JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Mechanisms of Cuticular Uptake of Xenobiotics into Living Plants: Evaluation of a Logistic−Kinetic Penetration Model

W. ALISON FORSTER,*,† JERZY A. ZABKIEWICZ,† AND MARKUS RIEDERER§

Plant Protection Chemistry$_{NZ}$, P.O. Box 6282, Rotorua, New Zealand, and Julius-von-Sachs-Institute für Biowissenschaften, Lehrstuhl für Botanik II—Ökophysiologie und Vegetationsökologie, Universität Würzburg, Julius-von-Sachs-Platz 3, D-97082 Würzburg, Germany

The objective of this study was to determine whether a logistic−kinetic penetration model could be applied to whole plant uptake. Uptake over 24 h was determined for three model compounds, applied in the presence and absence of surfactants, into the leaves of two plant species. Data for two time intervals were used in the model to predict uptake at intermediate intervals and compared with experimental results. Overall, the model fit the whole plant uptake data well. The study confirmed that an increase (or decrease) in active ingredient (ai) concentration or an increase in contact area will have no effect on the penetration rate factor, *q*, within the normal working concentration range. This enabled uptake to be predicted at different times for concentrations of ai not already studied, having first derived *q* for one concentration of the formulation of interest and having 24 h (maximum) uptake results for all formulations and concentrations of interest. The advantages of the models and equations described are that few variables are required, and they are simple to measure.

KEYWORDS: Cuticular uptake; dose; mechanisms; kinetics, rate, *Chenopodium album*; *Hedera helix*; triethylene glycol monododecyl ether; hexaethylene glycol monododecyl ether; trisiloxane ethoxylate

INTRODUCTION

Pesticide spray efficacy depends on several processes, namely, deposition, retention, uptake, and translocation. A spray droplet landing on foliage rapidly becomes a quasi-solid deposit due to solvent evaporation, but uptake into the leaf can occur over many hours. Total uptake after 24 h can be the same for a compound formulated with different surfactants, but rates of uptake in the intervening period (and therefore rain-fastness and subsequent translocation to target sites) can be quite different. Therefore, there is a requirement to be able to model uptake over time into plant foliage.

There is general agreement that uptake of xenobiotics (e.g., pesticides) through the leaf cuticle is a diffusion process (*1*). However, Fick's first law of diffusion as modified for plant cuticles in vitro (*1*, *2*) may not be appropriate for in vivo situations when the applied quantity is a finite dose from a droplet deposit. Watanabe (*3*) reviewed available uptake models and found that the ones dealing with nonequilibrium transcuticular penetration kinetics did not fully represent all of the kinetic parameters involved in penetration from a droplet residue. This led him to develop a non-steady-state, nonequilibrium model (*3*), termed "the logistic−kinetic penetration model", using isolated cuticles in the development of the model. The objective of our study was to determine the uptake of model xenobiotics differing in lipophilicity into two plant species over time, applied alone and in the presence of a range of surfactants, to determine whether the logistic−kinetic penetration model as described by Watanabe (*3*) could be applied to in vivo uptake. In the current study, *Chenopodium album*, which has a thin cuticle, was compared with *Hedera helix*, which has a thick cuticle and has been widely used in isolated cuticle work (*4*).

MATERIALS AND METHODS

The materials and methods used have been described elsewhere (*5*). However, the following is a summary of the most pertinent features.

Plant Material. *C. album* (common lambsquarters) plants were grown from seed and raised under controlled environment conditions. *H. helix* plants were grown from cuttings raised in a glass house and used at 6−9 months of age. Two weeks prior to use, the *H. helix* plants were transferred into growth cabinets having controlled environment conditions that were the same as for the *C. album* plants.

Chemicals. *Model Compounds.* 2-Deoxy-D-glucose (DOG; Aldrich Chemical Co., Inc.; 99% purity), 2,4-dichlorophenoxyacetic acid (2,4-D; Dow Agrosciences (NZ) Ltd.; 92% purity) and (2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2-(4-fluorophenyl)oxiran-2-ylmethyl]-1*H*-1,2,4-triazole (epoxiconazole; BASF; 96% purity) were studied initially at one concentration each (0.75 g L^{-1} DOG, 1.09 g L^{-1} 2,4-D, 1.554 g L^{-1} epoxiconazole) providing a molar concentration (0.0045 mol L^{-1}; 1.1 nmol per 0.24 μL droplet) close to that of the surfactant's concentration. A second experiment on *C. album* determined the uptake of DOG, 2,4-D, and epoxiconazole, each at two concentrations (0.1107 and 54.29 nmol per 0.24 μL for DOG; 0.024 and 10.87 nmol per 0.24 μL for 2,4-D; 0.029 and 2.19 nmol per 0.24 μL for epoxiconazole) in the presence of the three surfactants. The solubilities in water (20 °C) of

* Author to whom correspondence should be addressed (e-mail alison.forster@ppcnz.co.nz; fax + 64 7 343 5811).
† Plant Protection Chemistry$_{NZ}$.
§ Universität Würzburg.

10.1021/jf0528916 CCC: $33.50
Published on Web 03/18/2006

DOG, 2,4-D, and epoxiconazole are 100, 0.620, and 0.0000663 g L^{-1} respectively (*6*, *7*); the log *P* values are −2.69, 2.62, and 3.44, respectively (*7*, *8*); and the molecular weights are 164, 221, and 330.

Surfactants. Silwet L-77 [TSE7.5, a trisiloxane ethoxylate with mean ethylene oxide (EO) content of 7.5, supplied by GE Advanced Materials—Silicones], triethylene glycol monododecyl ether ($C_{12}EO_3$), and hexaethylene glycol monododecyl ether ($C_{12}EO_6$), both from Fluka, were used. All surfactants were studied at an equimolar concentration (0.0044 mol L^{-1}, corresponding to 2.3 g L^{-1} TSE7.5, 1.4 g L^{-1} $C_{12}EO_3$, and 2.0 g L^{-1} $C_{12}EO_6$), approximating typical use rates. The molecular weights of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5 are 319, 451, and 517, respectively. All xenobiotics were studied alone and in the presence of each of the surfactants.

