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Permeation of Water Contaminative Phenols Through Hairless Mouse Skin

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Abstract. As a means of determining the risk of absorption of water contaminative phenolic compounds through the skin, the permeation of a number of phenols, all on the U.S. Environmental Protection Agency's list of priority pollutants, through hairless mouse skin has been studied, using *in vitro* diffusion cell methods. Experimentally determined permeability coefficients through intact skin and stratum corneum denuded skin and permeability coefficients derived therefrom for the viable tissue layer and the stratum corneum, which are the tissue's major contributing substrata, have been correlated with their log $K_{octanol/water}$ partition coefficients. Permeability coefficients for the whole skin and the stratum corneum systematically increased with increasing phenol lipophilicity to limiting values of about 0.15 and 0.30 cm/hr, respectively. The values of the permeability coefficients for the viable tissue were roughly the same for all compounds (≈0.36 cm/hr). Because of the inductive effects of Cl and NO_2 substituents on the aromatic ring, phenolic analogs containing these moieties are acidic and, consequently, their overall skin permeabilities were highly pH-dependent in the range of pH values seen for surface waters. High fluxes were noted for such phenols at low pH, where they exist essentially in a non-ionized state. Though low, fluxes of the compounds were measurable at pH's $\gg pK_a$'s, indicating that phenolic anions also pass through the skin. With the exceptions of relatively polar phenol and the mono-nitro phenols, the free acid forms of all the phenols studied permeated skin with ease and at rates approaching those of denuded skin. The intact skin permeability coefficient of the free acid form of 4-nitro phenol was exceptionally low, which suggests that it might associate intermolecularly.

Certain toxic compounds are found as contaminants of industrial waste streams and migrate into the aquifer and national watershed. By and large, it is unclear what risk trace levels of these pollutants present to individuals who unsuspectingly drink or bathe in the contaminated waters. The relative risk of absorption of such agents through the skin is especially difficult to put into proper perspective as the kinetics of skin permeation are unknown for all but a few of the compounds of interest. More than 100 compounds, many of them phenols, have been identified by the United States Environmental Protection Agency as significant waterborne pollutants and the potential extent of skin absorption relative to the potential of uptake by other modes of exposure needs to be established.

The present study deals with pollutant phenols and was undertaken: (a) to determine the kinetics of skin permeation of phenol and certain of its analogs, (b) to investigate the pH dependency of the permeability of these compounds, (c) to put these compounds into a structure-permeability framework for eventual toxic risk assessment of all compounds of structural similarity and, finally, (d) to provide estimates of the possible skin uptake of the investigated compounds. The studies were made

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with hairless mouse skin as the model skin for the assessment of the permeation kinetics. Permeability studies, using whole and stripped skin, were done to determine the separate diffusional resistance contributions of the stratum corneum and viable tissue layer to the skin permeation of each compound.

Experimental

Materials

Phenolic compounds of analytical grade quality included phenol, 4-nitrophenol, 2,4-dinitrophenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4-dimethylphenol and 4-chloro-3-cresol. Tritium-labeled phenol and 4-nitrophenol were purchased and used as received. Reagent grade buffer chemicals included citric acid · $1H_2O$, disodium phosphate anhydrous, monosodium phosphate · $1H_2O$, sodium chloride, sodium hydroxide and hydrochloric acid. Citrate-phosphate buffers were prepared for solutions between pH 2.0–4.5 and phosphate buffers for solutions between pH 4.5 and 8.0.

The compositions of the buffers were as follows:

a) Citric Acid · $1H_2O$ 11.58 g
Na_2HPO_4 12.75 g
NaCl 0.35 mg
NaOH(2N) ~3.00 L
Distilled water, up to 1 L
HCl 6N was added, if necessary, to get a desired pH.

b) Phosphate buffers:
$NaH_2PO_4 \cdot H_2O$ 9.195 g
NaCl 4.45 g
NaOH 2N ~30 L
Distilled water, up to 1 L

HCl(6N) was added, if necessary, to get a desired pH. All prepared buffer solutions were iso-osmotic with physiologic fluids.

Skin Preparation

Hairless mice (SKH-hr-1 strain), 60–100 days old, were used in this study. After sacrificing a mouse by spinal cord dislocation, its abdominal skin was wiped clean with a tissue moistened with normal saline. Rectangular sections of whole skin (or skin stripped of its stratum corneum) several cm in each dimension were excised with surgical scissors. Adhering fat and other visceral debris were removed carefully from the undersurface with tweezers. The excised skin was trimmed into oversized circles and mounted in a two-compartment diffusion cell whose construction and operation have been detailed earlier (Durrheim *et al.* (1980)).

