Aerobic metabolism and adsorption of pyrethroid insecticide imiprothrin in soil

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Imiprothrin, a unique type of pyrethroid having an imidazolidinyl ring in its alcohol moiety, was rapidly degraded in two aerobic U.S. soils. The half-lives of biologically active trans isomer were estimated by assuming the first-order kinetics to be 1.6–2.5 days, shorter than those of the cis isomer (3.3–12.5 days). The primary metabolic pathway was ester cleavage followed by instantaneous elimination of a hydroxymethyl group from the alcohol moiety to form PGH (1-propargylimidazolidine-2,4-dione). The opening of the imidazolidinyl ring with subsequent release of the carbamoyl group resulted in the formation of PG (N-propargylglycine). The soil adsorption coefficients (Kso) of the trans isomer, the main component of imiprothrin (80%), to the two soils were determined to be 376 and 428 (ml/g o.c.) by the batch equilibrium method and these lower values as compared with other pyrethroids were likely to stem from the hydrophilic character of the alcohol moiety. Based on the metabolic half-lives and Kso values, the groundwater concentration of imiprothrin was calculated to be 0.039 µg/L by using the screening simulation model SCI-GROW known to give a conservative groundwater concentration. This significant lower concentration clearly indicates that imiprothrin is most unlikely to contaminate the groundwater, mainly due to its rapid degradation in soil. © Pesticide Science Society of Japan

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The objective of this study is to determine the metabolic and adsorptive profiles of IP using typical U.S. agricultural soils in California and Mississippi. Furthermore, the possibility of groundwater contamination was concisely assessed based on the metabolic half-lives and Koc values of IP in each U.S. agricultural soil by using the SCI-GROW (Screening Concentration in Ground Water) simulation model provided by the Office of Pesticide Programs (OPP) in the Environmental Protection Agency (EPA).19)

Materials and Methods

1. Chemicals

(1R)-Cis and (1R)-trans isomers of IP, separately labeled with 14C at the 5-position of the imidazolidinyl ring (Fig. 4) were synthesized in our laboratory. The specific activities and radiochemical purities of both isomers were 5.04 MBq/mg and 100%, respectively. The non-radiolabeled isomers of IP, PGH (1-propargylimidazolidine-2,4-dione), CPG-Me (methyl N-carbamoyl-N-propargylglycinate) and PG (N-propargylglycine), whose chemical structures are shown in Table 1, were also synthesized in our laboratory according to the reported methods.14,20,21) The chemical purity of each standard was determined to be >93% by high-performance liquid chromatography.

2. Radioassay

The radioactivity in organic and aqueous fractions via the extraction of soils, bound residues and trapping media was individually determined by liquid scintillation counting (LSC) with a Packard Model 2000CA liquid scintillation spectrometer equipped with an automatic external standard in low potassium glass scintillation vials, using 10 ml of Packard Emulsifier Scintillator Plus™. The unextractable soil-bound residues were powdered after drying in a vacuum desiccator and their portion was subjected to combustion analysis using a Packard Model 307 sample oxidizer under the 14C recovery of >95%. Details of these measurements have been previously reported.22)

3. Chromatography

The aliquots from each soil extract were analyzed by reversed-phase high-performance liquid chromatography (HPLC) for the analysis and identification of IP and its soil metabolites. A Hitachi L-6200 pump equipped with a Sumipax ODS A-212 column (5 μm, 6-mm i.d. ×15 cm, Sumika Chemical Analysis Service, Ltd., Osaka) was operated by using mobile-phase stepwise changing as follows: 0 min, %A (acetonitrile)–%B (0.1% trifluoroacetic acid), 0:100, a flow rate of 1 ml min−1; 0–11 min, linear, 5:95 at 11 min, 1 ml min−1; 11–30 min, linear, 80:20 at 30 min, 1 ml min−1; 30–40 min, linear, 100:0 at 40 min, 1 ml min−1; 40–40.2 min, linear,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical structure</th>
<th>R t (min)a</th>
<th>Rf valuesb</th>
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<tbody>
<tr>
<td>(1R)-cis IP</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>37.6</td>
<td>0.64 0.64</td>
</tr>
<tr>
<td>(1R)-trans IP</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>37.4</td>
<td>0.63 0.65</td>
</tr>
<tr>
<td>PGH</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>15.3</td>
<td>0.55 0.30</td>
</tr>
<tr>
<td>CPG-Me</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>18.9</td>
<td>0.47 0.29</td>
</tr>
<tr>
<td>PG</td>
<td><img src="image" alt="Chemical structure" /></td>
<td>4.0</td>
<td>0.12 0.01</td>
</tr>
</tbody>
</table>

