Permeation of Tecnazene through Human Skin in Vitro as Assessed by HS-SPME and GC-MS

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Permeation of tecnazene into and through human cadaver skin in vitro was assessed using a GC-MS method employing HS-SPME for receptor solution analyses. Two doses of tecnazene dissolved in acetone, corresponding to 103 and 864 μg/cm^2 of tecnazene, were applied to skin mounted on Franz diffusion cells and placed in a fume hood. Cells were either occluded with aluminum foil or left unoccluded. Total absorption of tecnazene (dermis + receptor fluid) after 48 h was 2.2−6.1% of the applied dose for the unoccluded treatments and 22−33% for the occluded treatments. Potentially absorbed dose including all tecnazene that may have eventually permeated the skin ranged from 10% unoccluded to 42−53% occluded. Accumulation in the receptor solutions was satisfactorily described by a working diffusion model after upward adjustment of the partition coefficient for tecnazene in all skin layers by a factor of 5−16 versus *a priori* values. However, residual amounts of tecnazene in both the epidermis and dermis were higher than those estimated from the model, suggesting the existence of tissue binding not accounted for in the calculation. The results indicate that the diffusion model as presently calibrated may significantly underestimate both systemic absorption and skin concentrations of highly lipophilic compounds, as predicted from data generated from in vitro skin permeation assays. Model predictions could be improved by better accounting for partitioning into the epidermis and dermis.

Introduction

Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene, CAS no. 117-18-0) is a dual acting pesticide. It is used as a plant growth regulator to suppress sprouts on potatoes during storage and as a fungicide to prevent dry rot on the tubers. Human exposure is primarily through ingestion of food residues, with an Acceptable Daily Intake level of 0.01 mg/kg of body weight according to FAO/WHO guidelines (*1*). Tecnazene is commercially available as granules and as dispersible dust formulations. Although it has been studied extensively as a fungicide and its toxicology and metabolism in animal models including rats, dogs, and mice has been documented (*2, 3*), its disposition following application to human skin to our knowledge has not been studied. This paper describes the fate of tecnazene following topical application to human skin in vitro. Permeation results for two doses of tecnazene applied to skin in acetone solution are presented and interpreted in terms of a quantitative skin absorption model (*4–7*). We chose to study tecnazene to examine the extrapolation of the model, which was developed using databases comprised of small, moderately lipophilic compounds (*8, 9*), to a small, highly lipophilic, chlorinated pesticide. Although tecnazene is commonly applied to foods in powdered form, many other such chemicals are applied as aerosols that sometimes contact the skin of the worker; thus, application via solvent deposition is relevant to the compounds as a class. Unoccluded and occluded applications were studied in order to span the range of pesticide exposures encountered by field workers; furthermore, we wished to determine whether the effect of hydration on skin permeability for such a highly lipophilic compound was adequately predicted by the computational model. High specific doses of tecnazene (103 and 864 μg/cm^2) were used primarily due to analytical sensitivity limitations. The model facilitates extrapolation to lower doses (*10*).

A secondary objective of the study was to develop methods that could subsequently be used to study the skin absorption of complex chemical mixtures. After studying several options and experimenting with liquid−liquid extraction techniques, we selected headspace solid phase microextraction (HS-SPME) followed by GC-MS analysis. SPME is a simple and convenient sample preparation technique that uses a thin fused silica rod to preconcentrate analytes on to a polymeric fiber using one of the three possible modes−immersion, headspace, or membrane-protected mode (*11*). The preconcentrated analytes are then thermally desorbed into a heated injection port attached to a chromatographic device for separation and quantification. HS-SPME gave superior sensitivity to immersion SPME for tecnazene in our experience. Excellent reviews of SPME applications including environmental studies and biological monitoring are available (*12, 13*). The present report demonstrates the applicability of HS-SPME combined with GC-MS to determine in vitro human skin permeation of tecnazene.

