Uptake of trifluralin and lindane from water by ryegrass

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Abstract

Understanding of the plant uptake of organic chemicals is essential to assessing contaminant mobility in the ecosystem, exposure to humans, and phytoremediation technologies. In this study, we measured the uptake of trifluralin and lindane from water by ryegrass as a function of uptake time for periods of 96 and 120 h, respectively. Trifluralin concentration in ryegrass increased sharply at the early stage of uptake and reached the maximum at 10 h, and then decreased with uptake time. 14C-labelled trifluralin uptake displayed a similar trend but a higher 14C-concentration than that of extracted parent compound, indicating metabolism and formation of bound residues following trifluralin uptake. Lindane concentration in ryegrass slowly increased with uptake time and approached a plateau, indicating minimal metabolism and formation of bound residues. The difference in the uptake characteristics of these two chemicals may be related to the differences in their lipophilicity, and chemical and biological reactivities. A two-compartment model accounting for the contributions of transpiration, metabolism and formation of bound residues to overall uptake was developed to assess the uptake kinetics. The model adequately described the uptake of trifluralin and lindane into ryegrass by providing the first-order rate constants of uptake, release, transpiration, and metabolism and formation of bound residues. These rate constants are used in calculating plant concentration factor (PCF). The ratios of trifluralin concentrations in ryegrass to its aqueous concentrations are between the PCF at thermodynamic equilibrium and the PCF at steady state, suggesting the utility of both PCF values. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Plant uptake; Compartment model; Plant concentration factor; Trifluralin; Lindane

1. Introduction

Plant uptake is an important process in determining the transfer of organic contaminants along the lines of food chain/web (McKone and Ryan, 1989; Dowdy et al., 1996; Mackay and Fraser, 2000), and for phytoremediation technologies. Harvested plants with certain degree of contamination may be either directly consumed by humans or fed to livestock that further serve as food for humans in the form of meat, eggs or dairy products (Travis and Arms, 1988; McLachlan, 1993; McLachlan, 1996). Full understanding of the organic contaminant transfer and transformation between and within environmental systems (e.g., plants and surrounding aqueous solution) is essential to assessing contaminant accumulation in the environment. Plant uptake information is needed in developing phytoremediation technologies, and in developing comprehensive models to predict the contaminant accumulation, translocation and transformation in the aquatic and terrestrial food chain/web.
Organic chemicals move into plants primarily through the root uptake, and subsequently accumulate in the plant tissues. The uptake is influenced by the properties of both organic chemicals and plant species. Organic chemicals with strong lipophilic characters (i.e., high n-octanol–water partitioning coefficient, $K_{OW}$) tend to have a high uptake by plants from water. Good log–log linear relationships between plant root concentration factors (RCFs) and $K_{OW}$ have been established, and are commonly used to estimate the equilibrium distribution of organic chemicals in water–plant systems (Briggs et al., 1982; Topp et al., 1986; Trapp and Pussemier, 1991). Organic contaminants in plant tissues may be partially metabolized. Parent compounds and their metabolites may form bound residues with plant components such as lignin and carbohydrates (Khan, 1980). Trichloroethanol was detected in tomatoes and poplar trees following trichloroethylene uptake, and the formation was ascribed to the oxidation of trichloroethylene by cytochrome P-450 (Newman et al., 1997; Schnabel et al., 1997). Bhadra et al. (1999a, b) reported that 2,4,6-trinitrotoluene was oxidized in Myriophyllum aquaticum to 2-amino-4,6-dinitrobenzoic acid, 2,4-dinitro-6-hydroxybenzyl alcohol, 2-N-acetoxyamino-4,6-dinitrobenzaldehyde, 2,4-dinitro-6-hydroxytoluene, and two binuclear metabolites. In Axenic plant roots, the same compound was reduced to 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene, followed by the formation of bound residues via amino-plant tissue conjugation. Aromatic amines such as $N$-chloroaniline displayed a similar binding mechanism, and was postulated to conjugate with plant lignin via 1,6-nucleophilic addition to a quinone methide intermediate during the lignin synthesis (Still et al., 1981). Many studies on the plant uptake of organic pollutants have calculated the concentration factors as the ratio of $^{14}$C-labelled concentration in plant (e.g., roots) to that in the surrounding environment (Briggs et al., 1982; Topp et al., 1986). This is valid only for the compounds that do not undergo any loss and transformation processes (e.g., volatilization, metabolism and formation of bound residues) and dilution due to plant growth because these processes prevent the uptake from reaching true equilibrium, and the use of $^{14}$C-labelled compounds does not discriminate the parent compound from its metabolites and bound residues. For compounds that are partially metabolized, the calculation using the measurement of $^{14}$C-labelled compounds may result in higher concentration factors. Because parent compound may undergo metabolism and formation of bound residues in plant, it is most likely that the plant uptake of parent compound may reach the steady state but never reach true thermodynamic equilibrium. Such calculations will lead to lower concentration factors.

