Note

Relation between Penetration Rates of Pesticides and Partition Coefficients in Topical Application to *Spodoptera litura*

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INTRODUCTION

Penetration rate of insecticides into and through the insect cuticle is one of the important factors to determine their insecticidal activity¹⁾ and its modification is known to occur in the resistant strain of insects.^{2,3)} The behavior of the radiolabeled insecticides by a topical application to insect larvae⁴⁻⁷⁾ or in the diffusion cell experiment using the epidermal tissue⁸⁻¹⁰⁾ has been examined to know the mode of penetration, revealing that the process is not simply described by the Fick's law. The physicochemical properties of an insecticide such as hydrophobicity are considered to deeply correlate with the rate of penetration but the relation between them is still obscure.^{4,7,8)} In order to examine the relation, the penetration of eleven radiolabeled pesticides possessing the different structures and physicochemical properties when topically applied was studied by using the *Spodoptera litura* larvae.

MATERIALS AND METHODS

1. Chemicals

The radiolabeled (¹⁴C or ³T) pesticides listed in Table I and the corresponding nonlabeled standards were synthesized in our laboratory for penetration study. They were purified by the successive TLC (Kiesel gel 60F₂₅₄, 0.25-mm layer thickness; E. Merck) with the solvent systems of toluene/ethyl formate/formic acid (A; 5/7/1, v/v/v) and benzene saturated with formic acid/diethyl ether (B; 10/3, v/v). The radiochemical purity of each compound was determined >99% by radio-HPLC. For purity determination, the liquid chromatograph (Waters LC Module I) equipped with a Sumipax ODS A-212 (6 mm i.d. × 15 cm, Sumika Analytical Service Ltd.) column was operated at a flow rate of 1 ml min⁻¹ with a mobile phase of methanol/distilled water (C; 80/20, v/v). The peak was monitored both by a UV detector at 250 nm and by a radiodetector (Radiomatic Flo-one Beta

A-120). The partition coefficient of each pesticide between *n*-octanol and distilled water (log P) was obtained from ref. 11 and 12, and otherwise, was measured in our laboratory by a shake-flask method in a usual manner.

2. Penetration Study

The fifth instar larvae of common cutworm (*Spodoptera litura*) supplied from Agricultural Chemicals Research Laboratory of Sumitomo Chemical Co., Ltd. (Takarazuka) were reared at 27–28°C under a long-day photoperiod (16L - 8D) regimen by using an artificial diet in our laboratory. The average body weight was 0.5 ± 0.02 g. The rate of cuticular penetration was determined in five replicates by topically applying 2–4 µl of acetone solution of each radiolabeled pesticide to the dorsal part (5–7th segments) of the insect body using a 10 µl Hamilton syringe (Microliter #701). The dosage was about 0.2 µg per insect for every pesticide by considering the concentration dependency of the penetration rate being reported previously.^{6,13,14)} The insect was individually kept in a 20-ml glass vial at 25°C. The penetration was monitored up to 24 hr at the maximum after application and the death of insect due to the pesticide was not observed during the experiment.

At 8–10 sampling points with appropriate intervals, 5 ml of toluene was added to each vial followed by agitation for 10 sec with a vortex mixer to rinse out the radiolabeled compound from the body surface of the insect. After the same procedure was repeated, the 2-ml aliquot of the rinsed portions (% Surface Residue) was radioassayed in duplicate using Packard TR-1600 liquid scintillation counter (LSC). Both the insect after rinsing and the excreta (% Penetrated) were dried *in vacuo* overnight and combusted together with a sample oxidizer (Packard Model 306) to quantify the radioactivity in the insect as ¹⁴CO₂. The recovery of radiocarbons was >95%. The rate of penetration into and through the cuticle was assumed to be equal to the dissipation rate of the pesticide from the body surface of the insect. The penetration rate (k in hr⁻¹) was estimated by applying the least-square approximation method to the % Surface Residue data. Sigma-Plot® software (version 5.0, SPSS Inc., 1998) was utilized for the statistical analysis. In order to clarify the stability of the pesticide on the body surface during the experiment, the rinsed portions when about half of the applied radioactivity dissipated were combined and subjected to two-dimensional TLC (solvent systems A and B) and HPLC (mobile phase C) co-chromatographies with the corresponding standard.

RESULTS AND DISCUSSION

Radioactivity recovered from the insect body (% Recovered, sum of % Surface Residue and % Penetrated) gradually decreased with time, for example as shown in Fig. 1, and

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Table 1 Physicochemical properties and penetration rates of the pesticides.