Experimental Methods

Materials. Tecnazene in the form of PESTANAL was purchased from Reidel-de-Haen (Germany). The purity was estimated at 99.8% by GC analysis. Hexachlorobenzene, 99.99% purity, was purchased from Supelco (Bellefonte, PA). Polyoxyethylene-20 oleyl ether (Oleth-20), reagent grade sodium chloride, calcium-free Dulbecco's phosphate-buffered saline, and bovine serum albumin were obtained from Sigma-Aldrich (St. Louis, MO). Pesticide grade acetone and GC-Resolve methylene chloride were purchased from Fisher Scientific (Pittsburgh, PA). Split thickness (300 μm) human cadaver skin (back, thigh, and abdomen) was procured from U.S. Tissue and Cell (Cincinnati, OH). The skin was preserved in 10% glycerol and stored at −80 °C until use.

Physical Properties and Protein Binding. Physical properties of tecnazene relevant to skin absorption were obtained from the EpiSuite program available from the U.S. Environmental Protection Agency (*14*) unless otherwise noted. The extent of binding to extravascular albumin in the dermis was estimated from the ratio of the observed solubility in a

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2% solution of bovine serum albumin in phosphate-buffered saline at 37 °C to the estimated solubility in water at 37 °C. As a check, this value was also estimated by the computational method of Yamazaki and Kanaoka (*15*). Finally, the solubility of tecnazene in a 1% solution of Oleth-20 in phosphate-buffered saline at 37 °C was determined by visual inspection.

Skin Permeation Study. The excised skin tissue was mounted on modified Franz diffusion cells with a cross sectional area of 0.79 cm^2 (*16*). The receptor solution was Dulbecco's phosphate-buffered saline (pH 7.4) containing 1% (w/v) Oleth-20 (*17*) and 0.02% (w/v) sodium azide to inhibit microbial growth. Receptor solutions were maintained at 37 ± 2 °C by thermostatted heating and stirring modules, yielding a skin surface temperature of about 32 °C. The tissue was screened using a short duration ^3H_2O permeability test, and samples with water permeation greater than 1.2 $\mu L/cm^2$ were discarded (*18*). The exterior surface of the ground glass joint between the donor and receptor compartments was then sealed with Parafilm. The cells were randomized over treatments using previously established procedures (*19*). They were equilibrated overnight and dosed the following morning with 10 μL of either 1% (w/w) or 9% (w/w) tecnazene in acetone, resulting in an average dose of 103 or 864 $\mu g/cm^2$, respectively. Each dose was studied under open and occluded conditions, yielding a total of four treatments. The occluded cells were covered with aluminum foil, which was wrapped securely around the top immediately after dosing. Tests with an infrared thermometer showed that occlusion in this manner raised the skin surface temperature by less than 1 °C versus the unoccluded samples. The cells and the thermostatted blocks were placed in a fume hood with the sash raised to 18″ (*20, 21*). The experiment was allowed to proceed for 48 h after dosing to ensure that a steady-state flux of tecnazene into the receptor compartment was achieved. No wash off procedure was employed; thus absorption may be expected to be higher than that for a comparable study employing a skin wash. The entire receptor content (4.5 mL) was collected at 4, 8, 12, 24, and 48 h postdose. At this point the skin was dissected to obtain epidermis and dermis samples. The diffusion cells were rinsed with acetone, and the wash was collected in a separate vial. Parafilm and aluminum foil were also collected in individual vials. All samples were stored at −4 °C until analysis. The skin was obtained from four donors with 1−4 replicates per donor for each treatment. The data reported are the mean ± standard error for each treatment across all donors, with each sample given equal weight. Absorbed dose was calculated as the sum of the amounts in the receptor fluid (cumulative) and dermis after 48 h. Potentially absorbed dose was calculated as the sum of absorbed dose plus the amounts in epidermis and Parafilm. The Parafilm content was included in the potentially absorbed dose because we hypothesized the compound arrived in the Parafilm by lateral diffusion through the tissue clamped in the ground glass joint. The alternative of diffusing through the air following evaporation from the skin surface did not seem likely in the fume hood environment, but it could not be ruled out. Wash and tissue amounts were not obtained for Donor 1; hence, the averages for these parameters represent only three donors.