For some of the studies, the hairless mouse skin was stripped repeatedly (20 times) with cellophane tape to remove the stratum corneum. Substantial previous experience showed this to be sufficient stripping to functionally eliminate the stratum corneum's diffusional resistance. Each stripping was performed with fresh tape. The tape was placed firmly against the abdominal surface of a freshly sacrificed mouse and then peeled away. After the first several strippings, the skin became more and more an oozing surface with each succeeding peeling.

Skin Permeation Method

The skin sections as described above were mounted carefully between the half-cells of the diffusion cell and fastened with a spring clamp. The two half-cells were filled with normal saline, the whole system was immersed in a waterbath (37°C) and the stirring motors were connected. After the cell compartments were rinsed with normal saline, the donor compartment was charged with appropriate concentrations of the permeant; the complete donor solution was carefully removed and replaced with a concentration solution of a phenol (UV assays) or 100 μL was removed and replaced with an equal volume of a radiochemical concentrate. Stirring set at 150 rpm was initiated. At predetermined times, samples were withdrawn from the receiver compartment and analyzed by direct UV spectrophotometry, high pressure liquid chromatography (HPLC) with UV detection, or liquid scintillation spectrometry. Each sample was replaced with fresh saline with correction of the receiver concentration for the dilution of sampling. Samples were taken from the donor compartment at the beginning and end of each experiment to check for mass balance.

With the exceptions of phenol and 2,4-dinitrophenol, the phenols were assayed spectrophotometrically with a Beckman model DK-2A spectrophotometer and samples were withdrawn at predetermined times and absorbances measured at: 2-chlorophenol, 273 nm; 2,4-dichlorophenol, 245 nm; 2,4,6-trichlorophenol, 245 nm; *p*-chloro-m-cresol, 245 nm; 2-nitrophenol, 414 nm; 4-nitrophenol, 400 nm and 2,4-dimethylphenol, 414 nm. Samples were diluted for analysis, if necessary.

An HPLC assay system with a Waters model M-45 pump, Gilson model PWS2107FL holochrome detector and Micrometrics model 725 autoinjector was used to assay 2,4-dinitrophenol samples. The column was a reversed phase RP-18 (10 μm), 25 cm × 4.6 mm. The mobile phase was 50% acetate buffer (pH 4.3), 40% methanol and 10% water. 720 nL samples were withdrawn at predetermined times and assayed with necessary dilutions. Detection wavelength was set at 254 nm.

For radiolabelled phenol, 200 μL samples were withdrawn at predetermined times and added to 10 mL of aqueous counting scintillant (Amersham) and counts per minute were obtained with a Beckman LS 9000 liquid scintillation counter. For permeability-pH studies, the 4-nitrophenol was radiolabelled. The sample size and assay procedure were the same for phenol.

With the exception of permeability-pH studies with 4-nitrophenol, 2,4-dinitrophenol and 2,4,6-trichlorophenol, the receiver side pH was kept at 6.1, the pH of unbuffered normal saline. For the permeability-pH studies with 4-nitrophenol, 2,4-dinitrophenol and 2,4,6-tricholorophenol, the receiver side pH was maintained at 7.6 with phosphate buffer. With the exception of the pH-permeability studies, where donor pH was deliberately varied, the donor pH was maintained at pH < pKa of each compound with either citrate-phosphate or phosphate buffers.

Estimation of Permeability Coefficient in Whole and Stripped Skin from Experimental Data

Effective permeability coefficients (P_e, cm/hr) were calculated from quasi-steady state fluxes according to:

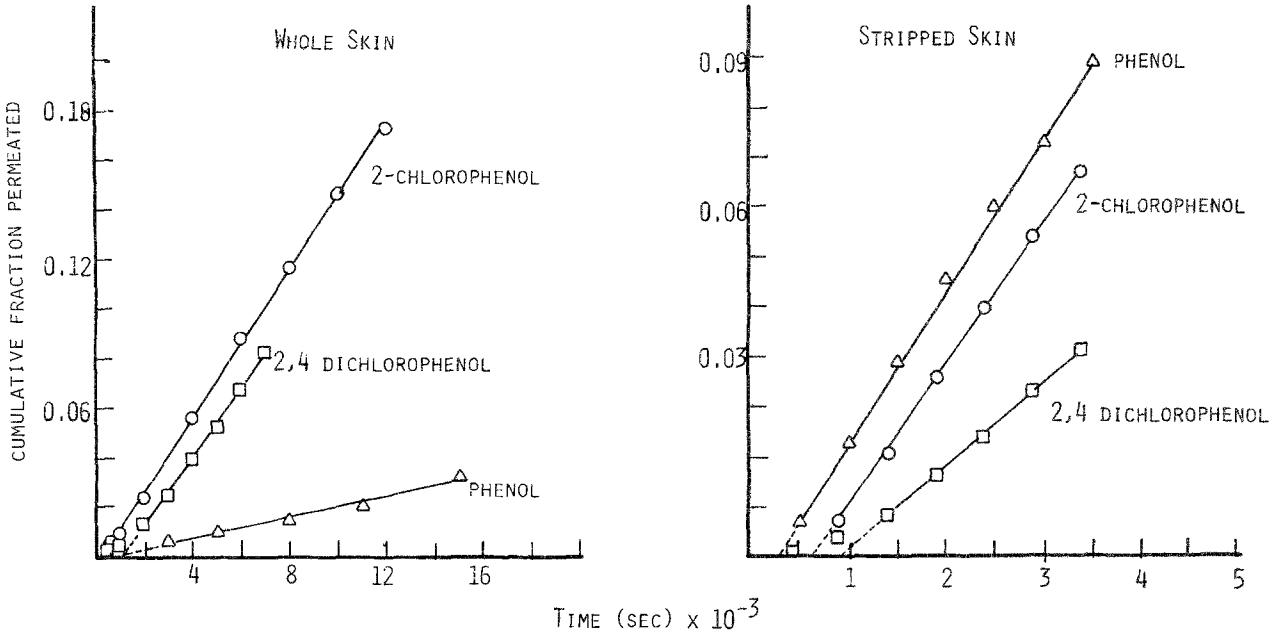

Fig. 1. Appearance kinetics of phenol and chloro-analogs in dilute solutions across whole skin and stripped skin; presented in terms of cumulative fractions of the applied phenols which have permeated to the receiver compartment

$$P_e = \frac{V}{A\,C_D} \cdot \left(\frac{dC_R}{dt}\right) \qquad \text{(Eq. 1)}$$

where

P_e = effective permeability coefficient
A/V = ratio of the diffusional area to receiver-side volume
C_D = initial concentration of permeant in the donor
dC_R/dt = steady-state appearance rate in the receiver

Sink conditions were maintained in the receiver by keeping the concentration at an inconsequential fraction of the donor concentration.

The steady-state exchange method developed previously for permeation studies with *n*-alkanol homologs (Flynn *et al.* (1981)) was also employed to obtain a separate estimate of the effective permeability coefficient 2,4 dichlorophenol. After multiple exchanges, effective permeability coefficients were calculated for the ninth exchange from donor depletion and receive accumulation data respectively from:

$$\ln \frac{C_{D,t}}{C_{D,O}} = -\frac{A\,P_e \Delta t}{V} \qquad \text{(Eq. 2)}$$

and

$$\frac{\Delta C_R}{\Delta t} = \frac{A\,P_e}{V}\,(\bar{C}_D - \bar{C}_R) \qquad \text{(Eq. 3)}$$

where

$C_{D,O}$ $C_{D,t}$ = donor concentrations at the beginning and end of each exchange, respectively
$\bar{C}_D$, $\bar{C}_R$ = average donor and receiver concentrations during a given exchange, respectively
$\Delta C_R/\Delta t$ = increase in receiver concentration per exchange period
Δt = exchange interval

