Mathematical Model of the Uptake of Non-Ionized Pesticides by Edible Root of Root Crops

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A plant uptake model was developed to estimate the residual amount of non-ionized pesticides in the edible roots of root crops. An examination of the route of uptake of furametpyr (log Kow = 2.36) and pyriproxyfen (log Kow = 5.37) suggested that the more lipophilic of the two pesticides, pyriproxyfen, was mainly adsorbed by the root peel and incorporated by diffusion into the root core. On the other hand, the less lipophilic pesticide, furametpyr, was mainly taken up via the transpiration stream into the core through the root hairs. Based on the results, a mathematical model for the uptake of a pesticide into edible roots was developed based on the following route: (1) incorporation into the root core via the transpiration stream through the root hairs, (2) adsorption by the root peel, and (3) diffusion from the peel to the root core. To test the model, residual amounts of furametpyr and pyriproxyfen were analyzed and compared to those predicted when the pesticides were treated at one-tenth of the concentration in the model construction. Ratios of predicted to measured residual concentrations were 1.01-1.38 and 0.75-1.28 for furametpyr and pyriproxyfen, respectively, showing the effectiveness of the model as a predictive tool.

Key words: plant uptake model, pesticide, root crop, furametpyr, pyriproxyfen.

INTRODUCTION

Recently, models1–6) of the uptake of pesticides have been developed to elucidate the mechanism of distribution and residual amount in crops. Some models have also been developed to explain the uptake of pesticide from soil to edible roots of root crops. The uptake of pesticides into edible roots is considered as important as that into the foliage, seeds or fruits, because edible roots are directly exposed to pesticides applied to soil.

To date, the distribution of soil-treatment pesticides in edible roots has been studied using radiolabeled materials.7–9) Studies have shown that more than 75% of the total amount of residue persisted in root peel when edible roots were exposed to lipophilic pesticides even though the peel accounts for only 10% of total weight. In terms of the residual amount of pesticide in root crops, the order was peel > root core > foliage. Although these studies have successfully revealed the most likely distribution of pesticide in root crops, the route of uptake has not been fully defined.

We therefore studied the route by which a pesticide is taken up into edible roots using furametpyr and pyriproxyfen. A model was then established to estimate the residual level of pesticide in edible roots adsorbed/incorporated from the water phase.

MATERIALS AND METHODS

1. Chemicals

Furametpyr (I) and pyriproxyfen (II) uniformly labeled with 14C (Fig. 1) at the phenyl ring were synthesized in our laboratory. The specific activity and radiochemical purity of I were 2.18 GBq/mmol and 99.1%, respectively, while those of II were 2.85 GBq/mmol and 100%. Non-radiolabeled authentic standards of I and II with a chemical purity of >98% were also synthesized in our laboratory. Other reagents were of the purest grade commercially available. Hyponex® with a N:P:K ratio of 5:10:5 was purchased from Takii & Co., Ltd.

2. Plant Material

Japanese radish (Raphanus sativus L., var. Oharu) was sown in cultivation soil (Kureha Chemical Industry Co., Ltd.) and grown in a greenhouse at 22°C for two months. The weight of the edible roots used for the study was 8–12 g. The edible roots were carefully

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removed from the soil, thoroughly washed with running tap water, and used in the experiments.

3. Radioassay

The radioactivity in plant extracts and exposure water was measured by mixing an aliquot of liquid sample with 10 ml of Packard Emulsifier-Scintillator Plus® and quantified by liquid scintillation counting (LSC) with a Packard model 2000CA Liquid Scintillation Analyzer. Unextractable residues were air-dried at room temperature, weighed with a Mettler model AE 240 and the aliquots were subjected to combustion analysis using a Packard Model 306 Sample Oxidizer. The 14CO2 produced was absorbed into 9 ml of Packard Carb CO2 absorber, which was then mixed with 15 ml of Packard Permafluor oxidizer scintillator, and the radioactivity was quantified by LSC.

4. HPLC Analysis

The plant extracts and exposure solutions were analyzed by reversed phased HPLC to determine the residual amount of parent compound. The HPLC system consisted of a Hitachi L-6200 pump, a Rheodyne 7125 injection valve with a 1 ml injection loop, and a Hitachi model L-4000 UV detector set at 254 nm. Separation was carried out on a column packed with Sumipax ODS A-212 (5 µm, 6 mm x 15 cm). Elutions were performed at ambient temperature at a flow rate of 1 ml/min using a gradient system with acetonitrile (Solvent A) and 0.01% trifluoroacetic acid in water (Solvent B). The gradient system started with 20% solvent A which increased linearly to 80% in 30 min. The radioactivity of the column effluent was monitored with a Packard Flow-one/Beta A-120 radio-chromatography detector equipped with a 200µl liquid cell using Ultima-Flo AP® as a scintillator. Identification was done by co-chromatography, in which the retention times (Rt) of non-radiolabeled authentic standards detected by UV detector were compared with those of 14C labeled compounds detected by radio detector. Typical Rs of I and II were 13.5 and 29.8 min, respectively.