Sample Preparation and Analysis. Aqueous samples were thawed at room temperature prior to analysis. The receptor fluid was transferred to a conical glass derivatization vial with a screw cap top fitted with a polytetrafluoroethylene-lined septum. The vial was placed in a thermostatted base maintained at 70 °C, and the fiber was exposed for a period of 15 min. Samples were analyzed using HS-SPME coupled with an HP 5980 gas chromatograph and a 5972 series mass selective detector. The response was noted, and the amount of tecnazene in the 4.5 mL was calculated using a single-point standard curve that was constructed separately for each fiber on each day of analysis. The high precision of the analysis justified this procedure, as demonstrated in the Supporting Information.

TABLE 1. Physical Properties of Tecnazene[a]

property	units	value
MW	g/mol	260.89
log K_{oct}[b]		4.38
mp	°C	99
bp^{760}	°C	304
P_{vp}[c]	torr	7.23×10^{-4}
ρ[d]	g/cm^3	2.01[e]
solubilities	mg/L	
water (32 °C)	mg/L	2.76[f]
water (37°C)	mg/L	3.09[f]
2% BSA in PBS (37 °C)	mg/L	50
1% Oleth 20 in PBS (37 °C)	mg/L	300
f_u[g]		0.06[h]
f_{non}[i]		1.00

[a] All properties except mp, bp, and solubility are estimated at 32 °C. Properties were obtained from ref *14* unless otherwise noted and extrapolated to 32 °C as required. [b] $\log_{10}$ (octanol/water partition coefficient). [c] Vapor pressure. [d] Density. [e] Estimated by Schroeder's method (*37*). [f] Extrapolated from measured value of 2.09×10^{-3} g/L at 20 °C. [g] Fraction unbound in a 2% albumin solution. [h] Estimated as the ratio of water solubility to solubility in 2% BSA. [i] Fraction non-ionized at pH 7.4.

Epidermis, dermis, Parafilm, and foil samples were extracted with methylene chloride and analyzed using a Varian 3800 ion trap gas chromatograph coupled with a Saturn 2000 GC/MS/MS detector and an 8200 Autosampler. The wash samples were directly analyzed without any modification. All samples were analyzed in triplicate, and the mean value was used for quantification of tecnazene. The injection volume for all samples was 2 μL.

A 100 μm polydimethylsiloxane (PDMS) fiber (Supelco) was used for receptor phase analysis using HS-SPME (*22, 23*). The fibers were preconditioned according to the manufacturer's recommendations. A blank analysis was performed at the beginning of each day to ensure absence of carry-over effect for all fibers used during the course of the analyses. The extraction conditions were optimized by modifying the salt content, time of exposure, and temperature of the receptor fluid. Precision and recovery from different matrices (epidermis, dermis, Parafilm, and aluminum foil) were also determined.

Further details of the analytical procedures are provided in the Supporting Information.

Diffusion Model. The disposition of tecnazene under the various conditions in the skin penetration study was estimated using the diffusion/evaporation model summarized in refs *5* and *6*. The diffusion/evaporation model is constructed as a three-layer, one-dimensional model with a diffusivity (D_i), partition coefficient (K_i), and thickness (h_i) for each skin layer−stratum corneum (i = sc), viable epidermis (i = ve), and dermis (i = de). A brief description of the model as well as governing equations and transport parameter formulas are included in the Supporting Information. Details are given elsewhere (*4, 8–10, 24, 25*). The model includes binding of permeants to proteins and lipids in each skin layer. The physical properties in Table 1 combined with the formulas in the Supporting Information were used to estimate transport parameters. Transient diffusion calculations were conducted using an Excel spreadsheet and associated add-in of our own devising (*4*). The method allowed for a comparison with both the amounts of tecnazene in skin and the amounts found in the receptor solutions during the in vitro skin penetration study. Two model