Results

Figure 1 shows the appearance of phenol and two chlorophenols in the receiver chamber with time. The kinetic profiles are typical for the range of chemical pollutants covered within this report. Steady states in permeation were reached in time and permeability coefficients were calculated from the steady state (technically, quasi-steady state) fluxes. In theory, retention of permeant within a membrane must be small and insignificant for one to calculate true permeability coefficients from quasi-steady state data; it was suspected that this condition might not be met for the more lipophilic phenols. Therefore, an exchange method, developed previously for homologous alkanols (Flynn *et al.* (1981)) which effectively cancels the effects of membrane permeant retention, was used to independently assess the permeability coefficient of 2,4-di-chlorophenol (Figure 2). Steady rates of membrane uptake and output were obtained after multiple exchanges. Moreover, mass balance analysis indicated the amount of solute leaving the donor chamber of the diffusion cell was essentially equivalent to the amount appearing in the receiver chamber after a half dozen exchanges. Effective permeability coefficients calculated for the ninth interval from donor depletion and receiver accumulation were 0.40 and 0.32 cm/hr, respectively. The average of these, 0.36 cm/hr, was several-fold larger than the value of 0.125 cm/hr determined by

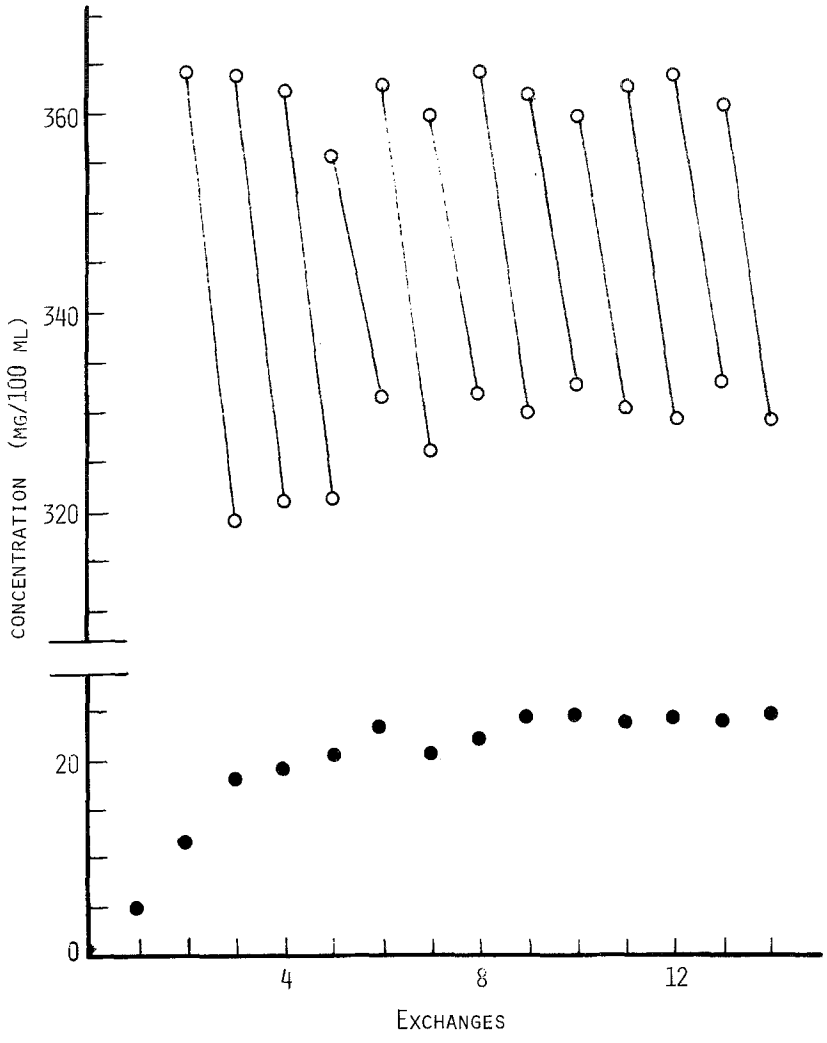

Fig. 2. Exchange method for 2,4-dichlorophenol over 2,000 sec intervals. Data (○) connected with a line represents the concentrations of the donor side at the beginning and end of each interval. Single point (●) represents the concentration of the receiver side of the end of an interval

the standard permeation procedure (Table 1). Although this indicated that sufficient 2,4-di-chlorophenol was retained in the skin to effect the estimated permeability coefficient by the conventional method, the effect of retention was not exaggerated. Since actual absorption rates of the phenols will also be affected by membrane retention, conventionally derived permeability coefficents are, nevertheless, the most appropriate for risk estimation.