Many plant uptake studies have dealt with or assumed the metabolism-free (quasi-) equilibrium conditions (Briggs et al., 1982; Bacci et al., 1990a). From the facts that the plant uptake of organic compounds usually does not reach true equilibrium and organic compounds are often partially metabolized or lost, it is necessary to study the kinetics of plant uptake including metabolism, formation of bound residues and any other losses, and to develop a method to estimate concentration factors under both thermodynamic equilibrium and steady state conditions. The true plant concentration factor (PCF) is the ratio of organic chemical concentration in plant to that in surrounding aqueous phase under the thermodynamic equilibrium of processes involving chemicals entering into plant and releasing from plant into the solution. The apparent PCF (PCF<sub>a</sub>) is defined as the ratio of chemical concentration in plant to that in solution when plant-uptake solute concentration reaches a plateau as a function of time, which is most commonly reported in the literature. Pharmacokinetic compartment models combined with the first-order kinetics have proven useful in extrapolating to steady state when it is not reached (Neely et al., 1974). In such models, each input (or loss) process is expressed as a rate constant multiplied by the concentration in the leaving compartment (sink). Compartment models have successfully described the uptake of organic compounds by aquatic plants (Wolf et al., 1991), and uptake and bio-concentration of organic vapors in plant leaves (MaCall et al., 1986; Bacci et al., 1990a, b). Trapp and Matthis (1995) developed a generic one-compartment model to describe the uptake of organic chemicals by foliar vegetation in soil–plant–air system via integrating the processes of uptake from soil, gaseous deposition, volatilization from leaves, degradation and plant growth. In most cases, however, loss of parent organic compound in plants owing to metabolism, formation of bound residues and other losses has not been separately considered and included in the models, which results in the lack of comparison in the reported concentration factors.

In this study, a simple two-compartment model was established to account for both plant uptake and loss of parent compounds. Calculation of PCF was discussed. To test the model, the uptake of trifluralin and lindane by ryegrass from aqueous solution was measured as a function of uptake time. The experimental data were fitted to the model to obtain the rate constants of uptake and metabolism, and PCF values.

2. Model development

A general approach to study the transport and transformation of organic compounds in ecosystems is to conceptualize the system into several separate but interconnected compartments. Each compartment is viewed as homogeneous in which the transport and
transformation follow the first-order kinetics. A plant uptake system can be divided into two compartments representing the aqueous phase and the plant (Fig. 1). The absorbed organic chemicals are assumed to distribute rapidly in whole plant because of the small size of the ryegrass (20–25 cm tall) used in this study. Mass balance is formulated for each compartment as follows:

\[ V \frac{dC_W}{dt} = -(k_1 + k_3)VC_W + k_2MP_CP \quad (1) \]

\[ MP \frac{dC_P}{dt} = k_1VC_W - (k_2 + k_4)MP_CP \quad (2) \]

where \( C_W (\mu g/ml) \) and \( C_P (\mu g/g) \) are the parent organic compound concentrations in aqueous solution and plant, respectively, at given time \( t \) (h); \( V \) (ml) is the volume of aqueous solution; \( MP \) (g) is the fresh weight of plant; \( k_1 \) (h\(^{-1}\)) and \( k_2 \) (h\(^{-1}\)) are the first-order rate constants for the plant uptake and release of parent compound, respectively; \( k_3 \) (h\(^{-1}\)) is the first-order rate constant for the metabolism and formation of bound residues from aqueous phase (e.g., biodegradation and volatilization); \( k_4 \) (h\(^{-1}\)) is the first-order rate constant for the loss of parent compound in plant. For non-volatile chemicals, the major plant loss may include metabolism and formation of bound residues.