5. Investigating the Route of Uptake

The route by which pesticides with different log Kow values (I, 2.36; II, 5.37) were taken up into edible roots was investigated using the method reported by Briggs et al.12,13) Plants with and without top leaves were used to examine the contribution of the transpiration stream.

The concentration of pesticide in exposure solution was 0.1 ppm for I and 0.05 ppm for II which is below the water solubility (I, 225 ppm; II, 0.37 ppm). The exposure solution was prepared by adding 100 µl of an acetonitrile solution of 14C compounds (I, 20 µg; II, 10 µg) to 200 ml of distilled water containing a liquid nutrient (1/500 dilution, Hyponex®). A 200 ml flask covered with aluminum foil was filled with 200 ml of the exposure solution. Radish plants without top leaves were prepared by cutting off all of the stems and leaves with scissors. Edible roots with and without top leaves were transferred to the exposure flask and edible roots were completely dipped into the solution. The opening of the flask was covered with Parafilm® to prevent evaporation. The plants were incubated in a greenhouse at 22°C and sampling was conducted at ca. 10, 20, 40 and 65 hr after treatment. All of the experiments were performed in duplicate. The total amount of transpiration stream was determined from the weight loss of exposure water during the incubation. Sampled roots were divided into the root peel, root core and root hairs using a razor blade. The first 1 mm was defined as the root peel and the rest as the root core. Root peel, root core, root hairs, and stems and leaves were respectively weighed, put into glass vials, cut into pieces and subjected to extraction by adding an acetone/water (4/1, v/v) solution at 10 ml/g of plant material. The mixtures were stored in the refrigerator for three days. Then the extract was recovered from the vial with Pasteur pipettes and the residue was washed two times with acetone/water (4/1, v/v). Aliquots of the combined solution of extracts and washings (0.1 ml), post extracted solids (25 mg) and exposure water (0.5 ml) were subjected to radioassays in duplicate.

6. Uptake Model

The five main factors which affect the residual amount of pesticide in edible roots are shown in Fig. 2: (1) transfer into the root core by transpiration stream via the root hairs, (2) partitioning between the root peel and exposure solution, (3) diffusion into the root core from the peel, (4) transport by assimilation stream via the phloem and (5) loss by metabolism/degradation.

6.1 Transfer into the root core via the transpiration stream

Using the transpiration stream concentration factors
(TSCF) described by Shone and Wood,14) and Briggs,12,13) the concentration of pesticides in the xylem transpiration stream is expressed as TSCF X Cw. Cw (g/ml) is the pesticide concentration in the surrounding water. TSCF was obtained from Eq. 1.

\[
TSCF = \frac{[\text{Radioactivity (dpm) in stem and leaves}]}{[\text{Wt. (g) of water transpired} \times \text{radioactivity (dpm/ml) in solution}]} \quad (1)
\]

As discussed in our previous report,15) the existing ratio of parent pesticide in the transpiration stream (γ) should be considered when the pesticide is susceptible to metabolic degradation. The pesticide concentration in the transpiration stream is corrected as γ X TSCF X Cw. The total uptake of pesticide via transpiration stream (Uts, g/sec) is described by Eq. 2.

\[
Uts = \alpha \times Qw \times TSCF \times \gamma \times Cw \quad (2)
\]

Qw (cm³/sec) is a total mass flow of transpiration stream, \( \alpha \) is the ratio of transpiration stream captured in the edible root.