Permeability coefficients through intact hairless mouse skin obtained under the condition that pH < pK for each phenol are given in Table 1; studies for several of the phenols were performed as a function of concentration initially placed in the donor chamber. Similarly, data for denuded skin (20 times stripped skin) are given in Table 2. Tables 1 and 2 show that, except where pH-effects themselves were being explored, permeability coefficients were assessed for stripped and whole skin under conditions which kept ionization to a minimum. Consequently, the bulk of the data represent intrinsic permeabilities of the non-electrolyte forms. Unbuffered normal saline was generally used as the receiver medium; any receiver ionization favorably influenced sink conditions and, therefore, could be ignored. Data gathered to explore the pH-permeability sensitivities of 4-nitrophenol, 2,4-dinitrophenol and 2,4,6-trinitrophenol are given in Table 3. These data were obtained with the receiver buffered to pH 7.6 (phosphate), which distinguishes them from the data in Tables 1 and 2, for which the receiver medium was unbuffered saline, nominally at a pH of 6.

An early concern in this work was whether the phenols could enhance their own permeabilities by denaturing the stratum corneum, as is known to happen with phenol (Roberts *et al.* (1977, 1978) and Behl *et al.* (1983). At trace radiolable concentrations, phenol's permeability coefficient (Table 1) was the same as previously reported (Behl *et al.* (1983)) but, as also shown previously, phenol at

Table 1. Permeability of phenolic compounds through whole skin

Permeant	pK_a[a]	Donor[d] pH	Concentration (g/100 mL)	Permeability coefficient, P_e, Apparent (±s.d.) × 10^3 (cm/hr)	Average lag time (min)
Phenol	9.92	5.82	3.30	171. (12.3)	10.3
		6.19	Trace	18.8 (3.0)	10.5
4-Nitrophenol	7.15	3.46	0.05 radiochemical assay	12.7 (2.8)	35.3
			0.50 UV assay	20.9 (1.2)	30.3 (6.4)
			0.05 UV assay	23.1 (2.2)	8.3 (3.3)
2-Nitrophenol	7.17	3.46	0.05	101. (6.9)	4.2
2-Chlorophenol	8.49	5.25	0.50	140. (13.5)	6.3
		5.50	0.19	116. (9.5)	10.3
		5.74	0.05	107. (10.5)	21.9
2,4-Dichlorophenol	7.85	5.15	0.40	125. (5.6)	18.9
		5.15	0.40	361.[c]	—
		5.30	0.20	107. (9.0)	23.2
		5.54	0.05	115. (12.7)	32.0
2,4-Dinitrophenol	3.96	2.0	0.05	151.	21.5
2,4-Dimethylphenol	10.59[b]	6.31	0.05	110.	10.1
4-Chloro-3-methyphenol (p-chloro-m-cresol)	9.56[b]	6.18	0.05	119. (1.8)	35.8
2,4,6-Trichlorophenol	6.0	5.0	0.05	174. (1.0)	29.7

[a] Literature values found in Barlin and Perrin (1966)
[b] Calculated from Hammett σ constants
[c] Average of the two methods of assessment by the exchange method (see text)
[d] The receiver pH was 6.2 in all experiments (pH of the unbuffered, normal saline)

Table 2. Permeability coefficients of phenolic compounds through stripped skin

Permeant	Concentration (g/100 mL)	Donor[a] pH	Permeability coefficient of Stripped Skin, P_{ss} (±s.d.) × 10^3 cm/hr	Lag time (min)
Phenol	Trace	6.19	264 (22)	3.7
4-Nitrophenol	0.500	3.4	173 (27)	6.7
	0.050	3.46	257 (11)	2.7
2-Chlorophenol	0.496	5.25	214 (30)	8.5
	0.186	5.50	276 (28)	5.5
	0.049	5.7	253 (4.7)	11.5
2,4-Dichlorophenol	0.400	5.15	136 (15)	3.3
	0.050	5.54	154 (23)	16.9
4-Chloro-3-cresol	0.055	6.2	241 (22)	14.6
2,4-Dinitrophenol	0.041	2.0	44 (34)	2.6