a) Typical HPLC retention time. b) TLC Rf values with indicated solvent systems. A, n-butanol/acetic acid/water (6/1/1, v/v/v); B, chloroform/acetonitrile/acetic acid (9/1/1, v/v/v).
identified by HPLC co-chromatography with the corresponding non-radiolabeled authentic standards being detected at 254 or 220 nm with a Hitachi model L-4200 UV detector. Two-dimensional thin-layer chromatography (TLC) was conducted using pre-coated silica gel 60F 254 thin-layer chromatoplates (20×20 cm, 0.25-mm layer thickness, E. Merck, Germany) with the solvent systems of n-butanol/acetic acid/water (6/1/1, v/v/v; A) and chloroform/acetonitrile/acetic acid (9/1/1, v/v/v; B). Autoradiograms were prepared by exposing a TLC plate to a BAS-III, Fuji imaging plate (Fuji Photo Film Co., Ltd.) for several hours, and the radioactivity in each spot and diffuse region was quantified using a Bio Imaging Analyzer BAS-1500 (Fuji Photo Film Co., Ltd.). The non-radiolabeled reference standards were detected by exposing TLC plates to ultraviolet light, iodine vapor or by utilizing the ninhydrin reaction. The typical retention times (Rt) and Rf values beled reference standards were detected by exposing TLC samples were placed in a 3-liter glass jar and incubated at 25±1°C in darkness. The humidified CO₂-free air was passed over soil samples into two gas-washing bottles each containing 350 ml of ethylene glycol and 0.5 M NaOH solution in sequence to trap volatile ¹⁴C. The soil moisture content was adjusted to its original level by the addition of distilled water once a month.

At appropriate intervals, the incubated soil was extracted three times with 100 ml of 0.1 M HCl–acetonitrile (1/5, v/v) by mechanical shaking for 10 min, followed by centrifugation at 5000 rpm for 10 min. After radioassay of the combined extract (1 ml), acetonitrile was removed by evaporation and the remaining aqueous portion was extracted three times with 100 ml of ethyl acetate. After extraction, a 1-ml aliquot of either the aqueous or organic layer was separately radioassayed in duplicate by LSC. The unextractable soil-bound residues were dried in vacuo and its portion (approximately 300 mg) was combusted using a sample oxidizer prior to LSC. The bound residues (10 g) at day 0 were exhaustively extracted using Soxhlet apparatus with 100 ml of acetic acid (1 M) at 140°C for 2 hr. After radioassay of the extract (1 ml), the concentrated extract was subjected to HPLC co-chromatography with non-radiolabeled reference standards. The first-order rate constant and half-life (DT50) of each IP isomer were calculated by the usual least-squares approximation method. Additionally, degradation rates in the aerobic soils were calculated by the curve fitting method in accordance with the Gustafson’s equation²³ as follows:

\[
C = C_0 (1 + \beta / \alpha)^{-\alpha}
\]

Where C and C₀ are the percent of the remaining and initial concentrations of IP, respectively, t is the incubation period in days, and α and β are constants. These calculations were performed with Microsoft Excel (Ver. 7) and Sigma plot 2000 (SPSS, Inc. Ver. 6) programs.

5. Soil adsorption

Since both geometrical isomers were most likely to have almost the same hydrophobicity as evidenced by their close retention times in HPLC as listed in Table 1, the soil adsorption coefficients (Kₐ) of IP in the two U.S. soils were conveniently examined using the trans isomer of [¹⁴C]-IP according to the batch equilibrium method recommended by OECD.²⁴ Although any sterilization method is known to affect soil struc-
ture, autoclaving soil at 121°C for 20 min was convenient to estimate adsorption profiles of the trans isomer being very susceptible to biotic degradation. The soil-to-solution ratio was adjusted to 1:2 (w/v) as the adsorbed amount of trans-IP exceeded 20% of the applied 14C for both soils in accordance with the OECD guideline. Each sterilized soil sample (5.0 g) was added to glass centrifuge tubes with a Teflon-lined screw cap containing sterile 0.01 M CaCl2 (10 ml), and the tubes were mechanically shaken at 25°C in darkness for 16 hr. After pre-equilibration, the appropriate volume of stock solution of the trans isomer of [14C]-IP (0.011 or 0.203 μg/μl) was aseptically added in duplicate to give nominal concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/L. After shaking for 16 hr at 25°C in darkness, the glass tubes were centrifuged at 2500 rpm for 20 min. The 1-ml aliquot of the supernatant, separated by decantation and passed through a 0.2-μm membrane filter, was radioassayed in duplicate. The remaining soil was extracted and analyzed similarly as the metabolism study.

