKow, TSCF)
x11()
plot(Species, Residuals)
x11()
plot(LogKow, Residuals)
x11()
plot(ChemicalName, Residuals)
x11()
plot(Age, Residuals)
x11()
plot(log(Solubility), Residuals)
x11()
plot(RootWeight, Residuals)
x11()
plot(FoliarWeight, Residuals)
Appendix B: Parameters, Nomenclature, and Dimensions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>Flux</td>
<td>(mass length$^{-2}$ time$^{-1}$)</td>
</tr>
<tr>
<td>A</td>
<td>Area</td>
<td>(length$^2$)</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>(time)</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Chemical concentration in “i”</td>
<td>(mass length$^{-3}$) or (mass mass$^{-1}$)</td>
</tr>
<tr>
<td>x</td>
<td>Length</td>
<td>(length)</td>
</tr>
<tr>
<td>BCF</td>
<td>Bioconcentration Factor</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$K_{OW}$</td>
<td>Octanol/water partition Coefficient</td>
<td>(unitless)</td>
</tr>
<tr>
<td>TSCF</td>
<td>Transpiration Stream Concentration Factor</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$M_i$</td>
<td>Mass of “i” compartment</td>
<td>(mass$^{-1}$)</td>
</tr>
<tr>
<td>$T_w$</td>
<td>Volume of Water Transpired</td>
<td>(length$^3$)</td>
</tr>
<tr>
<td>Activity</td>
<td>Activity of concern</td>
<td>(various)</td>
</tr>
<tr>
<td>$C_n$</td>
<td>Contribution coefficient</td>
<td>(various)</td>
</tr>
<tr>
<td>$P_i$</td>
<td>Structural parameter</td>
<td>(various)</td>
</tr>
<tr>
<td>C</td>
<td>Absolute amplitude</td>
<td>(various)</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Time constant</td>
<td>(time$^{-1}$)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Units</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>y(t)</td>
<td>Signal as a function of t</td>
<td>(various)</td>
</tr>
<tr>
<td>y</td>
<td>Concentration in xylem/concentration in solution at time t</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$V_{xylem}$</td>
<td>Volume of expelled xylem sap</td>
<td>(length$^3$)</td>
</tr>
<tr>
<td>$Q_{xylem}$</td>
<td>Flow rate of xylem</td>
<td>(length$^3$ time$^{-1}$)</td>
</tr>
<tr>
<td>$A_{flux}$</td>
<td>Area perpendicular to the flux</td>
<td>(length$^2$)</td>
</tr>
<tr>
<td>$D_m$</td>
<td>Diffusivity of chemical through the membrane</td>
<td>(mass length$^{-2}$ time$^{-1}$)</td>
</tr>
<tr>
<td>$\frac{dC_m}{dx}$</td>
<td>change in concentration with distance</td>
<td>(mass length$^{-4}$)</td>
</tr>
<tr>
<td>$h$</td>
<td>Membrane thickness</td>
<td>(length)</td>
</tr>
<tr>
<td>$J_{in}$</td>
<td>Flux into the membrane</td>
<td>(mass length$^{-2}$ time$^{-1}$)</td>
</tr>
<tr>
<td>$J_{out}$</td>
<td>Flux out of the membrane</td>
<td>(mass length$^{-2}$ time$^{-1}$)</td>
</tr>
<tr>
<td>$J_{total}$</td>
<td>Total flux through the membrane</td>
<td>(mass length$^{-2}$ time$^{-1}$)</td>
</tr>
<tr>
<td>$K_D$</td>
<td>Octanol/water distribution coefficient</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$P_m$</td>
<td>Permeability of the membrane to the compound</td>
<td>(length time$^{-1}$)</td>
</tr>
<tr>
<td>$P_w$</td>
<td>Permeability of membrane to water</td>
<td>(length time$^{-1}$)</td>
</tr>
<tr>
<td>$P_{total}$</td>
<td>Permeability of the membrane to water including aquaporin bypass</td>
<td>(length time$^{-1}$)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Units</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Aquaporin bypass fraction</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\Theta_{lipid}$</td>
<td>Wet weight mass fraction of lipid in</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\beta_{lipid}$</td>
<td>Affinity of chemical to lipid material in root</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\Theta_{Carb}$</td>
<td>Wet weight mass fraction of carbohydrate in root</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\beta_{Carb}$</td>
<td>Wet weight mass fraction of carbohydrate in root</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\Theta_{Protein}$</td>
<td>Wet weight mass fraction of protein in root</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\beta_{Protein}$</td>
<td>Affinity of chemical to protein material in root</td>
<td>(unitless)</td>
</tr>
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<td>$Z_{xylem}$</td>
<td>Xylem fugacity of chemical</td>
<td>(mass time$^{-2}$ length$^{-1}$)</td>
</tr>
<tr>
<td>$Z_{Membrane}$</td>
<td>Membrane fugacity of chemical</td>
<td>(mass time$^{-2}$ length$^{-1}$)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Aquaporin bypass fraction associated with only soybeans</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>Affinity of chemical to and wet weight mass fraction of root lipid material associated with only soybeans</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$z$</td>
<td>species dependant factor</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\gamma_0$</td>
<td>Aquaporin bypass fraction associated with soybean and tomato</td>
<td>(unitless)</td>
</tr>
<tr>
<td>$\alpha_o$</td>
<td>Affinity of chemical to and wet weight mass fraction of root lipid material to soybean and tomato</td>
<td>(unitless)</td>
</tr>
<tr>
<td>REIG</td>
<td>GETAWAY descriptors - first eigenvalue of the R matrix</td>
<td>(unitless)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Units</td>
</tr>
<tr>
<td>Descriptor</td>
<td>Description</td>
<td>Units</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
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<td>Mor18m</td>
<td>3D-MoRSE descriptor - signal 18 / weighted by atomic masses</td>
<td>(unitless)</td>
</tr>
<tr>
<td>Mor22u</td>
<td>D-MoRSE descriptor - signal 22 / unweighted</td>
<td>(unitless)</td>
</tr>
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<td>Mor32m</td>
<td>3D-MoRSE descriptor - signal 32 / weighted by atomic masses</td>
<td>(unitless)</td>
</tr>
<tr>
<td>MATS2v</td>
<td>2D autocorrelations descriptor - Moran autocorrelation - lag 2 / weighted by atomic van der Waals volumes</td>
<td>(unitless)</td>
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<td>Mv</td>
<td>constitutional descriptors - mean atomic van der Waals volume (scaled on Carbon atom)</td>
<td>(unitless)</td>
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<td>Jhetv</td>
<td>topological descriptors - Balaban-type index from van der Waals weighted distance matrix</td>
<td>(unitless)</td>
</tr>
<tr>
<td>R2u</td>
<td>GETAWAY descriptors - R autocorrelation of lag 2 / unweighted</td>
<td>(unitless)</td>
</tr>
<tr>
<td>Mor06v</td>
<td>3D-MoRSE descriptors - signal 06 / weighted by atomic van der Waals volumes</td>
<td>(unitless)</td>
</tr>
</tbody>
</table>
FIGURE C-1. Mechanical drawings of pressure chamber used in study.
FIGURE C-2 Mechanical drawings of pressure chamber lid assembly used in study.
Appendix D: Analytical Methods