Compound ^a	Chemical structure	Specific activity ^b (in MBq mg ⁻¹)	log P ^c	NW ^d	Penetration rate (k in hr ⁻¹)
(I)	Fenitrothion	15.7 (Ph)	3.27	3	0.4665
(II)	Fenitrooxon	16.7 (Ph)	1.69	4	1.0309
(III)	Cyanophos	4.6 (Ph)	2.65	3	0.8259
(IV)	Cyanophos-oxon	4.9 (Ph)	0.84	4	1.8241
(V)	Parathion	2.4 (Ph)	3.83	3	0.5317
(VI)	Cyanofenphos	4.5 (CN)	4.29	3	0.3268
(VII)	Pyriproxyfen	12.6 (Ph')	5.31	1	0.1677
(VIII)	(1R)-trans-Tetramethrin	19.0 (Cyc)	4.73	6	0.0471
(IX)	Diethofencarb	8.9 (Ph)	3.02	5	0.1004
(X)	Fenpropathrin	4.3 (Cyc)	6.00	2	0.1287
(XI)	RH-5849	12.7 (Ph)	2.45	7	0.0545

^aChemical structure of each compound is shown below. ^bIn GBq mg⁻¹ for (XI). Designations in the parentheses mean the ¹⁴C-labeled (³T for (XI)) positions. Ph, uniformly at the phenyl ring; Ph', uniformly at the phenoxyphenyl ring; CN, at the cyano carbon; Cyc, at the 1-position of the cyclopropyl ring. ^cCited from ref. 11 or measured by a usual shake-flask method. ^dNumber of the hydrogen-bonding sites defined in ref. 21. NW for a carbamate moiety (NHCOO) was defined to be 5. ^eCoefficient of correlation assuming the 1st-order kinetics.

amounted to 80.1–96.0% of the applied dose at the end of the study, as listed in Table 2. The poor recovery of ¹⁴C (about 80%) was similarly detected for (III) and (VIII) whose vapor pressure was greatly different (105 mPa at 20°C and 0.944 mPa at 30°C, respectively).¹⁵⁾ Since the better recovery was obtained for (I) and (V) with the similar or larger vapor pressure against (III) (15 mPa and 0.89 mPa at 20°C, respectively),¹⁵⁾ the loss of radioactivity due to vaporization was considered unlikely. The adhesion of ¹⁴C onto the inner surface of the vial was also unlikely since it was collected through the rinsing process of the insect. The TLC and HPLC analyses showed that most of the radioactivity collected by rinsing was due to the parent compound unchanged (91.6–98.0% on the remained basis), showing that the metabolic change of the pesticide on the insect integument was scarcely. Meanwhile, the radiocarbons bound to the insect body (% Penetrated) gradually increased and exhibited almost the plateau at the end of the study. In the case of (IV) showing the largest k value, % Penetrated was found to slightly decrease after the maximum at 30 min (Table 2). Although ¹⁴C in the insect body was not analyzed further by

TLC and HPLC, it was probable that the part of them consisted of metabolites on the basis of the previous studies.^{2,16–18)} Therefore, the loss observed was considered to stem from carbon dioxide and/or the volatile metabolites not being trapped in this study.

In this study, the first-order penetration rate (k in hr⁻¹) was estimated as listed in Table 1 with a coefficient of correlation (r) of 0.91–0.99. In contrast, the biphasic profiles of % Surface vs. time plot have been previously reported^{2,4,6,7,13)} and it was suggested that the penetration was not a simple process controlled by the Fick's law. The faster penetration in the early period of the experiment was explained by pharmacokinetic models as diffusion process until saturation to the interior of the insect body via sink in the cuticle.^{6,13)} Therefore, the double-exponential equation based on the two compartment model,¹⁹⁾ % Surface Residue = a*exp(–k₁t) + b*exp(–k₂t) (a, b, k₁ and k₂ are constants), was applied to each penetration but the statistical analysis showed that the r-value was not significantly improved (data are not shown).

Meanwhile, the structure of insect integument is known to be complex but it is considered that the outer part usually

Fig. 1 Distribution of ^{14}C -Fenitrothion (I) in fifth instar larvae of *S. litura* in a topical application.

Table 2 Distribution of the radioactivity after topical application.