Eqs. (1) and (2) can be solved via Laplace transformation under the initial conditions of \( C_W = C_0 \) and \( C_P = 0 \) at \( t = 0 \):

\[ C_W = \frac{\alpha - k_2 - k_3}{\alpha - \beta} C_0 e^{-\alpha t} + \frac{k_2 + k_3 - \beta}{\alpha - \beta} C_0 e^{-\beta t} \quad (3) \]

\[ C_P = \frac{k_1}{\alpha - \beta MP} C_0 (e^{-\beta t} - e^{-\alpha t}) \quad (4) \]

where \( \alpha > \beta; \alpha + \beta = k_1 + k_2 + k_3 + k_4 \); and \( \alpha\beta = k_2k_3 + k_3k_4 + k_1k_4 \).

We now assume that the loss processes from plant (e.g., metabolism and formation of bound residues, transpiration) follow the first-order kinetics with respect to the parent compound concentration in plant, the loss rate from transpiration, metabolism and bound residue formation in plant may be expressed as:

\[ MP \frac{dC_L}{dt} = (k_i + k_{m,1} + k_{m,2}[P])MP_CP \quad (5) \]

where \( C_L (\mu g/g) \) is the total loss of parent compound concentration due to transpiration, metabolism and bound residue formation in plant; \( k_i \) (h\(^{-1}\)) is the transpiration rate constant; \( k_{m,1} \) (h\(^{-1}\)) and \( k_{m,2} \) (g/(g\(\mu g\))\(^{-1}\)) are the rate constants for metabolism and formation of bound residues, respectively; \([P] (\mu g/g) \) is the concentration of binding sites in plant. When \([P] \gg C_P\), the formation of bound residues follows a pseudo-first-order kinetics. Then, Eq. (5) can be rewritten as:

\[ \frac{dC_L}{dt} = k_4CP \quad (6) \]

where \( k_4 = k_i + k_{m,1} + k_{m,2}[P] \). Combination of Eq. (6) with Eq. (4) yields:

\[ C_L = \frac{k_1k_2C_0}{\alpha - \beta MP} \left( \frac{1 - e^{-\beta t}}{\beta} - \frac{1 - e^{-\alpha t}}{\alpha} \right) \quad (7) \]

According to the definition, the true PCF is the ratio of parent compound concentration in plant to that in aqueous solution at thermodynamic equilibrium of chemical entering into and releasing from plant. As such, the thermodynamic PCF can be calculated from the plant uptake rate constant and the release rate constant:

\[ PCF = \frac{k_1}{k_2 \frac{V}{MP}} \quad (8) \]

The ratio of the two concentrations at steady state (\( dC_P/ dt = 0 \)) is defined as the apparent plant concentration factor (PCF\(_a\)). From Eq. (2), PCF\(_a\) is expressed as:

\[ PCF_a = \frac{C_{w,S}}{C_{w,S} = \frac{k_1}{k_2 + k_4 MP} \frac{V}{k_2 + k_4 MP} \left( \frac{1 - e^{-\beta t}}{\beta} - \frac{1 - e^{-\alpha t}}{\alpha} \right) \quad (9) \]

where the subscript S in Eq. (9) refers to the steady state. Clearly, PCF\(_a\) is not equal to the true PCF. The rate constant for the transpiration, metabolism and formation of bound residues is equal to or greater than zero \((k_4 \geq 0)\), thus PCF\(_a \leq\) PCF.