Compound Specific Methods (Sulfolane)

Extraction (plant tissue)

1. Plant tissue is pulverized and homogenized in a food processor.
2. ~5 gram (wet weight) aliquot is transferred to a 40 mL Teflon centrifuge tube.
3. 20 mL of DDW is added to the centrifuge tube.
4. Teflon tubes are shaken on an orbital shaker for 60 minutes at 180 oscillation/minutes using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
5. Sample is centrifuged at 10,000 rpm for 10 minutes
6. Sample is passed through the 45 µm filter under vacuum into a new centrifuge tube
7. Sample solids are discarded
8. Add 5 gm NaCl to 20 mL Aqueous sample
9. 4 mL of Methylene Chloride is added to 20 mL of aqueous solution
10. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
11. Centrifuge tubes are centrifuged for 15 minute at 7500 rpm
12. The methylene chloride layer (bottom) is extracted from the water using a pipette after centrifugation and placed in a 20 mL sealed test tube
13. 4 mL of Methylene Chloride is added to the aqueous solution
14. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
15. Centrifuge tubes are centrifuged for 15 minute at 7500 rpm
16. Methylene chloride is extracted from the water using a pipette after centrifugation as in step 5
17. 4 mL of Methylene Chloride is added to the aqueous solution
18. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).

19. Centrifuge tubes are centrifuged for 15 minute at 7500 rpm

20. Methylene chloride is extracted from the water using a pipette after centrifugation as in step 5

21. Methylene chloride maybe concentrated under nitrogen if required.

22. Aliquots are transferred from test tube to a 2 mL GC vial and refrigerated until GC analysis

23. Blank, spike and triplicate extractions are conducted after every ten extractions.

24. Spikes consist of 25 µL of 1000 mg/L standards in water.
Extraction (aqueous samples)

1. Add 5 gm NaCl to 20 mL Aqueous sample
2. 4 mL of Methylene Chloride is added to 20 mL of aqueous solution
3. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
4. Centrifuge tubes are centrifuged for 15 minutes at 7500 rpm
5. The methylene chloride layer (bottom) is extracted from the water using a pipette after centrifugation and placed in a 20 mL sealed test tube
6. 4 mL of Methylene Chloride is added to the aqueous solution
7. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
8. Centrifuge tubes are centrifuged for 15 minutes at 7500 rpm
9. Methylene chloride is extracted from the water using a pipette after centrifugation as in step 5
10. 4 mL of Methylene Chloride is added to the aqueous solution
11. Centrifuge tubes are shaken on an orbital shaker for 20 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
12. Centrifuge tubes are centrifuged for 15 minutes at 7500 rpm
13. Methylene chloride is extracted from the water using a pipette after centrifugation as in step 5
14. Methylene chloride maybe concentrated under nitrogen if required.
15. Aliquots are transferred from test tube to a 2 mL GC vial and refrigerated until GC analysis
16. Blank, and spike extractions are conducted after every ten extractions.
17. Spikes consist of 25 µL of 1000 mg/L standards in water.
Instrument Analysis/Quantification

**Instrument**
Pre-extracted aliquots in methyl chloride are analyzed using a Shimadzu Model GC-14A equipped with a DB-5 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) flame ionization detector, AOC-1400 autosampler, and Agilent GC Chemstation Rev A.08.03 [847] data acquisition and analysis software.

**Temperature Program**
80°C (2 minutes hold) to 160 °C at 10 °C/minutes, then 40 °C/minutes to 220 °C (2 minutes hold).

**Carrier gas**
Nitrogen (10 mL/minutes).

**Quality control and quality assurance**
For every ten samples, one blank and one calibration curve verification (CCV) sample is also analyzed.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (1, 4-Dioxane)

*Extraction (plant tissue and soil)*

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place by wrapping in parafilm after weighing and before sampling.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier + cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis.
Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis
Instrument Analysis/Quantification

Instrument
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

Temperature Program
Headspace Platen Temperature
 80°C, 20 minute shake and equilibration time
Oven Temperature
 40 °C (2 minutes hold), 10 °C/minutes to 230 °C, total run time 22 minutes

Carrier gas
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

Quantization Ions
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 88, 58, 43

Quality control and quality assurance
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Tertiary-butyl Alcohol)

Extraction (plant tissue and soil)

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place after weighing and before sampling by wrapping in parafilm.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier +cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis.
Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 μm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature

80°C, 20 minute shake and equilibration time

Oven Temperature

40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

**Quantization Ions**
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 31, 41, 59

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Tertiary-amyl Alcohol)

Extraction (plant tissue and soil)

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place after weighing and before sampling by wrapping in parafilm.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier +cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis
Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature

- 80°C, 20 minute shake and equilibration time

Oven Temperature

- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

**Quantization Ions**
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 45, 57, 87

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Methyl tert-Butyl Ether)

Extraction (plant tissue and soil)

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place after weighing and before sampling by wrapping in parafilm.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier + cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis.
Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
Instrument Analysis/Quantification

Instrument
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

Temperature Program
Headspace Platen Temperature
80°C, 20 minute shake and equilibration time