TABLE 2. Distribution of Tecnazene 48 h after Topical Application to Human Skin in Vitro[a]

		mean dose 103 μg/cm^2		mean dose 864 μg/cm^2	
		open	occluded	open	occluded
evaporated	foil	N/A	10.6 ± 3.4	N/A	3.1 ± 0.6
	wash	0.4 ± 0.2	9.0 ± 3.2	9.2 ± 4.7	41.2 ± 7.9
in/on skin	Para	2.5 ± 0.8	17.6 ± 5.8	4.0 ± 2.2	7.2 ± 3.5
	epidermis	2.0 ± 0.5	3.2 ± 0.4	5.2 ± 3.2	12.8 ± 4.1
absorbed	dermis	1.0 ± 0.4	4.2 ± 0.7	0.6 ± 0.5	20.5 ± 3.4
	rec. fluid	5.3 ± 1.7	29.8 ± 4.7	1.8 ± 0.3	3.9 ± 0.6
total absorbed[b]		6.1 ± 1.7	33.2 ± 4.8	2.2 ± 0.4	22.7 ± 3.6
potentially absorbed[c]		10.2 ± 1.4	53.3 ± 3.7	10.2 ± 3.0	41.6± 4.8
total recovered		10.5 ± 1.3	71.1 ± 5.5	17.1 ± 4.5	82.5 ± 10.3
missing		89.5 ± 1.3	28.9 ± 5.5	82.9 ± 4.5	17.5 ± 10.3

[a] Mean ± SE of 4 donors, n = 1−4 cells per donor. Each value represents a minimum of 9 measurements. Values are expressed as a percentage of the applied dose given at the top of each column. [b] Sum of dermis and receptor fluid. [c] Sum of dermis, receptor fluid, epidermis, and Parafilm.

calculations were conducted. First, an *a priori* or predictive calculation was conducted using the default parameter values in the spreadsheet calculation based on prior calibration of the diffusion model (*5, 6*). This calculation corresponds to Model 1 in ref *4*. The effective air flow velocities in the evaporative mass transfer correlation (*26*) were lowered versus those given in ref *5* based on recent work with volatile solvents (*27*), as discussed in the Supporting Information. A second calculation was conducted in which the partition coefficients of tecnazene in the stratum corneum and viable epidermis/dermis were adjusted to match the calculated accumulation in the receptor solution to the experimental data. This was accomplished by a nonlinear least-squares method as previously described (*20, 21*). The parameters associated with the two calculations will be referred to as "*a priori*" and "fitted" parameters, respectively.

Results

Analytical Method. The limits of quantification for the developed methods were 0.8−1.0 μg/mL for HS-SPME and 1.6−11 μg/mL for liquid samples. Calibration curves were linear over the range 0.1−5.2 μg/mL for HS-SPME and 0.5−100 μg/mL for liquid samples. Percentage recoveries of tecnazene from the various matrices were 111.7 ± 9.3, 89.3 ± 8.8, 100.0 ± 1.5, and 51.6 ± 19.7 from epidermis, dermis, foil, and Parafilm, respectively (mean ± SE, n = 3). Additional details including representative chromatograms and standard curves may be found in the Supporting Information.

Physical Properties and Protein Binding. Table 1 gives the physical properties for tecnazene obtained from literature sources and the solubilities and protein binding affinity measured in our laboratory. The latter value, f_u = 0.06, indicates that about 6% of dissolved tecnazene is unbound (hence, 94% is bound) in a 2% albumin solution, considered to be representative of in vivo albumin concentrations in dermis (*9, 28*). The alternative calculation method (*15*) yielded f_u = 0.02. The agreement between these values is better than the average for the calculation (*15*). Tecnazene solubility in the 1% Oleth-20 buffer solution used in the receptor compartments for the skin penetration studies was 6-fold higher than in the 2% BSA solution and about 100-fold higher than its water solubility.