[a] The pH values are the same as for the whole skin when identical concentrations were used. As with whole skin, the receiver pH was 6.2, the pH of unbuffered normal saline, in all cases

high concentration, in this case 3.3% W/V, permeated roughly ten times faster due to horny layer impairment. In contrast, the permeability coefficient of 2-chlorophenol was assessed over a tenfold range of concentration with an upper concentration of 0.5%. While the value of 0.140 cm/hr at 0.5% is significantly greater than the value of 0.107 cm/hr at 0.05% (t-test, $0.1 > P > 0.05$), the absolute difference was not large. The value obtained at 0.19% falls between these values and is not significantly different from either. For 2,4-dinitrophenol, the 0.4% solution permeability coefficient, 0.125 cm/hr, is significantly greater than the 0.2% value, 0.107 cm/hr ($0.025 > P > 0.125$) and the 0.05% value of 0.115 cm/hr ($0.15 > P > 0.1$). Despite these differences, the effect of concentration on permeability is nominal, no doubt due to the fact that these latter phenols experience relatively little diffusional resistance in the unimpaired stratum corneum to begin with. Because of the limited nature

Table 3. Permeability of selected phenols as function of pH

Permeant	pK[a]	Donor[b] pH	Concentration (g/100 mL)	Apparent P_e (±)s.d. × 10^3 cm/hr
4-Nitrophenol	7.15	3.46	0.05	12 (2.8)
		6.20	0.05	11 (1.3)
		7.56	0.05	7 (0.7)
		10.16	0.05	0.5 (0.1)
2,4-Dinitrophenol	3.96	2.0	0.05	151
		3.5	0.05	116
		3.5	0.05	105 (18.2)
		4.35	0.05	50.6
		4.65	0.05	32.6 (2.3)
		6.0	0.05	3.15
		7.7	0.05	0
2,4,6-Trichlorophenol	6.0	5.0	0.05	174
		6.0	0.05	87
		7.4	0.05	40.9 (4.0)

[a] Literature values found in Barlin and Perrin (1966)
[b] Receiver pH was maintained at 7.6 with phosphate buffer

of these effects, it was decided that no appreciable concentration artifact would be introduced, so long as the concentrations of the phenols were kept low, and all subsequent work was at 0.05% or lower concentration.

Lag times are recorded in Tables 1 and 2. These were determined by extrapolating the steady state lines to the time axis in the usual way. Such values are notoriously sensitive to experimental error and, therefore, must be considered very approximate. In addition, while lag times have a straightforward relationship to diffisivity for simple isotropic membranes in the absence of boundary layer influences, the interdependencies of lag times, diffusion coefficients and sorptive binding constants in membranes as complex as the skin is totally obscure. Therefore, no attempt was made to reduce lag times to effective diffusivities. It is merely pointed out that lag times for the phenols are of short duration, with a few marginally longer than 30 min and most less than 20 min. This simply means diffusive gradients of the phenols are quickly established across the skin and the onset of absorption is well within the usual times swimmers spend in a pool or lake and even within the times taken by some to bathe or shower.

Discussion

The present work concerns the permeability of known water contaminative phenols through skin and potential risk which attends bathing, swimming, or other direct skin contact with phenol-polluted water. In making the risk assessments for the phenols, one has to account for the weak electrolyte nature of phenols in general and for the possible ability such compounds have to impair the skin barrier as revealed in other work (Roberts *et al.* (1977, 1978) and Behl *et al.* (1983)). Thus, aspects of the studies involve assessing pH effects on permeability and, to a more limited extent, concentration dependencies of permeability of some phenolic prototypes. Special emphasis has been placed on how permeability relates to lipophilicity of the free acid forms of the respective phenols.

Strata Permeability and Relationship to Permeant Lipophilicity

For all of the phenols, except 2,4-dimethylphenol and 2,4,6-trichlorophenol, data were obtained on skin sections from which the stratum corneum was removed by adhesive tape stripping (Flynn *et al.* (1981)) (Behl *et al.* (1983, 1984)). Permeability coefficients obtained on stripped skin were larger than found for whole skin but the degree to which removal of the horny layer raised the permeability was exceedingly variable. Phenol and 4-nitrophenol experienced 15-fold increases in permeability coefficients while, at the other extreme, the permeability coefficients of 2-chlorophenol, 2,4-dichlorophenol and 4-chloro 3-methylphenol were less than doubled.