Oven Temperature
40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

Carrier gas
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

Quantization Ions
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 43, 57, 73

Quality control and quality assurance
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (2,2,2-Trichloroethanol)

Extraction (plant tissue)

1. Plant tissue is pulverized and homogenized in a food processor.
2. ~5 gram (wet weight) aliquot is transferred to a 40 mL Teflon centrifuge tube.
3. 20 mL of DDW is added to the centrifuge tube and 7 mL of Methyl tert-butyl ether (MTBE) is added.
4. Teflon tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
5. Sample is centrifuged at 7,500 rpm for 15 minutes.
6. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation.
7. 7 mL of Methyl tert-butyl ether (MTBE) is added to water/tissue solution.
8. Teflon tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
9. Sample is centrifuged at 7,500 rpm for 15 minutes.
10. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation.
11. 7 mL of Methyl tert-butyl ether (MTBE) is added to water/tissue solution.
12. Teflon tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
13. Sample is centrifuged at 7,500 rpm for 15 minutes.
14. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation.
15. MTBE is added to bring 25 mL volumetric flask up to volume.
16. Aliquots are transferred to 2 mL GC vials and refrigerated until GC analysis.
17. Aliquots are analyzed using GC-ECD.
18. Spikes consist of 25 µL of 1000 mg/L standards in water.
**Extraction (aqueous samples)**

1. Approximately 5 g NaCl solid is added until saturation (~1 “scoop”) of 20 mL of aqueous solution
2. 7 mL of Methyl tert-butyl ether (MTBE) is added to solution
3. Centrifuge tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
4. Centrifuge tubes are centrifuged for 5 minutes at 2500 rpm
5. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation
6. 7 mL of Methyl tert-butyl ether (MTBE) is added to solution
7. Centrifuge tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
8. Centrifuge tubes are centrifuged for 5 minutes at 2500 rpm
9. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation mL of Methylene Chloride is added to the aqueous solution
10. 7 mL of Methyl tert-butyl ether (MTBE) is added to solution
11. Centrifuge tubes are shaken on an orbital shaker for 15 minutes at 180 oscillation/minutes using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
12. Centrifuge tubes are centrifuged for 5 minutes at 2500 rpm
13. MTBE supernatant is extracted from water into a 25 mL glass volumetric flask using a pipette after centrifugation
14. MTBE is added to bring 25 mL volumetric flask up to volume
15. Aliquots are transferred to 2 mL GC vials and refrigerated until GC analysis
16. Aliquots are analyzed using GC-ECD
Instrument Analysis/Quantification

Instrument
Pre-extracted aliquots in MTBE are analyzed using a Shimadzu Model GC-14A equipped with a DB-5 (30 M x 0.5 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) electron capture detector, AC-15 autosampler, and Agilent GC Chemstation Rev A.08.03 [847] data acquisition and analysis software.

Temperature Program
50°C (2 minute hold) to 220 °C at 10 °C/minute total run time 22 minutes

Carrier gas
Nitrogen (10 mL/minute).

Makeup gas
Nitrogen (20 mL/minute).

Quality control and quality assurance
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Chloroform)

Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature
- 50 °C, 20 minute shake and equilibration time

Oven Temperature
- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 10:1
Helium 0.7 mL/minute; split flow 7 mL/minute

**Quantization Ions**
Operation mode – Scan M/Z 50 -550
Quantization mode -Total Ion Current

**Quality control and quality assurance**
10% of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (1,2-Dichloropropane)

Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature

50 °C, 20 minute shake and equilibration time

Oven Temperature

40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 10:1

Heelim 0.7 mL/minute; split flow 7 mL/minute

**Quantization Ions**
Operation mode – Scan M/Z 50 -550

Quantization mode -Total Ion Current

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (1,2-Dichloroethane)

Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
Instrument Analysis/Quantification

Instrument
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

Temperature Program
Headspace Platen Temperature

50 °C, 20 minute shake and equilibration time

Oven Temperature

40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

Carrier gas
Split ratio 10:1

Helium 0.7 mL/minute; split flow 7 mL/minute

Quantization Ions
Operation mode – Scan M/Z 50 -550
Quantization mode -Total Ion Current

Quality control and quality assurance
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (1,1,1-Trichloroethane)

Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature
- 50 °C, 20 minute shake and equilibration time

Oven Temperature
- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 10:1
Helium 0.7 mL/minute; split flow 7 mL/minute

**Quantization Ions**
Operation mode – Scan M/Z 50 -550
Quantization mode -Total Ion Current

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Trichloroethylene)

Extraction (plant tissue and soil)

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place after weighing and before sampling by wrapping in parafilm.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier +cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis.
Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis
Instrument Analysis/Quantification

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature
- 80°C, 20 minute shake and equilibration time

Oven Temperature
- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

**Quantization Ions**
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 60, 95, 130

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
**Compound Specific Methods (2,4-Dichlorophenoxy Acetic acid)**

*Extraction (plant tissue)*

1. Plant tissue is pulverized and homogenized in a food processor.
2. ~5 gram (wet weight) aliquot is transferred to a 40 mL Teflon centrifuge tube.
3. 20 mL of DDW is added to the centrifuge tube.
4. Teflon tubes are shaken on an orbital shaker for 60 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
5. Sample is centrifuged at 10,000 rpm for 10 minutes
6. Remove available aqueous solution and place in 100 mL flask
7. 20 mL of DDW is added to the centrifuge tube.
8. Teflon tubes are shaken on an orbital shaker for 60 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
9. Sample is centrifuged at 10,000 rpm for 10 minutes
10. Remove available aqueous solution and place in 100 mL flask
11. 20 mL of DDW is added to the centrifuge tube.
12. Teflon tubes are shaken on an orbital shaker for 60 minutes at 180 oscillation/minute using an Eberbach Model 6010 reciprocal shaker (Ann Arbor, MI).
13. Sample is centrifuged at 10,000 rpm for 10 minutes
14. Sample is passed through the 45 µm filter under vacuum and solution is added to 100 mL flask and is extracted using aqueous extraction technique.
15. Blank, spike and triplicate extractions are conducted after every ten extractions.
16. Spikes consist of 25 µL of 1000 mg/L standards in water.
Extraction (Aqueous samples)