Uptake. Radiolabeled 2-deoxy-D-(U-^{14}C)glucose (DOG), 2,4-dichlorophenoxyacetic acid-carboxy-^{14}C (2,4-D), and (2*RS*,3*SR*)-3-(2-chlorophenyl)-2-(4-fluorophenyl-2-[1*H*-1,2,4-triazol-1-yl)methyl]oxirane-[chlorophenyl-U-^{14}C] (epoxiconazole) were incorporated into treatments (added at ∼1400 dpm per droplet) prior to use. All solutions were made up in water + acetone (1:1 by volume). The use of 50% acetone/water for model uptake experiments is common, and this mixture is considered to have no significant effect on the uptake of the active ingredient (ai) (*9*). This enabled higher concentrations of lipophilic xenobiotics to be studied, as well as the xenobiotic in the absence of a surfactant. Droplets of each solution (0.24 μL, ∼770 μm diameter) were applied to the upper surface of the youngest fully expanded leaves of *C. album* and *H. helix* (14 per leaf) on five separate plants per species, within 4 h of the start of the illumination period. Treated leaves were excised at 0.5, 2, 4, and 6 h after treatment. A previous study (*5*) had already determined 24 h uptake. In the second experiment, treated leaves were excised at 2 and 6 h after treatment. Percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

Droplet Spread Area Determination. The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using V^{++} for Windows image analysis software, with added Blankophor-P fluor (Bayer NZ) to treatments containing DOG or epoxiconazole and UVITEX NFW 450 (Ciba Geigy) to treatments containing 2,4-D.

Statistical Analyses. The statistical software package Statistix was used to analyze the data, with least significant difference (LSD) tests used to compare treatments. Stabilizing transformations were performed, when required, prior to analysis.

RESULTS AND DISCUSSION

The intention of this study was to validate the logistic−kinetic penetration model, and hence formulation differences due to the surfactants and xenobiotics studied are not discussed in the conventional sense. However, **Tables 1** and **2** show actual percent uptake, along with statistical significance for all of the formulations tested.

Validation of a Logistic−Kinetic Penetration Model to in Vivo Systems. The model by Watanabe (*3*) is

$$f = U[K/(K + e^{-qt})](1 - e^{-qt}) \quad (1)$$

where *f* is the amount penetrated at a given time, *U* is the total amount of penetration (i.e., the maximum uptake), *K* is the integral constant (*K* = 0.6 or 0.7 is postulated for linearity or slight convexity, respectively, in the initial period of penetration), *q* is the penetration rate factor, and *t* is time.

$$U = VCAP_u \quad (2)$$

where *V* is the volume of the droplet applied, *C* is the concentration (molar) of the xenobiotic, and *A* is the contact (spread) area of the droplet. The unit partition ratio (P_u) of the pesticide is the ratio of the amount of pesticide partitioned from the droplet into the cuticular membrane (CM) relative to the amount applied (VC) per unit contact area, as defined by $P_u = U/(VCA)$. Thus, eq 1 can be transformed to

$$f = VCAP_u[K/(K + e^{-qt})](1 - e^{-qt}) \quad (3)$$

All of the variables in eq 1, except *q*, are known for each rate study described in the methods. Rearranging eq 1 to solve for *q* produces

$$q = -1/t \ln[K(U - f)/UK + f] \quad (4)$$

In most cases, *U* (total uptake) was taken as the uptake at 24 h. This was considered to be valid as the authors have found that in most cases the majority of uptake into whole plants has occurred by 24 h. A value of 0.6 was used for *K*, and in the majority of cases *f* (uptake at a given time) at 2 h was used. The exception was 2,4-D uptake into *C. album*, for which at 2 h most of the uptake had already taken place, necessitating *f* to be taken at an earlier time (10 min, 600 s). In the case of DOG applied alone to *C. album*, uptake was very low, with no significant difference in uptake over time. However, although uptake was not significantly different, the quantity at 2 h (0.11 nmol or 10% uptake) was larger than that at 24 h (0.07 nmol or 7% uptake). Hence, *U* was taken at 6 h and *f* at 30 min for illustrative purposes. After the value for *q* had been derived, uptake over time (*f*) was calculated using eq 3. The units used were *V* (μL), *C* (nmol μL^{-1}), *A* (mm^2), P_u (mm^{-2}), *q* (s^{-1}), and *t* (s). In all cases the volume applied was 0.24 μL, and the concentration was ∼4.6 nmol μL^{-1}, except for 2,4-D with *H. helix* for which the concentration was ∼5.1 nmol μL^{-1}. The values of *t*, *A*, P_u, and *q* used in eq 3 **a**re shown in **Tables 1** and **2**, for uptake into *C. album* and *H. helix*, respectively. These tables also show calculated and actual uptake (both in nanomoles and by current convention, by percentage). **Figures 1−6** graph the actual uptake (nanomoles) values over time, along with the calculated curves for the two species, three surfactants, and three xenobiotics used. Overall, agreement between calculated and actual values is remarkably good, particularly where a plateau has been very obviously reached within 24 h **(**86400 s; **Figures 1−3**). This highlights the fact that *U* in the Watanabe (*3*) model is defined as total uptake, meaning maximum uptake possible, and if maximum uptake, or a plateau, has not been reached at 24 h, then P_u and *q* cannot be derived correctly. This may be the case for uptake into *H. helix* of some of the formulations, in particular DOG, when applied in the presence of $C_{12}EO_6$ (**Figure 4**). However, in this particular case, if *f* at 4 h is used to derive *q*, rather than *f* at 2 h as has been used throughout, then the data points correspond much better (data not shown) even though the calculated *f* at 2 h is not greatly different from the actual *f* (3 versus 1% uptake at 2 h). This changes *q* (the penetration rate factor) to 0.00006 rather than the 0.00002 in **Table 2**. This demonstrates the importance of obtaining very accurate uptake data for the steep portion of the curve when using the logistic−kinetic model to predict uptake at different times. The penetration rate factor, *q*, is overall markedly lower for DOG and 2,4-D uptake into *H. helix*, compared with *C. album*, whereas the difference in *q* is much less for epoxiconazole **(Tables 1** and **2)**.