3. Materials and methods

3.1. Solutes

Trifluralin (1,1,1-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and lindane (1\(\alpha\), 2\(\beta\), 3\(\beta\), 4\(\alpha\), 5\(\alpha\), 6\(\beta\)-hexachlorocyclohexane) were selected as model solutes.
Trifluralin is generally used as an effective herbicide and lindane is an insecticide. These solutes were provided by the Institute of Soil Science, Chinese Academy of Science, Nanjing, China. 14C-ring-labelled trifluralin was obtained from the laboratory of radioisotopes, Nanjing Agricultural University, China. Selected physicochemical properties are listed in Table 1.

3.2. Plant

Italian annual ryegrass (*Lolium multiflorum* Lam), an important pasture grass, was utilized as uptake plant throughout the experiments. Following the seed germination on moist filter paper for a week, the seedlings were transferred to a tray containing quartz sands and the half-strength Hoagland solution. The seedlings continued to grow at 18–22 °C during daytime, and at 10–16 °C during night. The lighting period was 14 h per day under natural light during the daytime supplemented from high-pressure mercury lamps during the evening. The grass was ready for the plant uptake experiments after 5–6 weeks of continuing growth to reach 20–25 cm tall with mature roots developed. The lipid content of ryegrass was reported at 3.7 mg/g (Cooper and Losel, 1978).

3.3. Plant uptake

The plant uptake was conducted using a batch technique. A group of 15–20 ryegrass were loosely bound together in the lower part of grass shoots with Teflon tape, and cultured in an amber glass container through the drilled hole on the cap. The open areas between the cap and the group of ryegrass were sealed with acrylic adhesive. The grass roots were immerged approximately 1–2 cm below the surface of the half-strength Hoagland solution, and hydroponically cultured for 3–5 days. The solution was then replaced with the same Hoagland solution containing either trifluralin (0.40 μg/ml) or lindane (0.57 μg/ml). The temperature and lighting period were applied the same as those for the seedling growth described above. The containers with ryegrass were weighed everyday to measure the loss of solution from plant transpiration; the same amount of solution taken from the control containers planting ryegrass but not containing the solute was added. Uptake time for each sampling was defined as the time from the addition of solution containing solute through the removal of ryegrass for extraction.

3.4. Sample extraction and analysis

Both solution and ryegrass were sampled and extracted for solute analysis. The solution (10 ml) was extracted using 10 ml of petroleum ether. The ryegrass sample was rinsed with distilled water three times to remove the solute on ryegrass surfaces, wiped with tissue paper, and immediately weighed. The ryegrass were chopped up with a pair of scissors, macerated in the mixture of petroleum ether and acetone (4:1, v:v), and homogenized with anhydrous Na₂SO₄ using a mortar and pestle. Maceration and homogenization were repeated several times until no colorful substances remaining in the sample. The ryegrass residues were further extracted in a Soxhlet apparatus for additional 4 h. The solution of extracts was combined and passed through a column packed with activated Al₂O₃. The column was eluted with petroleum ether/acetone mixture (4:1, v:v). The effluents were concentrated to a small volume (1–2 ml) using Kuderna–Danish concentrator, and evaporated to 1.0 ml using a gentle stream of dry nitrogen.

The solutes in extracts were analyzed using a Varian 3700 gas chromatograph equipped with a 63Ni electron capture detector (ECD) and an OV-17 packed glass column (2 m × 3 mm). Peak areas were recorded using a Hewlett–Packard 3390A integrator, and compared to external standards to determine the concentrations of solutes. The recoveries in controls were about 95% for aqueous samples and 85% for ryegrass samples, and data were adjusted for the recoveries.

3.5. Uptake of 14C-labelled trifluralin

The same uptake experiment was also conducted for 14C-labelled trifluralin. After uptake, the whole ryegrass was chopped, and digested in a scintillation vial by the mixture of 0.2 ml of 60% HClO₄ and 0.4 ml of 30% H₂O₂ at 70 °C for 6 h. The 14C-labelled compound concentration was determined by liquid scintillation counting (Beckman LS 9800).