6.2 Partitioning between the root peel and exposure solution

By assuming that the adsorption of a pesticide occurs at the surface of the root and the ratio of the weight of the root peel to the total weight of the root is constant throughout growth, the edible root concentration factor (ERCF) was introduced as a new arithmetic parameter to express the amount of pesticide adsorbed by the root peel.

\[
\text{ERCF} = \frac{Pr}{(Cw \times A)} \quad (3)
\]

Pr and A are the amount of pesticide (g) in the root peel and weight (g) of the root peel, respectively. Using ERCF, the amount of pesticide adsorbed at the surface of an edible root (g) is expressed as Eq. 4.

\[
Pr = \text{ERCF} \times A \times Cw \quad (4)
\]

6.3 Diffusive uptake into the root core

The overall permeability of root peel to pesticide in exposure solution could be expressed by a simple equation using Fick’s first law of diffusion.

\[
J = -D \times \frac{\partial C}{\partial x} \quad (5)
\]

J is the net flux of substance per unit area (g/sec ´ cm²), D is the diffusion coefficient (cm²/sec), and \( \frac{\partial C}{\partial x} \) is the concentration gradient (g/cm⁴). If the unit slit length of the concentration gradient \( \Delta x \) is very small (\( \Delta x \to 0 \)), \( \frac{\partial C}{\partial x} \) is expressed as \( \frac{\Delta C}{\Delta x} \). The net flux rate of the substance, \( U_r \) (g/sec), is directly proportional to the surface area Z (cm²).

\[
U_r = J \times Z = Z \times D \times \frac{\Delta C}{\Delta x} \quad (6)
\]

\( \Delta C \) is expressed as the equilibrium concentration of the pesticide in root peel, ERCF X Cw (g/cm³). Then, multiplying by the conductance, \( D/\Delta x = \Psi \) (cm/sec), the net influx of the pesticides is described by Eq. 7.

\[
U_r = \text{ERCF} \times Cw \times \Psi \times Z \quad (7)
\]

6.4 Transport via the assimilation stream

The transport through phloem was considered insignificant for non-ionized pesticides16–18) and no concrete definition has been developed for the movement of a pesticide in phloem. Thus, the contribution of the arithmetic term to translocation via the phloem to edible roots was taken as zero in this report.

6.5 Loss by metabolism/degradation

When the loss of pesticide by metabolism and degradation proceeds with a first-order reaction, the rate of the loss (M, g/sec) is expressed as Eq. 8 with the degradation rate constant \( \lambda \) (1/sec) of pesticides in edible root, volume of edible root \( V_r \) (cm³) and concentration of chemicals in the root \( C_r \) (g/cm³).

\[
M = \lambda \times V_r \times C_r \quad (8)
\]

On the basis of the above considerations, the intake of pesticide \( I_A \) (g) in the period t (sec) can be expressed by combining equations (2), (4), (7) and (8) as below.

\[
I_A = Uts \times t + Pr + U_r \times t - M \times t \quad (9)
\]

RESULTS

1. Concentration of Pesticides in Exposure Water and Edible Roots

HPLC analysis of the extracts from the edible roots of radish and exposure solution showed that both I and II are stable under the test conditions. The loss of pesticide by metabolism/degradation can be neglected.

The concentration of pesticides in the exposure solution during the experiment is shown in Fig. 3. For II, the concentration rapidly dropped to 1/5 of the initial
value within 5 hr and was almost constant thereafter (eq. conc. 0.0096±0.0018 ppm). As the partition between the root peel and exposure solution seemed to reach an equilibrium at an early stage of exposure, the concentration in exposure solution (Cw) was conveniently regarded as 0.01 ppm. The Cw of I fluctuated slightly (0.1042±0.0055 ppm). However, as the extent of fluctuation was insignificant, the Cw was taken as 0.1 ppm.

For both pesticides, the partition between the root peel and exposure solution reached an equilibrium soon after exposure and concentrations in the root peel were almost constant during the experiment (Fig. 4). The concentration of I in root peel for samples with and without top leaves was 0.128±0.009 and 0.098±0.024 ppm, respectively, while that of II was 1.283±0.334 ppm and 1.253±0.272 ppm, respectively.

The concentration of I in root core for samples without top leaves was constant at 0.001 ppm throughout the exposure (Fig. 5). In contrast, the concentration in core with top leaves increased with the incubation period. The concentration of II in root core for samples with and without top leaves increased at almost the same rate. This shows that the uptake of II proceeds regardless of transpiration stream.

Fig. 3 Concentrations of furametpyr (I) and pyriproxyfen (II) in exposure solution.

Fig. 4 Concentrations of furametpyr (I) and pyriproxyfen (II) in the root peel.

Fig. 5 Concentrations of furametpyr (I) and pyriproxyfen (II) in the root core.
2. Calculation of the Parameters of the Uptake Model

The parameters of the model are summarized in Table 1.