These results show that the equation developed by Watanabe for uptake through isolated cuticles can be used to calculate uptake over time into whole plants, with either thin or thick cuticles. The logistic−kinetic transcuticular penetration model (*3*) in most cases correctly predicts a linear increase in the penetration rate for the initial period, followed by a gradual decrease in penetration rate, with maximum penetration being approached asymptotically.

Table 1. Watanabe Model Parameters[a] and Calculated and Actual Uptake into *C. album* Foliage over Time of DOG, 2,4-D, and Epoxiconazole, Applied in the Absence or Presence of Surfactants

formulation	time* (s)	total uptake (U)* (nmol)	spread area (A)* (mm^2)	unit partition ratio (P_u)* (mm^{-2})	q* (s^{-1})	calcd uptake (f) (nmol)	actual uptake (nmol)	calcd uptake (%)	actual uptake[b] (%)
DOG	1800	0.0793	0.69	0.1058	0.00248	0.07688	0.07688	7.08	7.08 fgh
	7200					0.07926	0.10564	7.30	9.73 efgh
	14400					0.07926	0.05780	7.30	5.32 h
	21600					0.07926	0.07926	7.30	7.30 fgh
	86400					0.07926	0.07219	7.30	6.65 fgh
DOG + $C_{12}EO_3$	1800	0.3831	13.76	0.0256	0.00022	0.05808	0.13802	5.35	12.71 def
	7200					0.22394	0.22394	20.62	20.62 bcd
	14400					0.34095	0.25910	31.4	23.86 bcd
	21600					0.37369	0.06577	34.4	6.06 gh
	86400					0.38305	0.38305	35.3	35.28 b
DOG + $C_{12}EO_6$	1800	0.9304	1.21	0.7082	0.00036	0.23431	0.17778	21.58	16.37 cde
	7200					0.76084	0.76084	70.07	70.07 a
	14400					0.91577	0.92091	84.34	84.81 a
	21600					0.92924	0.85980	85.58	79.19 a
	86400					0.93038	0.93038	85.69	85.69 a
DOG + L-77	1800	0.3951	31.13	0.0117	0.00013	0.03673	0.24480	3.38	22.55 bcd
	7200					0.14980	0.14980	13.80	13.80 defg
	14400					0.27223	0.30842	25.07	28.41l bc
	21600					0.34195	0.35798	31.49	32.97 bc
	86400					0.39508	0.39172	36.39	36.39 b
2,4-D	300	0.6359	0.87	0.6533	0.0019	0.13961	0.08373	12.85	7.48 jk
	600					0.27669	0.28511	25.48	25.48 ghi
	1200					0.47644	0.51426	43.88	45.96 e
	86400					0.61709	0.63589	56.83	57.82 de
2,4-D + $C_{12}EO_3$	300	1.0552	3.4	0.2766	0.0025	0.30654	0.07219	27.32	33.16 g
	600					0.58953	0.58953	52.55	52.55 ef
	1200					0.92001	0.74633	82.00	66.52 cd
	1800					1.0223	0.92756	91.11	82.67 b
	7200					1.0552	1.0490	94.05	93.49 a
	14400					1.0552	1.0592	94.05	94.4 a
	21600					1.0552	1.0850	94.05	96.71 a
	86400					1.0552	1.0552	94.05	94.05 a
2,4-D + $C_{12}EO_6$	300	1.1005	1.31	0.7469	0.0007	0.09504	0.09504	8.45	6.79 k
	600					0.19316	0.19316	17.17	12.89 ij
	1200					0.38894	0.38894	34.58	26.51 ghi
	1800					0.56880	0.56880	50.57	68.38 c
	7200					1.0873	1.0873	96.67	92.59 a
	14400					1.1005	1.1005	97.84	96.88 a
	21600					1.1005	1.1005	97.85	97.47 a
	86400					1.1005	1.1005	97.85	97.85 a
2,4-D + L-77	300	1.0498	44.13	0.02111	0.0010	0.11871	0.20953	10.53	18.59 i
	600					0.24123	0.24123	21.40	21.40 hi
	1200					0.47741	0.35456	42.36	31.46 gh
	1800					0.67433	0.78568	59.83	69.71 c
	7200					1.0473	1.0046	92.92	89.13 ab
	14400					1.0498	1.0302	93.14	91.40 ab
	21600					1.0498	1.0555	93.14	93.69 a
	86400					1.0498	1.0498	93.14	93.14 a
Epoxi	1800	0.44237	0.69	0.57587	0.0001	0.04576	0.16735	4.11	15.03 gh
	7200					0.18541	0.18541	16.65	16.65 fg
	14400					0.32691	0.36499	29.36	32.7 cd
	21600					0.39814	0.34440	35.76	30.94 cd
	86400					0.44236	0.44237	39.73	39.73 bc
Epoxi + $C_{12}EO_3$	1800	0.52443	5.93	0.07933	0.0002	0.07304	0.23817	6.55	21.36 ef
	7200					0.28602	0.28602	25.66	25.66 de
	14400					0.45197	0.38638	40.54	34.66 bcd
	21600					0.50596	0.36149	45.38	32.43 cd
	86400					0.52443	0.52443	47.04	47.04 b
Epoxi + $C_{12}EO_6$	1800	0.24107	1.31	0.16515	0.0003	0.04406	0.14024	3.95	12.59 h
	7200					0.1623	0.16230	14.57	14.57 gh
	14400					0.22641	0.20564	20.32	18.45 fg
	21600					0.23874	0.20600	21.42	18.49 fg
	86400					0.24107	0.24107	21.63	21.63 ef
Epoxi + L-77	1800	1.0260	33.8	0.02731	0.0004	0.2818	0.39738	25.35	35.75 bcd
	7200					0.87385	0.87385	78.61	78.61 a
	14400					1.0158	0.97333	91.37	87.55 a
	21600					1.0254	0.99095	92.24	89.14 a
	86400					1.0260	1.0260	92.29	92.29 a

[a] Required inputs for Watanabe model (*3*) are marked with an asterisk. [b] Treatments within each xenobiotic series with no letter in common are significantly different ($p = 0.05$).