The permeability coefficient of the stratum cor-

Table 4. Permeability coefficients for whole skin, viable tissue and stratum corneum

Permeant	Experimental permeability coefficient, whole skin $P_e \times 10^3$ (cm/hr)[a]	Estimate of viable tissue permeability coefficient $P_{vt} \times 10^3$	Estimated stratum corneum permeability $P_{sc} \times 10^3$ (cm/hr)	Log PC[b]
Phenol	18.8	338.	20.2	1.46
2,4-Dinitrophenol	151.	705.	228.	1.52
2-Nitrophenol	101.	—	—	1.77
4-Nitrophenol	22[d]	327.	25.4[d]	1.96
3-Nitrophenol	—	—	—	2.00
2-Chlorophenol	106.	320.	182.	2.15
2,4-Dimethylphenol	110.	—	—	2.30
4-Chlorophenol	—	—	—	2.39
2,4-Dichlorophenol	115. (361.)[c]	177.	453. (*)	3.08
4-Chloro-3-cresol	119.	302.	235.	3.10
2,4,6-Trichlorophenol	174.	—	—	3.69

[a] These values not adjusted for boundary layer effects
[b] Intrinsic partition coefficients in n-octanol/water from Hansch and Leo (1979)
[c] Derived from exchange method
[d] Based on UV results
* P_{sc} could not be calculated directly but is at least 20× greater than P_{vt}

neum can be assessed by difference from the two sets of data and knowledge of the total boundary layer resistance of the diffusion cell. The latter is known (Flynn *et al.* 1981) for the cells in question. The total resistance of the laminate, the reciprocal of the effective permeability coefficient, P_e, is described by a series of resistances as (Flynn *et al.* (1981)):

$$\frac{1}{P_e} = \frac{1}{P_{sc}} + \frac{1}{P_{vt}} + \frac{2}{P_{aq}} = \frac{1}{P_{sc}} + \frac{1}{P_{ss}} \qquad \text{(Eq. 4)}$$

where

P_e = effective permeability coefficient of the skin found in Table 1
P_{ss} = permeability coefficient of the stripped skin found in Table 2
P_{sc} = permeability coefficient of the isolated stratum corneum layer
P_{vt} = permeability coefficient of the isolated viable tissue layer
P_{aq} = permeability coefficient of an aqueous boundary layer on both sides of the skin

The P_{aq} value determined previously is 2.4 cm/hr. Note the actual permeability coefficient of the viable tissue layer, P_{vt}, is estimated from the permeability coefficients of stripped skin given in Table 2 by correcting them for the aqueous boundary layers. One estimates P_{sc}, the stratum corneum permeability coefficient, directly from the difference in whole skin and stripped data. The overall permeability coefficient and the derived values for P_{sc} and P_{vt} are given in Table 4 and also plotted in a log-log fashion against literature n-octanol/water partition coefficients of the respective phenols (Hansch and Leo (1979)) in Figure 3. It is evident from the widely spaced positions of P_{sc} and P_{vt} of phenol and 4-nitrophenol that permeability of these two compounds is controlled by the stratum corneum.

Estimated permeability coefficients for the viable tissue evidence some variability but are centered around 0.36 cm/hr, consistent with past experience with low molecular weight alkanols (Flynn *et al.* (1981)). It is notable, both from Table 4 and Figure 3, that the permeability coefficients of the viable tissue and stratum corneum are of comparable magnitude for the more hydrophobic phenols. This is in accord with past experience (Flynn *et al.* (1981)) (Roberts *et al.* (1978)). It is evident that for the more hydrophobic phenols, the living, cellular epidermis and the dermis, limit absorption. These living layers also exert the sole resistance and offer a minimal protection from absorption of all compounds across damaged skin. The actual limiting permeability coefficient of the living stratum in man is likely to be 2 to 3 times higher than seen in these studies, or about 1 cm/hr, due to the fact that blood flowing through the superficial capillary plexus effectively reduces the thickness of the living tissue to be diffusionally negotiated.

With the exception of data for the nitrophenols, the overall data fit a pattern which suggests the rate