Skin Permeation Study. Table 2 and Figure 1 give the results of the in vitro skin permeation study. The mean recovery of tecnazene from all samples ranged from 10.5% to 17.1% for the unoccluded treatments and from 71.1% to 82.5% for the occluded treatments. From these results, supported by the calculations described later, it may be inferred that a substantial amount of tecnazene evaporated from the skin surface in the unoccluded treatments. Absorbed dose ranged from 2.2% for the high dose unoccluded treatment to 33.2% for the low dose occluded treatment. Potentially absorbed dose including also the amounts in epidermis and Parafilm samples ranged from 10.2% unoccluded to 53.3% occluded. Further analysis of these data shows that the receptor fluid measurements were the most consistent in the study, with a median coefficient of variation of 61%. Median coefficients of variation for epidermis, dermis, and Parafilm samples exceeded this value by factors of 1.5, 1.6, and 2.4, respectively. These high levels of variability reflect the difficulty of performing low level analyses of volatile and highly lipophilic compounds in skin in vitro, even when the tissue has been screened for integrity as in the present study.

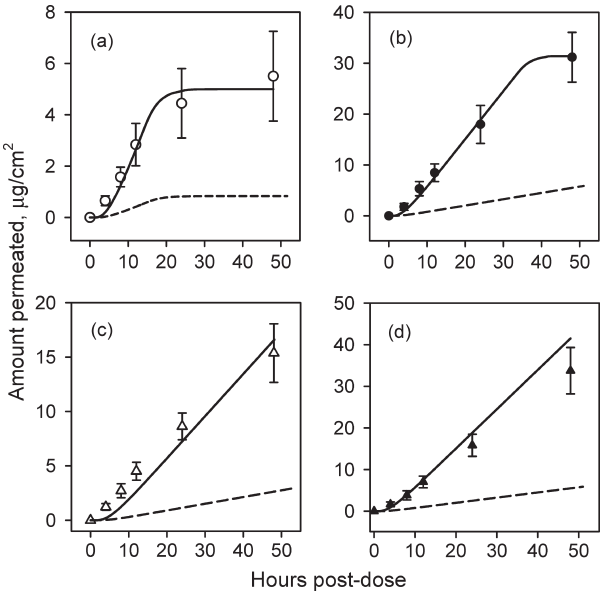

FIGURE 1. Accumulation of tecnazene in the receptor solution following application from acetone solutions to human cadaver skin *in vitro* (mean ± SE of 4 donors, n = 2−4 cells per donor) (○, •) 103 μg/cm^2 and (Δ,▲) 864 μg/cm^2. The open and closed symbols represent unoccluded and occluded conditions, respectively. The dashed line represents the diffusion model prediction using the *a priori* parameter values in Table 3, and the solid line represents the fitted model calculation using the partition coefficient correction factors given in Table 4.

Figure 1 shows the cumulative permeation of tecnazene into the receptor solutions, along with model calculations of these values. The data are shown as mass transported per unit area to emphasize the fact that the major difference between the low and high dose results was the duration of sustained absorption rather than the permeation rate. It should be evident that equivalent amounts of tecnazene permeated for low and high doses correspond to different

TABLE 3. Model Parameters for *a Priori* Calculation of Tecnazene Absorption through Human Skin in Vitro[a]

parameter	units	value: unoccluded	value: occluded
		Stratum Corneum	
h_{sc}	μm	13.4	43.4
h_{dep}	μm	1.34	4.34
D_{sc}	$cm^2\ s^{-1}$	5.34×10^{-11}	1.41×10^{-9}
K_{sc}	-	203.3	71.2
		Viable Epidermis	
h_{ed}	μm	100	
D_{ed}	$cm^2 s^{-1}$	6.17×10^{-8}	
K_{ed}	-	18.00	
		Dermis	
h_{de}	μm	300	
D_{de}	$cm^2 s^{-1}$	6.17×10^{-8}	
K_{de}	-	18.00	
k_{de}	s^{-1}	0	
		Environmental Factors	
T	°C	32	32
u	m/s	0.72	0.141[c]
		Derived Parameters[b]	
f_{dep}	-	0.1	0.1
C_{sat}	$\mu g/cm^3$	560	196
M_{sat}	$\mu g/cm^2$	0.075	0.085
k_p	cm/h	0.0226	0.0454
k_g	cm/h	766	215
$k_{evap}\rho$	$\mu g/cm^2$ h	7.59	2.13
χ	-	94.15	9.27