1. One Oasis solid phase extraction cartridge should be placed on a vacuum manifold block for each sample.
2. Place waste container in manifold to catch effluent.
3. Attach vacuum.
4. Pass 5 mL of Tetrahydrofuran (THF) through each cartridge at 5 mL/minute.
5. Immediately add 5 mL of DDW to cartridge following methanol.
6. Pass the DDW through at 5 mL/minute until only 1 mL remains in the cartridge.
7. Acidify sample with 1 mL of 1N H₂SO₄ to sample.
8. Add sample reservoir to cartridge.
9. Add 40 mL of sample to reservoir.
10. Pass sample through at 5 mL/minute until reservoir is depleted.
11. Continue to pull a vacuum for one full minute after the cartridge is empty to dry the solid phase.
12. Shut off vacuum, remove waste containers and replace with separate 5 mL volumetric flasks.
13. Line up drip tubes with volumetric flasks and replace vacuum manifold lid.
14. Pass 4 mL of THF through cartridges at 5 mL/minute.
15. Shut off vacuum and remove the volumetric flasks.
16. Dilute the Flasks to mark with THF.
17. THF may be concentrated under nitrogen if required.
18. Transfer the methanol extract from the volumetric flasks to 2 mL GC vials.
19. Label vials!
20. Seal vials and refrigerate until further analysis.
21. Aliquots are analyzed using a HPLC.
**Instrument Analysis/Quantification**

**Instrument**
Pre-extracted aliquots in Tetrahydrafuran are analyzed using an Agilent Model 1100 High Performance Liquid Chromatograph equipped with a Lichrospher RP 18 (250 mm x 4.6 mm, 5 µm particles) column (Spelco) diode array detector 1100 autosampler, and Agilent Chemstation LC 3D Rev A.10.01 [847] data acquisition and analysis software.

**Elution Program**
Isocratic 54% Water, 45 % Methanol, 1 % Glacial Acetic Acid
Flow rate – 1 mL/minute

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Carbon Tetrachloride)

Extraction (aqueous samples)

1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis
**Instrument Analysis/Quantification**

**Instrument**  
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 μm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**  
Headspace Platen Temperature  
- 50 °C, 20 minute shake and equilibration time  
Oven Temperature  
- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**  
Split ratio 10:1  
Helium 0.7 mL/minute; split flow 7 mL/minute

**Quantization Ions**  
Operation mode – Scan M/Z 50 -550  
Quantization mode -Total Ion Current

**Quality control and quality assurance**  
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.  
The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Tetrachloroethylene)

Extraction (plant tissue and soil)

1. 10 mL of matrix modifier (saturated salt solution pH adjusted to 2 with phosphoric acid) is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial weights with matrix modifier and cap are recorded.
4. Vial caps are temporarily held in place after weighing and before sampling by wrapping in parafilm.
5. Parafilm is removed at sampling and ~5 g of plant tissue is placed into each vial and the vials are labeled.
6. Vial caps are immediately crimped onto the vials to prevent analyte loss.
7. Vials containing samples are reweighed to determine exact amount of tissue added by subtracting the vial + modifier +cap weight from the final weight.
8. Samples are stored at 4 °C and kept dark until analysis
1. 2.6 g of NaCl is added to 20 mL headspace vials.
2. Caps are placed on the headspace vials but not sealed.
3. Vial caps are temporarily held in place before sampling by wrapping in parafilm.
4. Parafilm is removed at sampling and 10 mL of aqueous solution sampled by syringe is placed into each vial and the vials are labeled.
5. Vial caps are immediately crimped onto the vials to prevent analyte loss.
6. Samples are stored at 4 °C and kept dark until analysis.
**Instrument Analysis/Quantification**

**Instrument**
Headspace vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5970 equipped Mass Selective Detector with a DB-624 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Tekmar Dohrmann Headspace autosampler 7000 HT, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

**Temperature Program**
Headspace Platen Temperature
- 80°C, 20 minute shake and equilibration time

Oven Temperature
- 40 °C (2 minute hold), 10 °C/minute to 230 °C, total run time 22 minutes

**Carrier gas**
Split ratio 2:1
Helium 0.7 mL/minute; split flow 1.4 mL/minute

**Quantization Ions**
Operation mode – Single Ion Monitoring
Quantization mode - Total Ion Current
Primary Ions – 94, 129, 166

**Quality control and quality assurance**
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.

The quantification is by linear regression analysis of external standards.
Compound Specific Methods (Nonylphenol)

Extraction (plant tissue)

1. Record wet plant tissue weights
2. Air dry tissue on foil for 7 days;
3. Place tissue into a desiccator and leave for 24 hours;
4. Weigh dry tissue, record weights
5. Determine fractional water content by 1-drywt/wetwt
6. Grind tissue in a coffee grinder;
7. Label and tare 125ml Erlenmeyer flasks;
8. Place 25/80mm single thickness cellulose extraction thimbles with plant tissue into Soxhlet Extractor
9. Add enough hexane into thimbles to cover tissue;
10. Attach extractors to condensers and flasks containing 100ml of hexane
11. Extract for 20 hours (evaporate down to 5 mL)
12. Separate flasks from extractors
13. Clean up sample by alumina (SW 846-3610) or flourisil (SW 846-3620b)
14. Transfer contents of flask to 10 mL volumetric flasks and dilute to mark with hexane
15. Transfer 2 mL of sample to from volumetric flask to GC vials
1. One Oasis solid phase extraction cartridge should be placed on a vacuum manifold block for each sample.
2. Place waste container in manifold to catch effluent.
3. Attach vacuum.
4. Pass 5 mL of Tetrahydrofuran (THF) through each cartridge at 5 mL/minute.
5. Immediately add 5 mL of DDW to cartridge following methanol.
6. Pass the DDW through at 5 mL/minute until only 1 mL remains.
7. Add sample reservoir to cartridge.
8. Add 40 mL of sample to reservoir.
9. Pass sample through at 5 mL/minute until reservoir is depleted.
10. Continue to pull a vacuum for one full minute after the cartridge is empty to dry the solid phase.
11. Shut off vacuum, remove waste containers and replace with separate 5 mL volumetric flasks.
12. Line up drip tubes with volumetric flasks and replace vacuum manifold lid.
13. Pass 4 mL of THF through cartridges at 5 mL/minute.
14. Shut off vacuum and remove the volumetric flasks.
15. Dilute the Flasks to mark with THF.
16. THF maybe concentrated under nitrogen if required.
17. Transfer the methanol extract from the volumetric flasks to 2 mL GC vials.
18. Label vials!
19. Seal vials and refrigerate until further analysis.
Instrument Analysis/Quantification

Instrument
Extract vials are analyzed using an Agilent Technologies 6890N Gas Chromatograph with an Agilent 5973N equipped Mass Selective Detector with a DB-5 (30 M x 0.45 mm, 0.25 µm film thickness) column (J&W Scientific, Folsom, CA) Agilent Auto sampler, and Agilent Enhanced Chemstation G 1701A-A version A0.300 data acquisition and analysis software.