The Freundlich adsorption coefficient (K_F) was estimated by the following equation:

\[
\log C_s(eq) = \log K_F + \frac{1}{n} \times \log C_a(eq)
\]

Where \(C_s(eq)\) is the concentration of the trans isomer of IP adsorbed on soil at equilibrium (μg/g), \(C_a(eq)\) is that in the aqueous phase (μg/ml) and \(n\) is the constant. Constants \(K_F\) and \(1/n\) were calculated by least-squares regression of a log \(C_a(eq)\) vs. log \(C_s(eq)\) plot. The \(K_{soil}\) value was calculated by normalizing the \(K_F\) value to the content of soil organic carbon (% o.c.) as below.

\[
K_{soil} = K_F \times 100/\% o.c.
\]

Results

1. Aerobic soil metabolism of IP

The distribution of radioactivity among volatile, HCl/acetoni-trile extracts and unextractable soil-bound residues with total recovery of 14C in each soil system is shown in Fig. 1.

The total 14C recovery was 86.4–107.5% of the applied 14C. As with incubation, extractable 14C gradually decreased finally to 6.1–12.9% at day 60 with a significant formation of CO2 amounting to 51.0–68.0%. The amount of soil-bound residues also increased with time and peaked at day 30 (24.8–32.6%) but slightly decreased afterwards. There were no significant differences in 14C distribution between the soils and isomers. Further Soxhlet extraction of the unextractable soil residues at day 60 released only 1.7–5.9% of the applied 14C, most of which was found to originate from PG.

HPLC analysis of the soil extracts showed rapid degradation of each IP isomer, amounting finally to 1.5–3.5% of the applied 14C at day 60 (Fig. 2). The half-lives of cis and trans isomers assuming the first-order kinetics were estimated to be 3.3 and 1.6 days for California soil and 12.5 and 2.5 days for Mississippi soil, respectively. The correlation coefficient in the regression analysis was lower for trans isomer (\(r^2 = 0.81–0.87\)) than cis isomer (\(r^2 = 0.92–0.98\)) due to slower degradation in the later stage of incubation. In contrast, Gustafson’s
equation afforded better correlation of both isomers (0.993–1.000), and the corresponding half-lives of cis and trans isomers were calculated to be 2.2 and 0.22 days for California soil and 5.1 and 1.3 days for Mississippi soil, respectively.

2. Degradation products of IP
Several degradates were identified in soil extracts by HPLC analysis and three of them individually amounted to greater than 5% of the applied 14C. No significant differences appeared in the formation and degradation of the metabolites from both isomers. Furthermore, cis-trans isomerization of IP was not observed throughout the study. With the rapid degradation of IP, PGH was concomitantly formed with maximum amounts of 6.5–48.7% after 1–3 days and gradually decreased afterwards to <0.5% at day 60. The primary degradate formed via cleavage of the ester linkage (3-hydroxymethyl-1-propargylimidazolidine-2,4-dione) could not be detected throughout the study, possibly due to its instability and rapid degradation similar to the hemiaminal derivative reported in the hydrolysis of tetramethrin.25 The isolation of CPG (N-carbamoyl-N-propargylglycine) failed due to its instability under experimental conditions. CPG was identified as the methylated derivative (CPG-Me) due to its instability under experimental conditions.

3. Adsorption of IP on each soil
HPLC analysis for each intact soil/0.01 M CaCl2 (1/2, w/v) suspension showed significant degradation of IP through 3-hr shaking with PGH formation amounting to 4.9–8.7% of the applied 14C. On the other hand, the stability of trans isomer under sterile conditions was demonstrated by HPLC analysis of the supernatant and soil extract where more than 92% of 14C was recovered as the trans isomer with the unextractable soil residue amounting to less than 1%. The adsorbed amount of 14C under sterile conditions was periodically monitored and the test system was found to reach equilibrium after 16 hr for both soils (data not shown). No isomerization to the cis isomer was observed even after shaking for 16 hr. The plots of C(t) versus C(eq) for two soils under these conditions are summarized in Table 3.
shown in Fig. 3. The Freundlich adsorption coefficients (K_F and K_o) were calculated to be 2.18 ml/g and 376 ml/g o.c. for California soil and 1.24 ml/g and 428 ml/g o.c. for Mississippi soil, respectively.