[a] Formulas for the transport parameters may be found in refs *5* and *6*. The unoccluded column reflects the properties of partially hydrated skin and the occluded column reflects those of fully hydrated skin. [b] Formulas for derived parameters (*10*) : $f_{dep} = h_{dep}/h_{sc}$; $C_{sat} = K_{sc}S_w$; $M_{sat} = h_{dep}C_{sat}$; $k_p = [(h_{sc}/(D_{sc}K_{sc})) + (h_{ed}/(D_{ed}K_{ed})) + (h_{de}/(D_{de}K_{de}))]^{-1}$; $k_g = 6320\ MW^{-1/3}\ u^{0.78}$; $k_{evap}\rho = k_g[(P_{vp}MW)/(0.76RT)]$; $\chi = h_{sc}k_{evap}\rho/D_{sc}C_{sat}$. [c] Not a true *a priori* value. Effective value of u was estimated from fitted evaporation rate in absence of a wider body of data under the occluded conditions.

TABLE 4. Parameters Resulting from Least Squares Fit of Diffusion Model to the Experimental Accumulation of Tecnazene in the Receptor Solutions (Figure 1)

parameter	units	correction factor[a]	
K_{sc}	-	5.36	
K_{ed} and K_{de}	-	15.9	
derived parameters[b]	**units**	**unoccluded**	**occluded**
f_{dep}	-	0.1	0.1
C_{sat}	$\mu g/cm^3$	3002	1052
M_{sat}	$\mu g/cm^2$	0.401	0.456
k_p	cm h^{-1}	0.143	0.349
χ	-	17.57	1.73
statistics	**units**	**unoccluded**	**occluded**
n	-	20	
s	% of dose	0.59	
r^2	-	0.993	

[a] Values in Table 3 were multiplied by the correction factor listed to obtain the fitted parameters. [b] Revised values based on the corrected values of the partition coefficients. Parameter values not listed are the same as Table 3. See Table 3 for formulas.

percentages of dose permeated, with the low dose having the higher percentage permeation. Following a time lag of 1−3 h, the permeation rates became nearly constant, peaking at 8−12 h postdose. At the lower dose, permeation slowed after 12 h (unoccluded) or 24 h (occluded). The decline in permeation rate was consistent with depletion of tecnazene from the skin, as evidenced by the solid lines in Figure 1(a) and 1(b). This phenomenon was not observed at the higher dose (Figure 1(c) and 1(d)).

The two model calculations are shown in Figure 1. The dashed curves represent calculations performed using the *a priori* parameters in Table 3. These curves are a true prediction, as only the tecnazene physical properties in Table 1 and previously established formulas (Supporting Information) were used to calculate the parameter values. The *a priori* calculation underestimated tecnazene permeation through the skin in each of the four test conditions by a factor ranging from 5.7 to 6.6. Examination of these results indicated that the discrepancy between model calculations and experiment could be largely eliminated by upwardly adjusting two of the model parameters, corresponding to the partition coefficients of tecnazene in the stratum corneum and viable epidermis/dermis layers. This choice was dictated by a combination of several factors: (1) Modification of the transport parameters in all skin layers was required to obtain a suitable fit to the data. (2) Since skin layer thicknesses are well-known and fairly consistent for broad body surfaces, the choices for each layer were diffusivity and partition coefficient. (3) Diffusivities for compounds the size of tecnazene in skin are well established by the current model calibration (*8, 9*). (4) Tecnazene is at the border of the current model calibration range in terms of lipophilicity. This reasoning made modification of the tissue/water partition coefficients the logical choice for obtaining a fit. Multiplicative correction factors for each partition coefficient were determined by the method of least-squares and are reported in Table 4. The values of these factors were 5.36 and 15.9 for stratum corneum and viable tissues, respectively. These are significant corrections that warrant attention prior to applying the diffusion model for dermal risk assessments of highly chlorinated aromatic compounds.