Compound	Period ^a	% of the applied radioactivity		
		Surface Residue ^b	Penetrated ^c	Recovered
(I) Fenitrothion	1	43.6 ± 2.9 (40.9) ^d	51.2 ± 0.6	94.8 ± 2.9
	6	10.8 ± 2.9	71.0 ± 10.2	81.8 ± 11.5
(II) Fenitrooxon	0.5	44.9 ± 5.2 (42.7)	51.5 ± 5.9	96.4 ± 3.4
	3	25.3 ± 2.2	62.4 ± 2.2	87.8 ± 6.7
(III) Cyanophos	1	37.4 ± 4.2 (34.3)	39.2 ± 4.5	76.6 ± 5.6
	6	8.1 ± 0.9	72.8 ± 4.0	80.9 ± 3.3
(IV) Cyanophos-oxon	0.5	31.2 ± 7.0 (29.3)	65.5 ± 4.6	96.7 ± 3.2
	1	34.5 ± 10.8	55.4 ± 4.3	89.9 ± 9.0
(V) Parathion	1	60.6 ± 5.4 (56.8)	35.0 ± 7.8	95.6 ± 3.6
	6	10.9 ± 3.5	78.9 ± 4.9	89.8 ± 5.6
(VI) Cyanofenphos	3	39.9 ± 9.8 (37.4)	56.5 ± 13.0	96.4 ± 4.4
	8	11.3 ± 2.7	84.6 ± 6.3	96.0 ± 4.5
(VII) Pyriproxyfen	4	56.4 ± 7.1 (55.3)	40.3 ± 6.7	96.7 ± 0.7
	24	7.6 ± 3.3	76.4 ± 8.5	84.0 ± 7.1
(VIII) (1R)-Trans-Tetramethrin	8	63.7 ± 7.2 (60.3)	21.2 ± 3.6	84.9 ± 4.5
	16	44.9 ± 9.2	35.2 ± 8.6	80.1 ± 12.1
(IX) Diethofencarb	3	56.6 ± 10.2 (52.6)	31.9 ± 5.5	88.5 ± 7.5
	16	18.1 ± 3.3	63.5 ± 6.8	81.7 ± 4.3
(X) Fenpropathrin	2	69.2 ± 10.5 (66.5)	13.1 ± 1.2	82.3 ± 9.8
	4	68.8 ± 18.1	22.0 ± 5.5	90.8 ± 18.0
(XI) RH-5849	8	52.3 ± 5.0 (50.2)	- ^e	-
	24	38.4 ± 3.3	-	-

^aIn hr. ^bCollected by rinsing the insect body. ^cTotal radioactivity in the insect and excreta. ^dPercent of the parent unchanged. ^eNot determined.

consists of the lipophilic waxes and sclerotin which are the first barrier to the penetration of a pesticide.^{1,20)} Therefore, the log P value representing hydrophobicity of a pesticide was first taken as a descriptor to clarify the relation of the penetration rate (k) with the physicochemical properties of pesticides. The regression analysis between log k and log P showed that the simple correlation coefficient at the confidence level of 95% was 0.320. There are the several studies discussing the relation between the penetration rates of pesticides through the insect cuticle and their physicochemical properties.⁴⁻⁸⁾ The negative correlation between the rate and log P has been observed for organophosphorus pesticides⁶⁾ and ureas,⁸⁾ while the correlation has become poor if the various type of pesticides are taken for analysis.^{4,5,7)} Furthermore, no significant correlation has been found when the diffusion cell system is applied to the common cutworm epidermal tissues.¹⁰⁾ It was considered that the rates for the series of pesticides possessing the different skeletons would make the analysis complex. Therefore, the regression analysis was conducted for the six organophosphorus pesticides (I-VI) and the following negative correlation was obtained.

$$\log k = -0.199(\pm 0.024) \log P + 0.400(\pm 0.074) \quad (1)$$

$$(r = -0.971, s = 0.072, n = 6)$$

This equation means that less hydrophobic organophosphorus pesticide penetrates more quickly into the cuticle of 5th instar larvae of *S. litura*. Although the similar statistical analysis has not been apparently reported, the results using the 5th instar armyworm larvae (*Leucania separata* Walker)

clearly show the similar profiles for methyl parathion, phenthoate, diazinon and fenitrothion.⁶⁾ These results implied that the cuticle was rather hydrophilic on the whole and hence the hydrophobic wax layer did not positively act as a barrier to penetration under the tested conditions. This might be due to the existence of organic solvent such as acetone in the topical application which eliminated the intrinsic role of the wax layer. The slower penetration of the more hydrophobic pesticides implied that the wax layer was a sink for such pesticides being hard to move to the deeper region of the cuticle, as stated previously.¹⁰⁾