Table 2. Watanabe Model Parameters[a] and Calculated and Actual Uptake into *H. helix* Foliage over Time of DOG, 2,4-D, and Epoxiconazole, Applied in the Absence or Presence of Surfactants

formulation	time* (s)	total uptake (U)* (nmol)	spread area (A)* (mm^2)	unit partition ratio (P_u)* (mm^{-2})	q* (s^{-1})	calcd uptake (f) (nmol)	actual uptake (nmol)	calcd uptake (%)	actual uptake[b] (%)
DOG	1800	0.04058	0.48	0.07785	0.00023	0.00644	0.02866	0.59348	2.64 ef
	7200					0.02461	0.02461	2.2663	2.27 ef
	14400					0.03668	0.09365	3.3781	7.93 bcd
	21600					0.03977	0.03197	3.6631	2.94 ef
	86400					0.04058	0.04058	3.7369	3.74 def
DOG + $C_{12}EO_3$	1800	0.11289	1.65	0.06301	0.00012	0.00922	0.04983	0.84908	4.59 cde
	7200					0.03780	0.03780	3.4815	3.48 def
	14400					6.5214	0.04409	6.5214	4.06 de
	21600					8.4875	0.07016	8.4875	6.07 cde
	86400					10.396	0.11289	10.396	10.40 bc
DOG + $C_{12}EO_6$	1800	0.20765	1.33	0.14379	0.00002	0.00255	0.02131	0.2343	1.96 ef
	7200					0.01029	0.01029	0.9475	1.17 f
	14400					0.02083	0.06638	1.9186	6.11 de
	21600					0.03155	0.07252	2.9054	6.68 bcde
	86400					0.12160	0.20765	11.199	17.38 a
DOG + L-77	1800	0.14901	18.23	0.00753	0.00013	0.01346	0.03608	1.2397	3.32 ef
	7200					0.05498	0.05498	5.0636	5.06 cde
	14400					0.10065	0.04691	9.2695	4.32 de
	21600					0.12745	0.04236	11.738	3.90 de
	86400					0.149	0.14901	13.723	13.72 ab
2,4-D	1800	0.37865	0.678	0.45046	0.00012	0.03011	0.02879	2.4288	2.32 j
	7200					0.12357	0.12357	9.9668	9.97 ghij
	14400					0.23271	0.0991	18.770	7.99 hij
	21600					0.30496	0.08662	24.598	6.99 ij
	86400					0.37860	0.37865	30.538	30.54 def
2,4-D + $C_{12}EO_3$	1800	0.91370	1.557	0.48090	0.00011	0.07138	0.07219	5.8497	6.17 ij
	7200					0.29305	0.29305	24.015	24.02 ef
	14400					0.55382	0.61384	45.385	50.30 bcd
	21600					0.72907	0.67957	59.746	55.69 abc
	86400					0.91357	0.91371	74.865	74.88 a
2,4-D + $C_{12}EO_6$	1800	0.7697	1.188	0.52572	0.00012	0.06128	0.07992	4.9720	6.48 ij
	7200					0.25145	0.25145	20.402	22.54 fgh
	14400					0.47353	0.29071	38.415	23.59 ef
	21600					0.62029	0.50512	50.329	40.98 cde
	86400					0.76964	0.76974	62.447	70.89 ab
2,4-D + L-77	1800	0.30226	13.696	0.01812	0.00026	0.05580	0.11825	4.5810	9.71 ghij
	7200					0.20493	0.20493	16.825	16.82 fghi
	14400					0.28451	0.25624	23.358	21.04 fg
	21600					0.29949	0.11328	24.588	9.30 ij
	86400					0.30226	0.30226	24.815	24.82 def
Epoxi	1800	0.28736	0.986	0.26011	0.00030	0.06104	0.20412	5.4476	18.22 bcdef
	7200					0.21370	0.21369	19.072	19.07 bcdef
	14400					0.27754	0.19194	24.770	17.13 bcdefg
	21600					0.28622	0.20308	25.545	18.12 bcdef
	86400					0.28736	0.28736	25.647	25.64 bcd
Epoxi + $C_{12}EO_3$	1800	0.52183	1.647	0.28107	0.00012	0.04180	3.7080	3.7080	15.56 defgh
	7200					0.17148	15.213	15.213	15.21 cdefg
	14400					0.32249	28.610	28.610	17.43 bcdefg
	21600					0.42184	37.424	37.424	25.43 bc
	86400					0.52177	46.289	46.289	46.29 a
Epoxi + $C_{12}EO_6$	1800	0.3200	1.44	0.19989	0.00021	0.04718	0.03891	4.2437	3.50 i
	7200					0.18291	0.18291	16.453	16.93 bcdefg
	14400					0.28198	0.13491	25.364	12.13 efgh
	21600					0.31115	0.13294	27.988	11.96 efgh
	86400					0.32000	0.32000	28.785	28.78 b
Epoxi + L-77	1800	0.24085	4.41	0.04864	0.00014	0.02297	0.02297	2.0460	5.52 hi
	7200					0.09357	0.09357	8.3333	8.33 ghi
	14400					0.16889	0.16889	15.041	10.96 fghi
	21600					0.21059	0.21059	18.755	16.44 bcdefg
	86400					0.24084	0.24084	21.449	21.45 bcde

[a] Required inputs for Watanabe model (*3*) are marked with an asterisk. [b] Treatments within each xenobiotic series with no letter in common are significantly different ($p = 0.05$).