Discussion

According to the premises of the computational model (*5, 6*), the disposition of a volatile organic compound on skin, applied either neat or in an even more volatile solvent, can be estimated from the physical properties listed in Table 1. These properties are used to calculate the diffusivities (D_i) and partition coefficients (K_i) for each skin layer. The values of these parameters change with the hydration state of the skin. In the present study we considered the stratum corneum to be partially hydrated (water content ∼ 31% w/w) in the unoccluded experiments and fully hydrated (water content ∼ 73% w/w) in the occluded experiments. A 3-fold difference between these states in microscopic stratum corneum lipid-phase diffusivities is assumed in the model. The lower skin layers are not subject to this difference. The evaporative mass transfer coefficient k_{evap} is derived from a combination of physical properties and environmental factors of temperature (T) and wind velocity (u) according to the formulas in Table 3. The model employs the concept of a deposition region in the stratum corneum of thickness h_{dep} into which topically applied chemicals rapidly penetrate (*10*). The amount of the chemical required to saturate this region is designated M_{sat}, and the saturation concentration in the stratum corneum is C_{sat}. The ratio of the applied dose M_0 to M_{sat} distinguishes a small dose ($M_0/M_{sat} \leq 1$) from a large dose ($M_0/M_{sat} > 1$). Transient disposition on skin is largely a function of this ratio combined with the dimensionless volatility parameter χ (Table 3). Higher values of χ favor evaporation over absorption. For a simple, one-layer model with zero deposi-

tion depth the value $\chi = 1$ leads to equal amounts evaporated and absorbed (*10*); however, in a more complex model this relationship is not exact. The value of χ is nevertheless a guide as to the ultimate disposition of the compound; in engineering parlance it may be considered to be a Biot number for the system (*27*).

In the case of tecnazene permeation through skin, the two doses were similar in their behavior despite the difference in the doses. This can be explained by considering the ratio of the doses (M_0) of 103 and 864 μg/cm^2 to the M_{sat} values, which according to the fitted model are in the range 0.4 to 0.5 μg/cm^2 (Table 4). Thus, with regard to the saturation of the compound in the upper stratum corneum, both doses were large and differed only in the duration of absorption. Variations in the skin concentrations between the low and high doses, particularly those in the occluded samples, are not as easily explained and may be associated with the high levels of variability in the measurements.

The *a priori* model parameters in Table 3 led to an approximately 6-fold under prediction of tecnazene accumulation in the receptor solutions for all four cases (Figure 1, dashed curves). The calculated residual amounts in the skin tissues after 48 h (data not shown) were also substantially below the measured values given in Table 2. These results indicate that skin permeability to and binding affinity for tecnazene were significantly underestimated by the model. It was found that increasing the partition coefficient for tecnazene in the stratum corneum by a factor of 5.36 and that in the lower skin layers by a factor of 15.9 (Table 4) led to accurate estimates of receptor solution accumulation (Figure 1, solid curves); however, the tissue levels predicted by the fitted parameter set were still substantially below the measured values. Part of this difference may be ascribed to the presence of surfactant (1% Oleth-20) in the receptor solutions. Permeation of surfactant micelles into the dermis and viable epidermis very likely increased solubilization and, hence, the partition coefficient of tecnazene in these tissues relative to the physiological albumin solution plus a small amount of lipid (0.0007 volume fraction) assumed in the model (*9*). An estimate based on the solubility data in Table 1 indicates that surfactant solubilization could account for a 4- to 5-fold increase in K_{ed} and K_{de} versus values calculated from the current dermis partition model (*9*). However, there was too much tecnazene found in all skin layers to be solely accounted for by surfactant solubilization. It seems likely that tecnazene binds to skin tissues, perhaps irreversibly, in ways not considered in the model calculation. It is not discernible from this in vitro study whether such bound material would become systemically available following a comparable in vivo exposure. This question will surely warrant attention if the model is to be used for risk assessment.