Temperature Program
Oven Temperature
55 °C 10 °C/minute to 250 °C (no hold time), total run time 29.5 minutes

Carrier gas
Splitless
Helium 0.7 mL/minute

Quantization Ions
Operation mode – Single Ion Monitoring
Quantization mode -Total Ion Current
Primary Ions – 107, 135, 149

Quality control and quality assurance
10 % of samples are QA/QC consisting of blanks and calibration curve verification (CCV) samples in equal proportion.
The quantification is by linear regression analysis of external standards.
Miscellaneous Analytical Methods

Lipid Determination in Plant Tissue

1. Record wet plant tissue weights
2. Air dry tissue on foil for 7 days;
3. Place tissue into a desiccator and leave for 24 hours;
4. Weigh dry tissue, record weights
5. Determine fractional water content by \( \frac{1\text{-drywt}}{\text{wetwt}} \)
6. Grind tissue in a coffee grinder;
7. Label and tare 125 mL Erlenmeyer flasks;
8. Place 25/80 mm single thickness cellulose extraction thimbles into labeled beakers and keep them in a desiccator for 24 hours prior to tare weight;
9. Add approximately 2 g of air dried/desiccated plant tissue into thimbles;
10. For spikes (add olive oil to thimble containing tissue and record additional weight)
11. Place thimbles loaded with plant tissue into Soxhlet Extractor;
12. Add enough ethyl ether into thimbles to cover tissue;
13. Attach extractors to condensers and flasks containing 100 ml of ethyl ether;
14. Extract for 20 hours;
15. Separate flasks from extractors, and leave them on hot plates until all ether evaporates.
16. Place flasks into a desiccator for 24 hours.
17. Weigh flasks containing extracted lipid, record weights.
18. Determine lipid content by dividing extracted lipid weight by added tissue weight
$^{14}$C and $^3$H$_2$O Analysis

Liquid Scintillation Counting

1. Liquid scintillation vials are weighed with screw caps in place.
2. 2 mL of liquid sample is collected in 7 mL liquid scintillation vials or 5 -10 mL is collected in 20 mL liquid scintillation vials.
3. Vials are weighed with sample. Sample volume is determined by subtracting the starting weight from the final weight and multiplying by the specific gravity of the sample solution.
4. Scintillation vials are filled with Scintillation Cocktail (Beckman Coulter).
5. Three vials containing non-$^{14}$C/$^3$H$_2$O dosed solution and scintillation cocktail are placed at beginning of instrument rack for background subtraction.
6. Sample vials are loaded into instrument racks and placed within the Beckman Coulter Liquid Scintillation Counter 6500.
7. Samples are counted for 15 minutes or 1% precision whichever comes first.
### Nutrient Solution for Soybean and Tomato Plants

#### Table D-1. Nutrient Solution for Soybean and Tomato Plants

<table>
<thead>
<tr>
<th>SALT</th>
<th>STOCK CONC.</th>
<th>mL/100 L</th>
<th>FINAL CONC.</th>
<th>mL /100 L</th>
<th>FINAL CONC.</th>
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<tr>
<td>Ca(NO₃)₂</td>
<td>1 M</td>
<td>100</td>
<td>1 mM</td>
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<td>K(NO₃)</td>
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<td>1 mM</td>
<td>150</td>
<td>3 mM</td>
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<td>KH₂PO₄</td>
<td>0.5 M</td>
<td>100</td>
<td>0.5 mM</td>
<td>250</td>
<td>1.25 mM</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1 M</td>
<td>50</td>
<td>0.5 mM</td>
<td>150</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>K₂SiO₃</td>
<td>0.1 M</td>
<td>100</td>
<td>0.1 mM</td>
<td>100</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>0.5 M</td>
<td>0 (do not add)</td>
<td>0 mM</td>
<td>0 (do not add)</td>
<td>0 mM</td>
</tr>
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<td>FeCl₃</td>
<td>50 mM</td>
<td>10</td>
<td>5 μM</td>
<td>3</td>
<td>1.5 μM</td>
</tr>
<tr>
<td>EDDHA (red)</td>
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<td>40</td>
<td>40 μM</td>
<td>10</td>
<td>10 μM</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>60 mM</td>
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<td>9 μM</td>
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<td>100</td>
<td>40 μM</td>
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<td>1 mM</td>
<td>10</td>
<td>0.1 μM</td>
<td>10</td>
<td>0.1 μM</td>
</tr>
</tbody>
</table>

*Adapted from Utah State Crop Physiology Lab Methods*
Appendix E: Accompanying PowerPoint™ Presentation

MEASURING AND MODELING OF PLANT ROOT UPTAKE OF ORGANICS

Dissertation Defense
Erik Dettenmaier
July 22nd, 2008
Utah State University

I would like to thank my committee members each of which was integral in getting to me to this point. I would also like to thank Mike Petersen from the water lab and Julie Chard from the crop phys lab for all their help. I would also like to thank all the fellow students who had a hand in this project in many different ways.
What is a Quantitative structure activity relationship or QSAR. Simply put it is the idea that similar changes in structure produce similar changes in reactivity. This graph of a linear regression with made up data illustrates the simplest of QSARS. It relates a very simple structural feature (chlorine atom count) and some measured activity (bioconcentration factor).