In comparison, a dynamic nonlinear simulation model that included an equation describing the cuticular sorption process, developed (*10*) and apparently validated (*11*) for whole plant transport of foliar-applied xenobiotics, predicts (*12*) a steady (fairly linear) increase over a 72 h period for hydrophilic, intermediate polarity, and lipophilic compounds, which is not

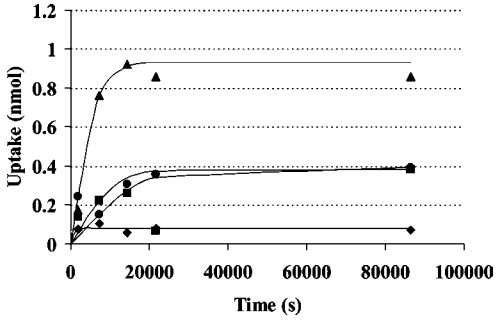

Figure 1. Uptake (nanomoles) over time of DOG into *C. album* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

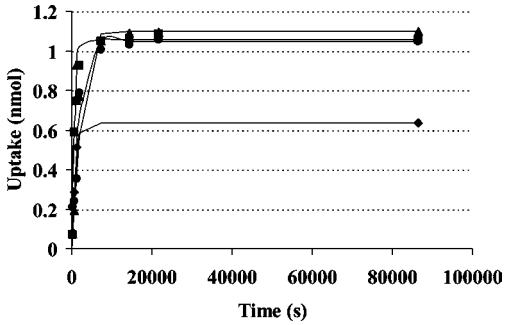

Figure 2. Uptake (nanomoles) over time of 2,4-D into *C. album* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

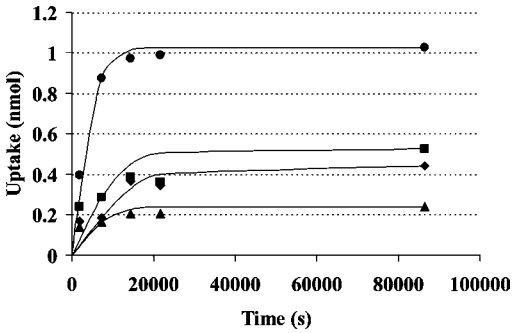

Figure 3. Uptake (nanomoles) over time of epoxiconazole into *C. album* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

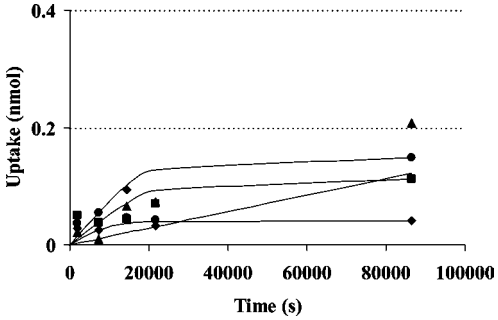

Figure 4. Uptake (nanomoles) over time of DOG into *H. helix* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

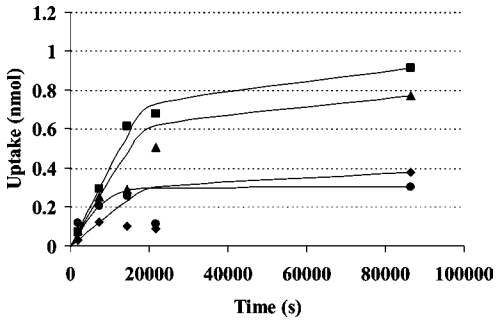

Figure 5. Uptake (nanomoles) over time of 2,4-D into *H. helix* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

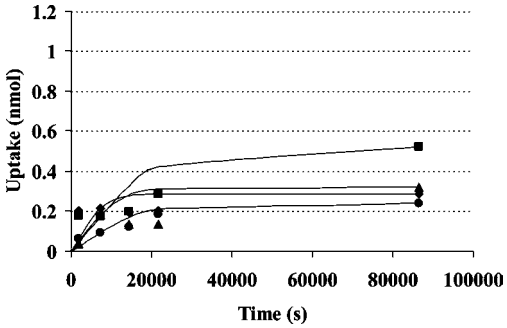

Figure 6. Uptake (nanomoles) over time of epoxiconazole into *H. helix* foliage, in the absence (◆) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eq 3.

what is found in practice. However, the advantage of the model by Satchivi et al. (*10*−*12*) is that it can be used to examine how different chemical and plant properties, as well as environmental factors, might affect the absorption and translocation of xenobiotic compounds. The disadvantage is the number of factors required, including many derived from isolated cuticle studies (e.g., xenobiotic diffusion coefficient, wax/water partition coefficient, cuticle/water partition coefficient, thickness of the limiting skin, xenobiotic concentration in the formulation residue and in the cuticular membrane, and the critical micelle concentration of the surfactant). Another method of modeling foliar uptake of pesticides (*13*) also requires numerous inputs such as diffusion coefficient, partition coefficient between droplet and cuticle and between cuticle and plant, cuticle thickness, droplet volume and diameter, and duration of the experiment. Species differences are taken into account in this model in the form of cuticle thickness, and prediction versus actual uptake appears to be good. A more recent study (*14*) again using isolated cuticles developed a one-dimensional, membrane diffusion model for cuticular penetration of a bioregulator (1-naphthylacetic acid) applied as a finite dose to a plant surface. The authors found satisfactory agreement over the experimental time course of 120 h, but for the first 10 h of penetration, the model predicted an overestimate of penetration. They concluded that the cause may be that the model is of a uniformly decaying form from the time transport begins. The authors considered that a model is required such that diffusivity starts at a relatively low value, increases to a peak, then decays uniformly with time. This type of model would simulate an initial increase in solute concentration as solvent evaporated, followed by a gradual transition to a hydrated residue state with a slower transport rate. The Watanabe logistic−kinetic penetration model describes this process. However, it cannot predict