In order to explain the penetration rates of all pesticides tested, the further analysis was conducted by introducing another descriptor. The most abundant constituent in the insect cuticle is a chitin, a polymer of *N*-acetylglucosamine.^{1,20)} Since the monomer unit consists of two hydroxyl groups, two ether moieties and one acetyl amino group, it is likely that the interactions of a pesticide with chitin through hydrogen bonds would greatly control the penetration rate. Therefore, the hydrogen-bond descriptor (NW) for each functional group,²¹⁾ which is defined as the total number of possible donor and acceptor sites of hydrogen bonds, was utilized and the following equation was obtained.

$$\log k = -0.317(\pm 0.046) \log P - 0.251(\pm 0.041) \text{NW} \quad (2)$$

$$+ 1.474(\pm 0.273)$$

$$(r = 0.938, s = 0.204, n = 11)$$

The correlation was quite good and Eq. (2) could describe well the penetration rates for the pesticides possessing the

different skeletons such as phosphorus ester, carbamate, ester, ether, imide and benzoylhydrazide used in the study. The coefficient for log P was negative similarly as obtained in Eq. (1) and the contribution of hydrophobicity to penetration was likely to be common for the pesticides tested even if their chemical structures were different. The negative coefficient of NW implied the retardation of penetration by the interaction of pesticides with the cuticle (perhaps chitin) via hydrogen bonding. Thus, the less hydrophobic pesticide possessing less hydrogen-bonding sites penetrated more rapidly into the cuticle.

The applicability of Eq. (1) was examined for the other organophosphorus pesticide. The penetration of diazinon *via* topical application to larvae of *S. litura* has been previously reported.¹⁴⁾ When diazinon was applied at a rate of 2.0 μg per insect, about 16% of the applied dose remained on the insect surface after 4 hr. Since log P of diazinon was 3.11²²⁾ and its NW value was estimated to be 5,²¹⁾ the first-order k value was calculated to be 0.604 hr^{-1} according to Eq. (1). The percent of diazinon remained on the surface was then estimated to be 8.9%, which was close to the observed value. In the case of non-organophosphorus pesticides, the penetration studies of flufenoxuron²³⁾ and carbaryl^{14,16)} were available. About 11% of flufenoxuron topically applied to 6th instar *S. litura* at a rate of 1 μg per insect penetrated into the insect body after 24 hr. Based on its log P and NW values (4.0¹⁵⁾ and 8²¹⁾), Eq. (2) afforded the k value of $1.51 \times 10^{-2} \text{ hr}^{-1}$ and then the percent of penetration assuming the first-order kinetics was estimated to be 30.5% being larger than the observed value. In the case of carbaryl, the extent of penetration was found to vary between the studies; the corresponding % Surface value was 29.3% after 2 hr at a rate of 0.1 μg per insect¹⁵⁾ and about 3% after 4 hr at a rate of 2 μg per insect.¹⁴⁾ The k value was similarly calculated by Eq. (2) to be 0.290 hr^{-1} (log P=2.36²²⁾ and NW=5²¹⁾), indicating the predicted values of 56.0% (2 hr) and 31.4% (4 hr). Meanwhile, the corresponding k value obtained in the diffusion cell experiment¹⁰⁾ was 0.401 (± 0.045) hr^{-1} at a rate of 0.1 μg and close to the predicted value. Judging from these results, Eqs. (1) and (2) seemed to be only applicable to data from the topical application especially when the penetration profiles followed the first-order kinetics, but they would afford the valuable structural information of a pesticide in considering penetration.

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要 約

ハスモンヨトウ幼虫への局所施用による農薬の浸透速度と分配係数との相関

片木敏行

有機リン剤、ピレスロイド等の11農薬の¹⁴C, ³Tラベル化合物を用いてハスモンヨトウ (*Spodoptera litura*) 5令幼虫における外皮からの浸透, 分布を検討した。6種の有機リン剤においては1次の浸透速度と各農薬の水-オクタノール分配係数との間には高い負相関が認められたが, 基本骨格の異なる全農薬を対象とした場合にはさらに農薬分子の水素結合能を表現するパラメータ(NW)を導入する事により浸透速度を表現できる相関式を得る事ができた。本相関式より農薬分子の疎水性が小さく, かつクチクラとの水素結合を形成しにくい化学構造を有する農薬ほど外皮からの浸透速度が速くなる事が示唆された。