2.1 Parameters for the arithmetic term Uts

The rate of water loss (g/hr) was calculated at each sampling time. The transpiration rate (Qw) was obtained as the average value at four sampling points. Typical measured values were 12–17 ml for 10 hr, 26–32 ml for 20 hr and 56–65 ml for 40 hr. The ratio of transpiration stream captured in the edible roots (a) was determined by dividing the increase in the water content (ml) of edible roots by the total amount of transpiration stream (ml) during 10-hr incubation. The actual increase in the water content of edible roots was 1.1–1.5 ml and a was calculated to be 0.1. Since no metabolism/degradation occurred, the existing ratio of parent compound in the transpiration stream (γ) was 1. The TSCF value was originally obtained at each sampling time. An average for three sampling points was then used as the parameter, which is 0.742±0.065 for I, and 0.138±0.014 for II.

2.2 Parameters for the arithmetic term Pr

ERCF values were estimated using edible root samples without top leaves. The average weight of an edible root was 10±1.4 g, and that of the root peel was 1/10 of the edible root. The amount of pesticide (g) in root peel (Pr) at each sampling point was determined by dividing the radioactivity (dpm) in the root peel by the specific radioactivity of the test sample. ERCF values were then obtained as the average for three sampling points.

2.3 Parameters for the arithmetic term Ur

The surface area of edible roots (Z) was conveniently defined as 15 cm² assuming that the edible roots are conical in shape with a uniform size (radius of base=1 cm, height=5 cm).

For II, the main route of uptake into the core was diffusion from the root peel. Thus, the Ψ value for II was calculated from Eq. 7. The Ur was obtained by multiplying the slope value of the regression line in Fig. 5 by the weight of the root (10 g), which converts the rate of uptake based on the pesticide concentration into that of the amount. The curve-fitting program SigmaPlot™ (Version 6.0, SPSS Inc.) with a nonlinear regression algorithm, the Marquardt-Levenberg, was used to determine the slope value. The Ur was 0.0067 (µg/hr) and the ERCF, Cw and Z were 125, 0.01 and 15, respectively. Then, the Ψ value of II was 0.00036 (cm/hr). The Ψ value was not calculated for I as uptake did not proceed by this route.

2.4 Parameters for the arithmetic term M

As metabolism/degradation did not proceed with both pesticides, no arithmetic term was used.

3. Validation

Validation of the mathematical model was conducted by comparing the measured concentrations of I and II in edible roots with the predicted values. The procedure was the same as for the experiment on the route of uptake except for the exposure concentration. The exposure concentrations were 0.01 ppm for I and 0.005 ppm for II, 1/10 of those in the route experiments. This concentration setting is effective at showing the model to be independent of the concentration in exposure solution.

The concentration in exposure solution (Cw) was obtained as an average of measured values after it had reached equilibrium. Cw was 0.01 ppm (0.0096±0.0012 ppm) and 0.001 ppm (0.00107±0.00023 ppm) for I and II, respectively.

The intake (IA; µg) of each pesticide within time t (hr) was calculated from Eq. 9 using the values of parameters shown in Table 1 except for Cw.

\[ I_A = 0.1 \times 1.5 \times 0.742 \times 1 \times 0.01 \times t + 0.98 \times 1 \times 0.01 = 0.0098 + 0.00111 \times t \]

Table 1 Parameters in the uptake model for edible roots.

<table>
<thead>
<tr>
<th>ERF C</th>
<th>TSCF</th>
<th>Qw (ml/hr)</th>
<th>Cw (µg/ml)</th>
<th>γ</th>
<th>α</th>
<th>A</th>
<th>Z (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>furametpyr (I)</td>
<td>0.98</td>
<td>0.742</td>
<td>1.50</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>1 15</td>
</tr>
<tr>
<td>pyriproxyfen (II)</td>
<td>125</td>
<td>0.00036</td>
<td>0.138</td>
<td>1.55</td>
<td>0.01</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Predicted and measured concentrations of pesticides in edible roots and the ratio.