Table 3. Calculated Uptake into *C. album* Foliage at 2 and 6 h of DOG, 2,4-D, and Epoxiconazole, Applied in the Presence of Surfactants, Using *q* Derived for Another Concentration of Each AI, and 24 h Uptake Data for the Predicted Concentration

formulation	nmol applied per 0.24 μL	time*[a] (s)	total uptake (*U*)* (nmol)	spread area (*A*)* (mm^2)	*q** (s^{-1})	calcd uptake (*f*) (nmol)	actual uptake (nmol)	calcd uptake (%)	actual uptake (%)
DOG + $C_{12}EO_3$	0.1107	7200	0.02088	10.92	0.00022	0.01223	0.0066	11.04	5.96
		21600				0.02038	0.00892	18.40	8.05
DOG + $C_{12}EO_3$	54.292	7200	24.736	2.31	.00022	14.488	3.2502	26.69	6.29
		21600				24.137	6.3478	44.46	16.00
DOG + $C_{12}EO_6$	0.1108	7200	0.04653	1.5	0.00036	0.03806	0.02755	34.34	25.37
		21600				0.04647	0.03296	41.94	29.74
DOG + $C_{12}EO_6$	54.292	7200	52.034	1.2	0.00036	42.558	2.3440	78.39	4.32
		21600				51.97	8.30	95.72	15.29
DOG + L-77	0.1106	7200	0.03284	46.51	0.00012	0.01134	0.03207	10.25	29.53
		21600				0.02723	0.06040	24.62	52.85
DOG + L-77	54.292	7200	11.99	32.5	0.00012	4.1405	5.8707	7.63	10.81
		21600				9.9402	8.1237	18.31	14.96
2,4-D + $C_{12}EO_3$	0.02403	7200	0.01568	2.35	0.0025	0.01568	0.01372	65.26	57.09
		21600				0.01568	0.01695	65.26	70.53
2,4-D + $C_{12}EO_3$	10.87	7200	7.9976	1.81	0.0025	7.9976	9.3676	73.56	86.16
		21600				7.9976	10.186	73.56	94.27
2,4-D + $C_{12}EO_6$	0.02396	7200	0.02186	1.61	0.0007	0.02149	0.00657	89.68	60.31
		21600				0.02186	0.01891	91.24	78.91
2,4-D + $C_{12}EO_6$	10.87	7200	8.9402	1.37	0.0007	8.7876	8.956	80.83	82.38
		21600				8.9402	9.8229	82.23	90.36
2,4-D + L-77	0.02512	7200	0.02341	33.29	0.001	0.02336	0.00776	93.19	71.23
		21600				0.02341	0.00981	93.19	90.07
2,4-D + L-77	10.87	7200	8.1320	3.66	0.001	8.1158	7.9534	74.64	73.15
		21600				8.1320	9.1700	74.79	84.34
Epoxi + $C_{12}EO_3$	0.02994	7200	0.02874	6.65	0.0002	0.01572	0.02822	52.51	94.27
		21600				0.02774	0.02858	92.66	95.45
Epoxi + $C_{12}EO_3$	2.1915	7200	0.49965	2.73	0.0002	0.27333	0.49012	12.47	21.83
		21600				0.48231	0.3924	22.01	16.97
Epoxi + $C_{12}EO_6$	0.02872	7200	0.02816	1.41	0.0003	0.02090	0.00810	72.76	74.28
		21600				0.02805	0.02774	97.65	96.60
Epoxi + $C_{12}EO_6$	2.1901	7200	0.41874	1.47	0.0003	0.31073	0.36387	14.19	16.61
		21600				0.41703	0.33263	19.04	15.19
Epoxi + L-77	0.02968	7200	0.02862	26.09	0.0004	0.02470	0.01004	83.23	92.08
		21600				0.02861	0.01055	96.39	96.56
Epoxi + L-77	2.1906	7200	1.7234	36.65	0.0004	1.4875	0.93170	67.90	42.53
		21600				1.7226	1.5138	78.63	69.10

[a] Required inputs for Watanabe model (*3*) are marked with an asterisk.

total or maximum uptake, and this value needs to be known, as well as uptake at a time on the steep portion of the uptake curve (e.g., uptake at 2 h), for all formulations, to derive *q* and use eq 3.

Application of a Logistic−Kinetic Penetration Model. Watanabe (*3*) states that an increase in ai concentration or an increase in contact area will have no effect on the penetration rate factor (*q*). A previous study (*5*) had determined uptake at 24 h for the same formulations as used in the preceding sections, but with a much wider range of concentrations for DOG, 2,4-D, and epoxiconazole. If the Watanabe concept is correct, and applicable to whole plant uptake, then the values for *q* derived in the current study, along with the 24 h uptake data determined in the previous study, should enable uptake to be predicted at any given time prior to 24 h using eq 1. This proposition was tested using two widely different concentrations of DOG, 2,4-D, and epoxiconazole, all applied in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5, onto *C. album*. The concentrations used were chosen to be well below and considerably above the original concentration used in the rate experiment described earlier. The concentrations chosen for DOG were 10 times lower and 50 times higher than the original concentration studied; for 2,4-D these were 100 times lower and 10 times higher; and for epoxiconazole they were 100 times lower and 2 times higher. Uptake at two intervals (2 and 6 h, 7200 and 21600 s) was predicted and then tested experimentally. The correlation between actual and predicted uptake was mainly poor (**Table 3**). Epoxiconazole showed the best correlation, followed by 2,4-D and then DOG. The largest discrepancy was with DOG in the presence of $C_{12}EO_6$, particularly at the higher concentration. This formulation of DOG spreads the least, meaning that the dose is highest. It has been rationalized (*15*) that actual uptake can be much lower than predicted due to a significant amount of crystallization of xenobiotic on the surface of the leaf (*15*). However, in the current case, contact phytotoxicity to the leaf surface is observed when DOG is applied in the presence of $C_{12}EO_6$, whereas none is observed when DOG is applied in the presence of $C_{12}EO_3$ or TSE7.5. A much better correlation is found between predicted and actual uptake of the highest concentration of DOG formulated with TSE7.5, which spreads the most, meaning that the dose per unit area is much less. This lends weight to the postulate that the reason for DOG