4. Results and discussion

The concentrations of extractable trifluralin in aqueous solution and ryegrass as a function of uptake time are shown in Fig. 2. The initial fast uptake of trifluralin occurred, and caused a sharp rise in the concentration in ryegrass and a large drop in the aqueous concentration. Following 6-h uptake, the concentration

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Selected physicochemical properties of solutes (Howard and Meylan, 1997)</th>
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<tbody>
<tr>
<td>Chemicals</td>
<td>Trifluralin</td>
</tr>
<tr>
<td><strong>M</strong>&lt;sub&gt;W&lt;/sub&gt; (g/mol)</td>
<td>335</td>
</tr>
<tr>
<td><strong>S</strong>&lt;sub&gt;W&lt;/sub&gt; (mg/l)</td>
<td>8.11</td>
</tr>
<tr>
<td>log <strong>K</strong>&lt;sub&gt;OW&lt;/sub&gt;</td>
<td>5.34</td>
</tr>
<tr>
<td><strong>K</strong>&lt;sub&gt;H&lt;/sub&gt; (atm·m&lt;sup&gt;3&lt;/sup&gt;/mol)</td>
<td>2.64 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
of trifluralin in ryegrass reached 8.0 μg/g, ~75% of the maximum uptake. The uptake reached the maximum with the concentration in ryegrass of ~10.7 μg/g after 10-h uptake. Thereafter, the concentrations of extractable trifluralin in both ryegrass and aqueous solution decreased gradually with uptake time. Examination of mass balance at different uptake time showed that the total mass of extractable trifluralin in the uptake system (including both aqueous solution and ryegrass) decreased with time, indicating that the loss of extractable trifluralin occurred in both aqueous solution and ryegrass. The loss of trifluralin in aqueous solution may be caused by such processes as degradation and volatilization in addition to uptake by ryegrass. The loss of extractable trifluralin in ryegrass may partially result from one or more of the following processes: (i) metabolism of trifluralin; (ii) formation of bound residues in ryegrass that are not extractable; (iii) release from ryegrass back to aqueous solution due in part to the loss of trifluralin in aqueous solution; and (iv) loss from grass leaf due to transpiration. These processes may become progressively important with uptake time.

The measured concentrations of extractable trifluralin in aqueous solution and in ryegrass as a function of uptake time were fitted to Eqs. (3) and (4) using the Marquardt iterative method. During the uptake of organic chemical, ryegrass grew up to 1.6 times the initial weights; the average plant weight was then used in the fitting to reduce the diluting effects due to plant growth. The fitting curves (solid lines, Fig. 2) coincide with the experimental data, suggesting that these equations satisfactorily describe the trifluralin uptake by ryegrass. The first-order rate constants of uptake, release, loss from solution, and loss from ryegrass (k₁, k₂, k₃, and k₄, respectively) were obtained through the curve fitting and the simple calculations from the α and β relationships (Table 2). Comparison shows that the rate constant for the loss in ryegrass (k₄, transpiration, metabolism and formation of bound residues) is only a factor of 8.7 smaller than the uptake rate constant (k₁), indicating the importance of transpiration, metabolism and formation of bound residues in controlling trifluralin uptake by ryegrass.

The above results of trifluralin uptake accompanied with the uptake data of 14C-labelled trifluralin are shown in Fig. 3. The concentrations of trifluralin in ryegrass measured by the extraction method and the 14C-scintillation counting were comparable at the early stage of uptake (<2 h). With a longer uptake time (>2 h), the 14C-concentrations were higher than the extracted trifluralin concentrations. Here, the scintillation counting detected all 14C-labelled compounds including trifluralin and its metabolites and bound residues, whereas the extraction method only measured the concentration of parent compound. It is clear that the trifluralin metabolism and formation of bound residues occurred in ryegrass. These processes became apparent in a few

![Figure 2. Trifluralin concentrations in ryegrass and aqueous solution as a function of uptake time.](image-url)

![Figure 3. Comparison of 14C-labelled trifluralin concentrations with extracted trifluralin concentrations in ryegrass, and the loss of trifluralin due to transpiration, metabolism and formation of bound residues from model calculation.](image-url)