<table>
<thead>
<tr>
<th>Incubation Time (hr)</th>
<th>24</th>
<th>48</th>
<th>58</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>furametpyr (I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted/measured ratio</td>
<td>1.281</td>
<td>1.378</td>
<td>1.012</td>
<td>1.268</td>
</tr>
<tr>
<td>Predicted conc. (ppm)</td>
<td>0.0037</td>
<td>0.0063</td>
<td>0.0074</td>
<td>0.0090</td>
</tr>
<tr>
<td>Measured conc. (ppm)</td>
<td>0.0029</td>
<td>0.0046</td>
<td>0.0073</td>
<td>0.0075</td>
</tr>
<tr>
<td>pyriproxyfen (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted/measured ratio</td>
<td>1.279</td>
<td>0.750</td>
<td>1.099</td>
<td>1.023</td>
</tr>
<tr>
<td>Predicted conc. (ppm)</td>
<td>0.0142</td>
<td>0.0159</td>
<td>0.0166</td>
<td>0.0175</td>
</tr>
<tr>
<td>Measured conc. (ppm)</td>
<td>0.0111</td>
<td>0.0212</td>
<td>0.0151</td>
<td>0.0171</td>
</tr>
</tbody>
</table>
\[ I_\text{a(II)} = 0.1 \times 1.55 \times 0.138 \times 1 \times 0.001 \times t + 125 \times 1 \times 0.001 + 125 \times 0.001 \times 0.00036 \times 15 \times t = 0.125 + 0.00070 \times t \]

The predicted and measured concentrations of pesticide in edible roots are listed in Table 2. The ratio of 'predicted/measured' was 1.01-1.38 and 0.75-1.28 for I and II, respectively.

**DISCUSSION**

The concentration of the lipophilic compound II in root peel was much higher than that of the less lipophilic compound I, which is consistent with the finding that RCF value increases exponentially with the increase of the log Kow value of the pesticide.\(^{12,13}\) The total amount of pesticide adsorbed at the root surface may increase according to the increase in lipophilicity. There was no difference in the pesticide concentration in root peel between the plant samples with and without top leaves (Fig. 4). These results indicate that the concentration in peel is not affected by the transpiration stream. In other words, the partitioning of pesticides between the root peel and exposure solution proceeds passively and rapidly reaches an equilibrium in the same manner as that between 1-octanol and water. This agrees well with the results of Golab,\(^7\) Businelli,\(^8\) and Wild\(^9\) obtained using carrots. We express this partitioning between root peel and exposure solution as the ERCF parameter. ERCF may differ among plant species because of heterogeneity in the constitutes of the root peel.

The route of uptake into the root core differed for I and II (Fig. 5). For I, the residual concentration in the root core of radish with top leaves increased according to the incubation time, but no increase was observed without top leaves. Considering that the total amount of transpiration stream increased in proportion to the incubation time and the uptake of I increased in proportion to the amount of transpiration stream, it is likely that the main route of uptake of I into the root core is via mass flow through the root hairs. By contrast, the concentration of II in the root core with top leaves was the same as that without top leaves. This indicates that the uptake of II does not proceed in accordance with the transpiration stream. Taking into account that the uptake of II into the root peel rapidly reaches an equilibrium at a relatively high concentration, it is proposed that II is incorporated into the core via diffusion from the peel.

In summary, the route of uptake of I and II differs. For the lipophilic pesticide II, the main route was likely to be partitioning to the root peel and further intake via diffusion into the core. For the less lipophilic pesticide I, the primary route seemed to be incorporation into the root core via the transpiration stream.

**CONCLUSION**

A new mathematical model for evaluating the amount of pesticide residue in edible roots was developed using modified concepts of the route of uptake. The amount of pesticide residue in the edible roots of crops can be calculated using just a few input parameters. The validation of the model was conducted using two pesticides, I and II. Some of the assumptions in the model should be investigated further to confirm the model's reliability.

**REFERENCES**


**要約**

非解離性農薬の根葉類根部による取り込みに関する数理モデル

藤澤卓生, 一瀬桂子、福島雅雄、片木敏行、瀧本善之
1-オクタノール/水分配係数（log Kow）の異なる非解離
性農業 furametpyr（log Kow = 2.36）及び pyriproxyfen（log Kow = 5.37）を使用し、水耕栽培にてダイコン根部への取り込み経路を調べたところ（1）蒸散流を介した根毛から根内部への移行、（2）暴露水から根部表層への分配吸着、（3）根部表層から根内部への浸透拡散、と大きく3種類に分類された。furametpyr は蒸散流とともに根毛から根内部に取り込まれるのに対して、pyriproxyfen は根部表層に分配吸着され、その後表層から内部へと浸透拡散すると思われ、log Kow に依存して取り込みの主要経路が変化することが示唆された。以上の結果をもとに partition-theory を原理として、新たに農薬の根薬類根部への取り込みを予測できる数理モデルを構築した。また、モデル構築時の 1/10 の濃度で処理された furametpyr および pyriproxyfen の根部取込み量を分析し、予測値と比較した。その予測値と実測値の比は furametpyr で 1.01-1.38、pyriproxyfen で 0.75-1.28 となり、本モデルの有効性を示す良好な結果が得られた。