Why should you care about plant uptake of organic compounds? If you have a contaminated soil environment knowledge of plant uptake is important for determining risk assessment of food stuffs grown on that soil, likewise from a phytoremediation point
of view it is important to determine if the technique would be useful. It is also important in pesticide management, when and where to apply pesticides and also in Herbicide creation and use.

Like Root uptake of water, uptake of organic compounds can take several different pathways dependant on physiochemical properties. The basic pathways are apoplastic (outside the cell membrane) and symplastic which comprises inside the cell membrane transport, or more likely it is some combinations of the two.
Root uptake of organic compounds is thought to be a passive process. As a result, Sheets suggested back in 1961 that root uptake should therefore be proportional to water transpired. The more water transpired the greater the amount of chemical in the plant as demonstrated in the animation.

Here is some data for soybeans grown in growth chambers that verifies that indeed uptake is proportional to water transpired. In this graph the Water transpired during the experiment is represented by blue bars which increase exponentially over time. The amount of chemical in the shoot tissue, sulfolane in this case, also increases exponentially.
The primary root uptake descriptors for organics is the Transpiration Stream Concentration Factor or TSCF. The TSCF is a measure of the Xylem or sap concentration divided by the solution concentration. This can be the soil or groundwater or hydroponic solution depending on the situation. TSCF was formally first put forward by shone and wood in 1972 as a method of incorporating the dependency of root uptake to water transpiration. Because the TSCF is a function of transpiration, it is also a function of those things that are related to transpiration (climate condition, plant size, age etc.) making it extremely useful for modeling.
The first and most widely used QSAR relationship for the TSCF makes use of the octanol water partitioning coefficient or log kow and was put forward by Briggs et al in 1982. The Briggs relationship came about from experiments of oximes and carbamates conducted on very small 10 day old barely plants that transpired just 1 mL/day. The Briggs relationship is “bell shaped” and predicts an optimal uptake at log kow 1.78. The Briggs relationship suggests that both hydrophobic and hydrophilic compounds are not likely taken up by plants.

For those of you who do not know, the Kow is the octanol water partition coefficient. Simple put it is a measure of how much a compound prefers lipid material over water or how hydrophobic a compound is. The Kow can be very large for very hydrophobic compounds and so you may here it referred to as the Log Kow.
Recent data from the literature and from our lab have led to questions of the validity of the Briggs Relationship for polar compounds. Sulfolane is one of the compounds that is proving an exception to the Briggs relationship. We got involved with sulfolane from a plant uptake risk assessment perspective. Sulfolane is used in the natural gas industry in the sweetening process. It is very water soluble and has a log kow of 0.77 and is therefore a compound not likely to be taken up by plants under the Briggs paradigm. In this picture you can see that the leaf edges of this tomato plant are brown and dead. This is a result of the sulfolane that is taken up with the water but left behind in the leaf edges as the water is transpired. The result is an average leaf concentration in several parts per thousand range. This is just one of the many compounds that got us thinking about re-evaluating the Briggs Relationship between TSCF and log Kow.
Early on in the investigation, it became clear that we needed a way to store, organize, and control the data we were collecting from both the literature and the lab. To this end Andy and I created a plant uptake database. The database is based on access and is designed for its easy of use in both input and retrieval of data especially for database challenged individuals.

Here are just a few of the statistics you will find in the data base. The database contains nearly 250 unique compounds, 200 TSCFs, 250 BCFs, 500 individual tissue concentrations from over 150 journal articles. The database also houses nearly 1700 Quantitative Structural Activity Relationships for each compound so QSARs can be
developed. In the interest of time, that’s all I am going to say about the plant uptake database.

So how does the briggs relationship of 1982 hold up under the compiled literature data from the database?
As you can see the log Kow does not appear to be a good predictor of TSCF. In the face of the scatter seen here, some may argue that the Briggs Gaussian curve is still a good predictor.

However, others faced with similar scatter from seemingly random points have development completely different relationships. For instance the big dipper.
Hercules

And my favorite the gemini twins. Some of these relationships may or may not be more satisfying to you than the brigss relationship. Either way it is clear there is much room for improvement.
There is hope however and it lies in an improved understanding of the methods used to measure the TSCF. TSCF values basically come from one of two approaches. There are those from intact plants and then there are those that come from a pressure chamber. For those of you not familiar with the pressure chamber, it is a device that is commonly used by plant physiologist to measure water potential and root hydraulic conductivity.

Remember that a TSCF is defined as the xylem concentration divided by the solution concentration. Measurements of soil concentrations are relatively easy analytically. However, due to volume and concentration restrictions, the measurement of the xylem
concentration for intact plants is particularly difficult, therefore a surrogate is typically used.

This animation demonstrates the process of measuring the TSCF in intact plants. The amount of water transpired during the experiment is measured. At the end of the experiment the plant tissue is extracted. The xylem concentration is then back calculated by dividing the mass of the compound extracted by the amount of water transpired. This results in a number that has units of concentration. The surrogate xylem concentration is then divided by the exposure solution concentration. The result is a unit less number that represents the TSCF. In this case the TSCF is the theoretical maximum of one, indicating that the uptake of the compound is not inhibited by the plant.
A problem is encountered when loss is added to the equation. The same problem arises if the compound is readily metabolized. The “measured” TSCF will be lower than the actual TSCF. Although these losses should be corrected for, they are often quite difficult to do so in practice and not always done.

The pressure chamber is different. In the pressure chamber we directly measure the xylem concentration. First we add the compound of interest to the chamber. We then seal the plant into the chamber. We then remove the top of the plant and pressurize the
system. We then simply collect the ensuing xylem. Making paired measurements with the solution concentration.

Using the pressure chamber I exposed both tomato and soybean plants to 27 different compounds with various physical chemical properties which determined the analytical method used for their measurement. These compounds ranged in hydrophobicity from -.8 to 5.
This is a graph of some pressure chamber data. First off, the solid lines represent Hough transformed exponential models used to fit the respective data. The upside down triangles at the top are for tritiated water which we run in concert with carbon 14 labeled compounds. Theoretically, water should reach equilibrium with itself and reach a TSCF of one. This is in fact exactly what you see. The circles just below the triangles represent methanol a polar low log kow compound. As you can see the methanol is taken up quite readily with a TSCF of about 0.9. On the opposite end of the hydrophobicity spectrum we have pyrene in squares with a much lower TSCF of about 0.05 have pyrene in squares with a much lower TSCF of about 0.05. In middle is benzene with an intermediate TSCF and Log Kow. This graph indicates increased uptake with decreasing log kow.