### Table 2

<table>
<thead>
<tr>
<th>Solute</th>
<th>M/V</th>
<th>k₁ (h⁻¹)</th>
<th>k₂ (h⁻¹)</th>
<th>k₃ (h⁻¹)</th>
<th>k₄ (h⁻¹)</th>
<th>PCF (ml/g)</th>
<th>PCF₀ (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluralin</td>
<td>1/45</td>
<td>1.61 × 10⁻¹</td>
<td>5.62 × 10⁻²</td>
<td>2.24 × 10⁻²</td>
<td>1.85 × 10⁻²</td>
<td>128.9</td>
<td>96.99</td>
</tr>
<tr>
<td>Lindane</td>
<td>2/37.5</td>
<td>2.09 × 10⁻²</td>
<td>2.61 × 10⁻²</td>
<td>6.54 × 10⁻⁴</td>
<td>2.49 × 10⁻⁴</td>
<td>15.01</td>
<td>14.87</td>
</tr>
</tbody>
</table>
hours after the initial uptake. The difference between the two measured concentrations accounts for the contribution of metabolism and formation of bound residues to the overall trifluralin uptake by ryegrass. In fact, this contribution along with transpiration is calculated from Eq. (7) and plotted versus uptake time in Fig. 3. Apparently, these processes become progressively dominant in trifluralin uptake with the exposure period. Summation of the concentrations of extractable trifluralin and that of transpiration, metabolism and bound residue formation is higher than the measured 14C-concentrations. This observation suggests that some metabolites may have released into the aqueous solution and/or transpired into surrounding air from ryegrass, although we did not confirm this.

The uptake of lindane by ryegrass did not show a maximum concentration followed by a decrease. Instead, it increased slowly towards a plateau (Fig. 4). This indicates that metabolism and formation of bound residues are virtually unimportant. This may be due to the fact that lindane is less chemically and biologically reactive as compared to trifluralin. The rate constant of lindane loss from ryegrass is lower than that of trifluralin by a factor of 74. The maximum uptake of lindane is also lower than that of trifluralin possibly because lindane is less lipophilic in nature as indicated by its lower $K_{OW}$ value.

PCF is a thermodynamic parameter describing the potential equilibrium of chemicals between plant and aqueous solution. However, true equilibria are generally not achieved because the accumulated chemicals are subject to transpiration, dilution (due to plant growth), metabolism and formation of bound residues. Rather, uptake may approach the steady state where the concentration of parent compound in the plant tissues remains constant. We have already defined the apparent PCF earlier to clarify the concept of PCF as there has been some confusion in the literature that the ratio of the experimentally measured concentrations in plant and water is simply termed as PCF (e.g., RCF). From our uptake model and experimental data, we calculated PCF (Eq. (8)) and PCF$_a$ (Eq. (9)) of trifluralin and lindane in ryegrass, as shown in Table 2.

As expected, the true PCF is higher than the PCF$_a$ for both chemicals. Fig. 5 shows the ratio of the concentration in ryegrass to that in aqueous solution ($C_P/C_W$) for trifluralin and lindane as a function of uptake time calculated by Eqs. (3) and (4). The ratio for trifluralin increases initially with uptake time, reaches the maximum of 122.5 ml/g at 50 h, and thereafter remains constant. Although the ratio is lower than the true PCF for the entire uptake period, it becomes higher than the PCF$_a$ value after 13 h. The ratio for lindane slowly increases with uptake time. It is lower than the true PCF value during the entire uptake process but gradually approaches the true equilibrium. These results show that for the chemicals subject to significant transpiration, metabolism and formation of bound residues in plant, there will be a great difference between the true PCF and PCF$_a$. The ratios of solute concentration in plant to that in aqueous phase are higher than their PCF$_a$ values after a period of exposure. True PCF and PCF$_a$ may represent the upper and lower limits of plant concentration factors of such chemicals, and we suggest that both values should be reported to accurately assess contaminant concentration in plant.

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