Table 4. Calculated Uptake into *C. album* Foliage at 2 and 6 h of Epoxiconazole, Applied in the Presence of Surfactants, Using q and P_u Derived from Another Concentration of Epoxiconazole

formulation	nmol applied per 0.24 μL	time*[a] (s)	total uptake (U)* (nmol)	spread area (A)* (mm^2)	q* (s^{-1})	calcd uptake (f) (nmol)	actual uptake (nmol)	calcd uptake (%)	actual uptake (%)
Epoxi + $C_{12}EO_3$	0.02994	7200	0.02874	6.65	0.0002	0.00864	0.02822	28.86	94.27
		21600				0.01525	0.02858	50.92	95.45
Epoxi + $C_{12}EO_3$	2.1915	7200	0.49965	2.73	0.0002	0.25963	0.49012	11.84	21.83
		21600				0.45814	0.3924	20.91	16.97
Epoxi + $C_{12}EO_6$	0.02872	7200	0.02816	1.41	0.0003	0.00496	0.00810	17.28	74.28
		21600				0.00666	0.02774	23.19	96.60
Epoxi + $C_{12}EO_6$	2.1901	7200	0.41874	1.47	0.0003	0.39454	0.36387	18.01	16.61
		21600				0.52952	0.33263	24.18	15.19
Epoxi + L-77	0.02968	7200	0.02862	26.09	0.0004	0.01825	0.01004	61.50	92.08
		21600				0.02114	0.01055	71.22	96.56
Epoxi + L-77	2.1906	7200	1.7234	36.65	0.0004	1.8925	0.93170	86.39	42.53
		21600				2.1916	1.5138	100.04	69.10

[a] Required inputs for Watanabe model (*3*) are marked with an asterisk.

Table 5. Calculated Uptake of Epoxiconazole, Applied in the Presence of Surfactants, into *C. album* Foliage at 2 and 6 h, Using q Derived for Another Concentration of Epoxiconazole and 24 h Uptake Data Predicted from Alternative (*5*) Dose Uptake Equation

formulation	nmol applied per 0.24 μL	time*[a] (s)	total uptake (U)* (nmol)	spread area (A)* (mm^2)	q* (s^{-1})	calcd uptake (f) (nmol)	actual uptake (nmol)	calcd uptake (%)	actual uptake (%)
Epoxi + $C_{12}EO_3$	0.02994	7200	0.03685	6.65	0.0002	0.02016	0.02822	67.33	94.27
		21600				0.03557	0.02858	118.80	95.45
Epoxi + $C_{12}EO_3$	2.1915	7200	0.71920	2.73	0.0002	0.39344	0.49012	17.95	21.83
		21600				0.69424	0.3924	31.68	16.97
Epoxi + $C_{12}EO_6$	0.02872	7200	0.02405	1.41	0.0003	0.01785	0.00810	62.15	74.28
		21600				0.02396	0.02774	83.41	96.60
Epoxi + $C_{12}EO_6$	2.1901	7200	0.61388	1.47	0.0003	0.45553	0.36387	20.80	16.61
		21600				0.61138	0.33263	27.92	15.19
Epoxi + L-77	0.02968	7200	0.05188	26.09	0.0004	0.04477	0.01004	150.86	92.08
		21600				0.05185	0.01055	174.70	96.56
Epoxi + L-77	2.1906	7200	1.3943	36.65	0.0004	1.2034	0.93170	54.93	42.53
		21600				1.3936	1.5138	63.62	69.10

[a] Required inputs for Watanabe model (*3*) are marked with an asterisk.

showing the poorest correlation between predicted and actual uptake is due to the 50 times higher concentration used. Considering the range of concentrations used, compared to the original concentration used to derive q, the correlations between actual and predicted uptake are remarkably good. More work is required, but the conclusion at this stage is that the Watanabe theory is largely correct; that is, a change in ai concentration or contact area (i.e., a change in initial dose) does not alter the penetration rate factor, q. However, a caveat needs to be added, to limit the concentration ranges over which specific xenobiotics should be estimated.

If eq 1 can be used to predict uptake, then can eq 3 also be used to predict uptake? Total uptake needs to be known to utilize eq 1, whereas total uptake is not required to utilize eq 3 if P_u is also constant across concentration or contact area. Using epoxiconazole data as an example (from **Table 3**), then **Table 4** shows predicted uptake using eq 3. It can be seen that the correlation between predicted and actual uptake is generally poor. This shows that although q remains constant, P_u, the unit partition ratio of the pesticide, changes when the concentration of the ai is changed. It would appear from these results that lowering the concentration of the ai increased P_u; that is, it would need a much higher P_u to increase the predicted uptake to a value closer to the actual uptake. Increasing the concentration results in a similar, if not lower, P_u. The lowest concentration of epoxiconazole used was 100 times less than that on which P_u (and q) was based, whereas the highest concentration was only 2 times greater, which would explain why the differences are not as large for the higher concentration of epoxiconazole.

In the current study it has been possible to predict the rate of uptake for concentrations of active ingredients not already measured (for 2,4-D and epoxiconazole, but not DOG), at different time intervals. However, q needs to be derived for one concentration of the formulation (into the specific species being considered), and maximum uptake needs to be known for all formulations and concentrations of interest. Recent studies (*5*, *16*) have shown that mass uptake at 24 h on a per unit area basis is related to the initial dose of xenobiotic applied, by an equation of the following form: $\text{uptake}_{(\text{nmol mm}^{-2})} = a[\text{ID}]^b$ at time $t = 24$ h, where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{\text{nmol xenobiotic applied}}/A_{\text{droplet spread area}}$) and a and b are constants specific to each xenobiotic applied to a specific species. Total mass uptake at 24 h could be calculated from an equation of the form $\text{total uptake}_{\text{nmol}} = a[\text{ID}]^b A$. Again, using epoxiconazole as the example, can this equation be used to predict U (total uptake) and then used to derive q and eq 1. In the case of epoxiconazole, Forster et al. (*5*) found that uptake (epoxiconazole in nmol) = $0.3103(\text{ID})^{0.745}$(spread area). When the predicted uptake value from this calculation is used in eq 1, the overall correlation between actual and predicted uptake at 2 and 6 h is variable (**Table 5**). There are some large discrepancies, and when

predicted uptake is well over 100%, actual uptake is close to 100%. Obviously, the equation using initial dose (ID) to predict uptake at 24 h needs to be refined further. The relative importance of each of the variables involved in uptake, that is, species, ai, ai concentration (g L^{-1}), and surfactant has also been established recently (*17*), and surfactant has been shown to be highly significant, even after taking spread area into account. Progress needs to continue in this area to produce a more accurate model for uptake at 24 h. However, using the equations based on initial dose provides a good rule of thumb for uptake at 24 h (*18*), and using these in conjunction with the Watanabe model has significantly advanced our understanding and ability to model xenobiotic uptake. The advantages of these models and equations are that few variables are required and they are simple to measure.