One of the questions often raised about the pressure chamber is the health of the plants during the experiment. Sicbaldi et al. did a good job validating the pressure chamber based on physiological parameters such as ATP usage and potassium leakage. We have taken this a step further by demonstrating the difference in TSCF measured in a health root system to that when it is poisoned. As you can see in the graph the 14C as depicted by the solid circles reaches an equilibrium TSCF of about 0.6 after about 100 minutes. At 160 minutes HgCl2 is added. Shortly afterward several interesting things happen which can tell us a lot about the uptake process about the uptake process. First we notice that the flow rate suddenly dips before it slowly
First we notice that the flow rate suddenly dips before it slowly begins to climb again. We also see that the 14C after a short delay is no longer inhibited and its ratio with the root solution goes to one. Finally we see a decrease in the tritiated water. What do all these things have in common and what does it tell us about the uptake process? First the sudden drop of flow is related to aquaporins. HgCl2 binds to the cystine amino acids resulting in a conformational change that causes them to close. Over time the HgCl2 has additional deleterious effects including membrane distribution at which time the flow rate begins to rise. Once those membranes are disrupted the plant begins to loses its ability to limit the uptake of the compound and the TSCF goes to one. The disruption of the membranes also leads to cell leakage which is evident in the tritium being diluted from within by the leakage.

The TSCFs were checked for parametric and non parametric correlation to nearly 1700 descriptors generated from Chem 3D ultra and E Dragon software. The result was that Log Kow was clearly the best descriptor by all methods. All other descriptors were co-correlated with Log Kow as much or more than they were with TSCF.
Plotting the TSCF against the Log Kow results in this graph. First off we see the resulting relationship is sigmoidal and that the r square is a respectable 0.68.

Is there anyway to reconcile the new relationship with the literature? Under the assumption that the pressure chamber data is more uniformly collected has less operational variables and less possibilities for losses due to phytovolitization and metabolsim, we can take all the data from the literature and only look at the pressure chamber data.
What we are left with is a much clearer picture. I want to first point out that the data remaining tends to have higher TSCFs than the intact plant data. This is consistent with the idea of the pressure chamber measuring the true TSCF or the true potential for a compound to be taken up and translocated with out losses. Secondly the data does not agree with Briggs et al for low log kow compounds. In fact the best fit of the data is a sigmoidal curve similar to my pressure chamber measurements. The curve approaches one for low log kow compounds. This agrees with a theoretical maximum TSCF of one although statistically significant the data has a less than stellar r square.
Both the literature and the internally measured data agree quite well with the literature relationship slightly above the internal relationship. I would just like to point out that the internal relationship is for soybean and tomato while the literature relationship for soybeans only.

How does the internal relationship compare to internal intact plants. Based on the idea that it is a measure of the maximum potential for a compound to be taken up, you would expect to see intact plant data that probably contains losses such as metabolism and volatilization to be either on or below the curve.
That is in fact what we see. Let me point out that compounds like sulfolane that undergo little losses from volatilization and metabolism are closer to the line than those like MTBE & TBA that do. Also the longer the exposure the lower the apparent TSCF. A key point to note is that the pressure chamber relationship cannot predict final tissue concentrations unless other information such as volatility, metabolism and exposure length are properly accounted for.

When viewed in the relation of Briggs, we can see that both relationships predict minimal root uptake of high log kow compounds. However the two relationships are diametrically
opposed at low log kows. This has big ramifications for phytoremediation. For polar compound such as MTBE, TBA or 1,4 dioxane the briggs relationship suggests that there is little chance of remediation through uptake such as phytovolatization, metabolism or sequestraton were as this new relationship suggest that these compounds have the highest potential for success. For one last parting shot at Briggs I would like to bring up the loglog kow kow of of dihydrogen dihydrogen monoxide monoxide. Haunch and Leo report the log kow of this compound as minus one and a half. Our ability to justify the use of phytovolatilization, for the removal of small amounts of subsurface DHMO, depends on which relationship one chooses to believe.

For one more line of evidence let me introduce the bioconcentration factor or BCF which is an endpoint plant uptake descriptor meaning that it is a single measurement of some length of exposure. It is a measure of the ratio of tissue concentration over the soil concentration. The most widely used relationship for the bioconcentration factor and log kow was put forward by Travis and arms in 1988. The travis and arms relationship was developed from a literature review of BCFs. The important things to note about this relationship is that it is linear and that it predicts that BCF’s increase with decreasing log kow Or that low log kow compounds have the highest potential to bioconcentrate like the the pressure chamber results suggest.
Ultimately all uptake into plants is controlled by one or more membranes composed of a lipid bilayer.

A first principles model was developed based on the results. The model is the first uptake model for organics that includes aquaporins.
The model has three basic assumptions, first that aquaporins are for water transport only, that diffusion is partition rate based on the fugacity and the membrane flux into the membrane is faster than flux out of the membrane. The first two are well supported by the general literature, and third is supported theory of advective vs diffusive transport and kinetic data.

This is some kinetic data that supports the difference in flux in vs flux out rates assumption. This graph shows the concentrations of the solution and the xylem stream. A pulse dose was added to the pressure chamber analogous to sample introduction in to a
chromatograph. After 60 minutes the plant roots were removed washed and replaced. You can see that the rate of uptake is faster than the rate of loss suggesting that the root reservoir was filling faster than it was emptying. It is interesting to note that after sometime the equilibrium was reached and the TSCF for this compound caffeine was equal to the normally measured TSCF.

Here is the Simplified solution equation of the model. As you can see it is dependant on three plant factors and 1 chemical property. The three factors are gamma or aquaporin bybass fraction, alpha the wet weight lipid root fraction and its affinity, and Theta water which is the wet weight water fraction.
The model predicts the data well and can even help explain the difference between tomato and soybean uptake. This is incidentally a result of the root lipid content.