The amount of lipid in the dermis partitioning model (0.0007 volume fraction) was chosen to match the experimentally determined dermis-water partition coefficients of 22 permeants having log octanol/water partition coefficients ranging from −3.24 to 4.06 (*9*). We assumed these lipids to be primarily phospholipids associated with cell membranes and to have partitioning properties similar to octanol. It is possible that by including a higher level of lipid in the dermis model (e.g., the 27 mg/g value measured by Pearce and Grimmer in postmortem human dermis (*29*)) and by the substituting of vegetable oil/water partition coefficients for octanol/water partition coefficients in the manner discussed by Poulin and Krishnan (*30*) a better fit to the entire data set including tecnazene might be obtained. It is furthermore possible that existing experimental dermis/water partition coefficients for highly lipophilic compounds are artificially low due to methodology problems associated with diffusible protein in the tissue. We are studying both of these possibilities.

Considering again the dermis, one significant anatomical feature that is not incorporated in the diffusion model is the pilosebaceous unit including hair follicle and sebaceous gland. Strictly speaking, the follicle is an invagination of the epidermis into the dermis; however, when skin layers are separated, most of the structure stays in the dermis. It can be a significant reservoir for lipophilic compounds (*31, 32*). According to the diffusion model, which assumes reversible binding of permeant to both extravascular albumin and dermis lipids, the largest amount of tecnazene present in the dermis at the 48 h study termination was 1.6 μg/cm^2, which corresponds to only 0.2% of the 864 μg/cm^2 dose. Yet the amounts found experimentally (Table 2) were 0.6% (unoccluded) and 20.5% (occluded). For the smaller dose the model predicts tecnazene to be almost completely cleared from dermis by 48 h (<0.03% of dose remaining), but levels of 1−4% were found in the tissue. It is likely that these differences will not be resolved without incorporating the pilosebaceous unit into the model. The importance of apocrine and eccrine sweat glands in dermal accumulation of lipophilic chemicals has not clearly been established; however, these appendages may also contribute to binding.

Another possible source of error in the *a priori* model calculation is the formulas used to estimate stratum corneum/water partition coefficients K_{sc}. The values given in Table 3 derive from a recent analysis of experimental partition coefficient data conducted in our laboratory (*24*). The data collected therein show large variability for highly lipophilic compounds. This phenomenon was previously noted by Anderson and co-workers, who showed that much of the variability could be explained by the different lipid contents of individual stratum corneum samples (*33, 34*). Based on the results reported here, it seems that the relationships for fully and partially hydrated K_{sc} given in ref *24* may have to be re-examined for highly lipophilic chemicals. It is important in the context of risk assessment that the calculations be conservative in nature, i.e., that any errors incurred tend to lead to higher estimated absorption and increased risk from a given exposure.

Since the primary route of human exposure to tecnazene is oral ingestion, it is unlikely the present results are directly applicable to tecnazene risk assessment. However, the diffusion model is applicable to a broad range of chemicals (*8*), and its predictive power for modeling transient in vitro exposures to the moderately lipophilic chemicals DEET (*4*) and benzyl alcohol (*21*) is acceptable. Calibration with volatile solvents is underway (*27*) and should benefit from comparison with existing skin:air partition coefficient data in full thickness skin (*35*). Because the numerical solution to the diffusion model yields a system of first-order ordinary differential equations, it is mathematically straightforward to couple it with physiologically based pharmacokinetic models (e.g. ref *36*), where it could serve as a sophisticated and flexible dermal input component.

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Supporting Information Available

Full details of the HS-SPME and GC/MS procedures and the mathematical formulas in the diffusion model. This material is available free of charge via the Internet at http://pubs.acs.org.

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