ACKNOWLEDGMENT

Silwet L-77 was supplied by GE Advanced Materials—Silicones. Epoxiconazole was supplied by BASF. We thank Madelein Hofstee for performing spread area measurements and Kevin Steele for maintenance of the CE facilities. We also thank Tadakazu Watanabe for helpful discussions and informal review of the manuscript.

LITERATURE CITED

(1) Price, C. E. A review of the factors influencing the penetration of pesticides through plant leaves. In *The Plant Cuticle*; Cutler, D. F., Alvin, K. L., Price, C. F., Eds.; Academic Press: London, U.K., 1982; pp 237−252.

(2) Schönherr, J.; Baur, P. Modelling penetration of plant cuticles by crop protection agents and effects of adjuvants on their rates of penetration. *Pestic. Sci.* **1994**, *42*, 185−208.

(3) Watanabe, T. Transcuticular penetration of foliar-applied pesticides—its analysis by a logistic-kinetic penetration model. In *Herbicide Classes in Development*; Böger, P., Wakabayashi, K., Hirai, K., Eds.; Springer-Verlag: Berlin, Germany, 2002; pp 319−340.

(4) Buchholz, A.; Baur, P.; Schönherr, J. Differences among plant species in cuticular permeabilities and solute mobilities are caused by differential selectivities. *Planta* **1998**, *206*, 322−328.

(5) Forster, W. A.; Zabkiewicz, J. A.; Riederer, M. Mechanisms of cuticular uptake of xenobiotics into living plants: 1. Influence of xenobiotic dose on the uptake of three model compounds, applied in the absence and presence of surfactants into *Chenopodium album*, *Hedera helix* and *Stephanotis floribunda* leaves. *Pest Manag. Sci.* **2004**, *60*, 1105−1113.

(6) *Fluka Laboratory Chemicals and Analytical Reagents Catalogue*, 1999/2000.

(7) *The Agrochemicals Handbook*, 3rd ed.; Kidd, H., James, D. R., Eds.; The Royal Society of Chemistry: Cambridge, U.K., .

(8) SRC's LOGKOW/KOWWIN Program; on-line at http://esc.syr-res.com/interkow/kowdemo.htm.

(9) Stevens, P. J. G.; Baker, E. A. Factors affecting the foliar absorption and redistribution of pesticides. 1. Properties of leaf surfaces and their interactions with spray droplets. *Pestic. Sci.* **1987**, *19*, 265−281.

(10) Satchivi, N. M.; Stoller, E. W.; Wax, L. M.; Briskin, D. P. A nonlinear dynamic simulation model for xenobiotic transport and whole plant allocation following foliar application I. Conceptual foundation for model development. *Pestic. Biochem. Physiol.* **2000**, *68*, 67−84.

(11) Satchivi, N. M.; Stoller, E. W.; Wax, L. M.; Briskin, D. P. A nonlinear dynamic simulation model for xenobiotic transport and whole plant allocation following foliar application II. Model validation. *Pestic. Biochem. Physiol.* **2000**, *68*, 85−95.

(12) Satchivi, N. M.; Stoller, E. W.; Wax, L. M.; Briskin, D. P. A nonlinear dynamic simulation model for xenobiotic transport and whole plant allocation following foliar application III. Influence of chemical properties, plant characteristics, and environmental parameters on xenobiotic absorption and translocation. *Pestic. Biochem. Physiol.* **2001**, *71*, 77−87.

(13) Lamb, S.; Bean, M.; Chapman, P. Foliar uptake of pesticides by mathematical modelling: a tool for influencing adjuvant selection. In *Proceedings of the 6th International Symposium on Adjuvants for Agrochemicals*; de Ruiter, H., Ed.; ISAA 2001 Foundation: Amsterdam, The Netherlands, 2001; pp 106−112.

(14) Brazee, R. D.; Bukovac, M. J.; Zhu, H. Diffusion model for plant cuticular penetration by spray-applied weak organic acid bioregulator in presence or absence of ammonium nitrate. *Trans. ASAE* **2004**, *47* (3), 629−635.

(15) Forster, W. A.; Zabkiewicz, J. A.; Riederer, M. Spray formulation deposits on leaf surfaces and xenobiotic mass uptake. In *Proceedings of the 7th International Symposium on Adjuvants for Agrochemicals*; Document Transformation Technologies; Cape Town, South Africa, 2004; pp 332−338.

(16) Forster, W. A.; Zabkiewicz, J. A.; Liu, Z. Q. Cuticular uptake of xenobiotics into living plants: 2. Influence of xenobiotic dose on the uptake of bentazone, epoxiconazole and pyraclostrobin, applied in the presence of various surfactants, into *Chenopodium album*, *Sinapis alba* and *Triticum aestivum* leaves. *Pest Manag. Sci.* **2006**, in press.

(17) Forster, W. A.; Zabkiewicz, J. A.; Kimberley, M. O.; Riederer, M. Statistical analysis to determine the relative importance of variables involved in foliar uptake. *Transactions of the ASABE*. **2006**, *49*(1), 25−31.

(18) Forster, W. A.; Zabkiewicz, J. A.; Riederer, M. Mechanisms of cuticular uptake into living plants: Influence of xenobiotic dose and surfactant on uptake. In *Proceedings of the 7th International Symposium on Adjuvants for Agrochemicals*; Document Transformation Technologies; Cape Town, South Africa, 2004; pp 83−91.

Received for review November 20, 2005. Revised manuscript received February 21, 2006. Accepted February 22, 2006. The New Zealand Public Good Science Fund provided the funding for this work. W.A.F. thanks the Allan and Grace Kay Overseas Scholarship and the Frank Sydenham Scholarship for support while studying toward a Ph.D. at the University of Würzburg. Financial support was also given by Fonds der Chemischen Industrie.

JF0528916