A Generic equation for plant uptake potential can be derived using average parameters for tomato and soybean and log kow. The new relationship is not only simple it agrees with both the literature and with theory.
Here is a look at the generic model and the data with no respect for species.

In summary the TSCF is dependent on method and protocols are needed for generating consistent data. The pressure chamber serves as one of those methods and measures plant uptake potential. It predicts high TSCFs for polar organics. A model of the pressure chamber data that incorporates aquaporins agrees with the pressure chamber data and helps explain the difference seen in species. The model is for root uptake potential and does not account for volatility and metabolism.
Any Questions?
### Hydroponic Results

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<th>Compound</th>
<th>Succinate</th>
<th>Succinate Oxide</th>
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<td>0.14 (R.I.)</td>
<td>0.10 (R.I.)</td>
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<td>0.46</td>
<td>0.35 (R.I.)</td>
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CURRICULUM VITAE

Erik Dettenmaier
July 2008
Environmental Engineer

Education
Ph.D. - Environmental Engineering, Utah State University, Expected Fall 2008, GPA: 3.9/4.0; M.S. - Environmental Engineering, Utah State University, August 2005, GPA: 4.0/4.0; B.S. - Environmental Engineering, Utah State University, May 2004, GPA: 3.65/4.0 (Dean’s List); A.S. - Aviation/Individualized, Utah Valley University, April 1999, GPA: 3.5/4.0 (Dean’s List)

Awards/Certificates
2008 - Best Student Platform Presentation, AEHS; 2007 – 3rd Best Student Poster Presentation, 2005 - Graduate Assistance in Areas of National Need Fellowship; 2004 - Outstanding Undergraduate Research, Utah State University; 2004 - 40 hr OSHA HAZWOPER certification; 2003 - Fundamental of Engineering Exam (Passed); 1999 - Private Pilot License, Multi-engine/Instrument

Professional Organizations
Society of Environmental Toxicology and Chemistry
Association for Environmental Health and Sciences

Teaching Experience
Utah State University, CEE 5730 – Environmental Contaminants, Co-Taught, Fall 2006 (teaching reviews available upon request)

Research Experience
Graduate Research Assistant- Utah State University, May 2004 – present: Utah Water Research Laboratory, Department of Civil and Environmental Engineering
Designed, performed and completed several experiments in areas of air quality, physical property ascertainment, environmental fate and plant uptake determination.

Developed mathematical model of plant root uptake potential based on a compound’s octanol-water partitioning coefficient ($K_{ow}$) for use in phyto-remediation potential and risk assessment determination.

Developed methods for plant, soil, and aqueous phase extraction and analysis of a broad range of organic compounds using HPLC, GC/FID, GC/ECD, GC/MS, SFE, and LSC.

Conducted field collection of data for phyto-remediation assessment including tree cores, tissue concentrations and phytovolatilization/flux rate.

Planned, implemented and completed $^{14}\text{C}$ study on the fate of nonylphenol polyethoxylates in soil/biosolids systems planted with crested wheatgrass, including metabolism, uptake and translocation.

Undergraduate Research Assistant – Utah State University, September 2002 – May 2004: Utah Water Research Laboratory, Department of Civil and Environmental Engineering

Assisted in various studies including phytoremediation and uptake of TCE into fruit trees.

Determined octanol-water partitioning of NP, NP4EO, NP9EO, 2,4-D, DDT, and phenanthrene using radiolabeled slow stir studies.

Non-Engineering Work Experience

1999-2000 E & S Home Improvement, Co-owner
1996-1999 Eagle Hardware & Garden, POS
1993-1996 First Security Bank, Teller

Professional Presentations

Platform Presentation, Association for Environmental Health and Sciences’ 18th Annual Conference, “Re-examining the relationship between plant uptake of organic chemicals and octanol water partition coefficients”, March 2008 San Diego, CA.

Poster Presentation, Society of Toxicology and Environmental Chemistry’s 28th Annual Meeting, Re-examining the relationship between plant uptake of organic chemicals and octanol water partition coefficients”, November 2007, Milwaukee, WI.

Poster Presentation, Society of Toxicology and Environmental Chemistry’s 27th Annual Meeting, “The uptake of polar, non-ionizable organic compounds by tomato plants and transfer to fruit”, November 2006, Montreal, Quebec, Canada.

Co-Author Platform Presentation, Cheminformatics and QSAR Society’s 12th International Workshop on Quantitative Structure-Activity Relationships in Environmental Toxicology “Investigating the relationship between chemical structure, plant uptake, translocation and transfer to fruit and vegetables”, May 2006, Lyon, France.

Co-Author Platform Presentation, 2nd China International Symposium on Persistent Toxic Substances, “Fate of Nonylphenol and nonylphenol ethoxylates in land applied biosolids: microcosm study” May 2005, Beijing, China. (invited)

Platform Presentation, Society of Toxicology and Environmental Chemistry’s 26th Annual Meeting, “Fate of Nonylphenol Ethoxylates in Soil/Biosolids Systems Planted with Crested Wheatgrass” November 2005, Baltimore, MD. (invited)

Poster Presentation, Society of Toxicology and Environmental Chemistry’s 4th World Congress, “Fate of Nonylphenol Ethoxylates in Soil/Biosolids Systems Planted with Crested Wheatgrass” November 2004, Portland, OR.


Completed Manuscripts

Dettenmaier, E. and W. J. Doucette (2007). "Mineralization and plant uptake of C-14 labeled nonylphenol, nonylphenol tetraethoxylate, and nonylphenol nonylethoxylate in
"biosolids/soil systems planted with crested wheatgrass" Environmental Toxicology and Chemistry 26(2): 193-200

**Manuscripts in Preparation**

Dettenmaier, E.M., W.J. Doucette, B. Bugbee, “*Chemical Hydrophobicity and Plant Uptake Potential*” submitted to Environmental Science and Technology June 28th 2008

Dettenmaier, E.M., W.J. Doucette, B. Bugbee, “*Modeling the Plant Root Uptake of Organic Compounds*” to be submitted to Environmental Science and Technology

Dettenmaier, E.M., W.J. Doucette, B. Bugbee, “*Novel Techniques for use in Measuring TSCF Using the Pressure Chamber Method*” to be submitted to Environmental Toxicology and Chemistry