**Discussion**

The rapid degradation of IP in intact soils during the adsorption study but its stability under sterile conditions clearly showed the involvement of microbial degradation processes in soil. Although IP was resistant to abiotic hydrolysis under acidic (stable at pH 5) and neutral (half-life of 58.6 days at pH 7) conditions,17,18) the soil metabolism study exhibited rapid microbial degradation with half-lives of 1.6–2.5 days and 3.3–12.5 days for trans and cis isomers, respectively. Previous studies5,10) have reported that the aerobic half-lives of synthetic pyrethroids such as phenothrin, permethrin and cypermethrin range from 1 to 14 days with the same isomorphic preference. Pyrethroids are known to undergo ester cleavage in the aid of various bacteria isolated from soils, and esterases are considered to participate in the stereoselective reaction.26,27) Since metabolic profiles in soil have been mostly taken for pyrethroids having a 3-phenoxybenzyl moiety used for agricultural purposes, it could not be easily determined whether the rapid degradation of IP originates from substrate selectivity in microbial esterases. More availability of IP to soil microbes due to its higher water solubility than other pyrethroids may account for this difference. Similar to rat metabolism15,16) and abiotic hydrolysis17,18) studies, the primary degradation product of IP was PGH, followed by the formation of CPG via ring opening. The more polar PG not detected in the abiotic hydrolysis study was formed as one of the main metabolites. The degradation mechanism is considered analogous to that in the bacterial metabolism of L-hydantoin-5-propionic acid to glutamic acid by the corresponding aminohydrolase.28)

The major degradation product from the acid moiety of IP by ester cleavage is most likely to be 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropanecarboxylic acid which undergoes rapid degradation to carbon dioxide and/or is bound to soils, as reported for other pyrethroids such as phenothrin.2 Based upon the decline and formation profiles of the identified degradates (Table 3), degradation pathways of IP under aerobic conditions are proposed in Fig. 4.

The distribution between solid and liquid phases of soil is one of the most critical factors in determining the potential mobility of pesticides in the soil environment.13) From the obtained KOC values for the trans isomer, IP can be classified as a “Medium mobility” category (150–500 ml/g o.c.)29) and these values are extremely lower than those of other pyrethroids (>105 ml/g o.c.).30) Incidentally, the water solubility of IP (93.5 mg/L at 25°C)17,18) is significantly higher than those of other pyrethroids (105–102 mg/L).30) The octanol-water partition coefficient (log P) value of the corresponding alcohols to typical pyrethroids can be estimated by KOWWIN™ (Ver. 3.10)31) to be -1.97 (IP), 2.08 (fenvalerate) and 3.13 (phenothrin). These results clearly show that lower IP hydrophobicity (log P=-2.9 at 25°C)17,18) would mainly originate from a more hydrophilic hydantoin moiety in its structure. Similar
tendencies of higher water solubility are also reported for
tetramethrin (1.83 mg/L at 25°C) and prallethrin (8 mg/L at
25°C),\textsuperscript{32} having tetrahydrophthalimide moiety and a
cyclopentenone ring in the alcoholic moiety, respectively. Therefore,
the significantly higher hydrophilic property of IP may
raise concern that IP has a higher potential for groundwater
contamination if released into the terrestrial environment
based on chemical and physical criteria in predicting the
leachability of a pesticide to groundwater as a result of
normal agricultural use in hydrogeologically sensitive areas: i.e.
 mobility criteria of $K_{oc}$ is less than 300–500 ml/g o.c.
and water solubility is more than 30 mg/L.\textsuperscript{13} Consequently,
prediction of the environmental concentration of IP would be
necessary even though its use patterns and application sites
are limited to indoor and non-food use.

In order to assess the risk of IP for groundwater contamination,
the Tier-1 screening simulation model developed by U.S.
EPA, SCI-GROW (Screening Concentration in Ground
Water)\textsuperscript{19} was conveniently utilized. The worst-case
application rate per year was assumed to be 9.10 lb/acre (1020 mg/
ca). 24-fold higher than that of this soil metabolism study,
by considering the full application of one commercial can
containing the highest amount of IP, that is 0.4% (w/w) of IP.
Incidentally, the average first-order half-life in the aerobic soil metabolism of both
isomers in two soils (4.98 days) was input to the simulation
model since the dissipation rate of pesticides in aerobic soil
would vary due to either soil characteristics or microbial ac-
tivity. The groundwater screening concentration of IP was
then calculated to be 0.039 $\mu$g/L, clearly indicating that IP is
most unlikely to contaminate the groundwater even when an
impractically large amount of aerosol spray was assumed to
be applied.

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