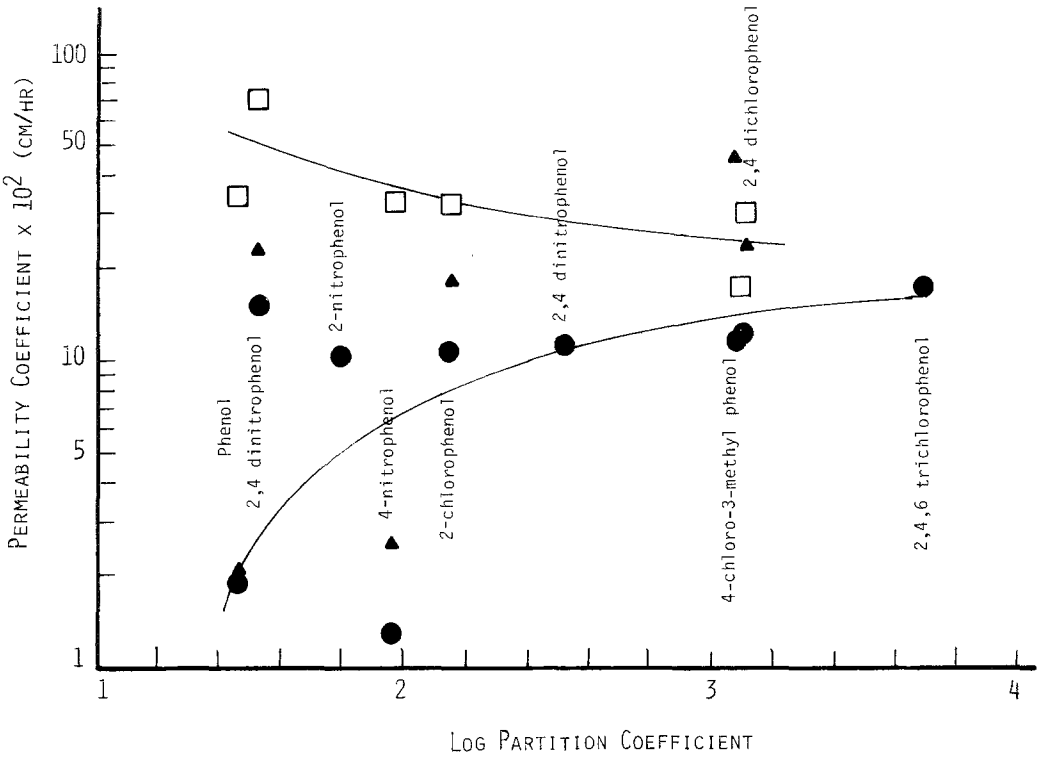

Fig. 3. Plot of log (permeability coefficient) vs log (partition coefficient, *n*-octanol/water); ●, whole skin; ▲, stratum corneum; and □, viable tissue. There is no appreciable partitioning dependency to the permeability coefficients obtained on tape denuded skin. However, the permeability coefficients of whole skin of the more polar phenols, phenol and 4-nitrophenol, are much lower than for the denuded, a sure sign of stratum corneum control. Stratum corneum permeability coefficients are calculated by difference

controlling portion of the skin membrane systematically shifts from the stratum corneum to the living tissues as the compounds become more hydrophobic. Previously, Roberts *et al.* (1978) showed with human epidermis that alkanol and phenol data are internally consistent and, when taken together, describe this transition well. Neither the 2,4-dinitro phenol nor the 4-nitro phenol permeability coefficient fits comfortably into their scheme however, the former being too high and the latter too low based on their positions on the octanol/water scale of polarity. It is impossible to say at this time why these two compounds are outliers to what is otherwise a highly systematic pattern. Octanol/water partition coefficients may not properly reflect the lipophilicities of this pair with respect to the lipoidal phases of the skin. Self-association of the phenols in octanol and/or water or in the skin's phases might also account for their anomalous behavior.

Permeability/pH Relationships

In recognition of the strong inductive electronic effects of Cl and NO_2 substituents on the acidities of phenols, 4-nitrophenol (pKa 7.15), 2,4-dinitrophenol (pKa 3.96), and 2,4,6-trichlorophenol (pKa 6.0) were selected as particularly useful phenol analogs to explore pH influences on permeability. Dissociations of these particular phenols will be more influenced by the natural or man-induced variations in surface water pH than will dissociations of phenols which have pKa values greater than 8.0. When these phenols were applied to the skin in buffered solutions covering a range of pH's bracketing the respective pKa values, they became less permeable as pH was increased (Figure 4), indicating ionization hinders mass transfer. Intrinsically lipophilic, acidic 2,4-dinitrophenol was highly permeable at pH 3 and nearly impermeable at pH 7. A 4-fold change in effective permeability coefficient for the highly lipophilic and less acidic trichlorophenol between pH 5 and 7.5 was noted; however, the permeability at pH 7.5 was still high. While permeability drops off more or less steeply as ionization of the phenols is induced, there is still measurable flux at the highest pH values studied. Apparently, on this basis, there is a limited flux of phenolic anions, although the data are not sufficient to factor out the contributions of free and ionic species to the flux. This suggests there is a means

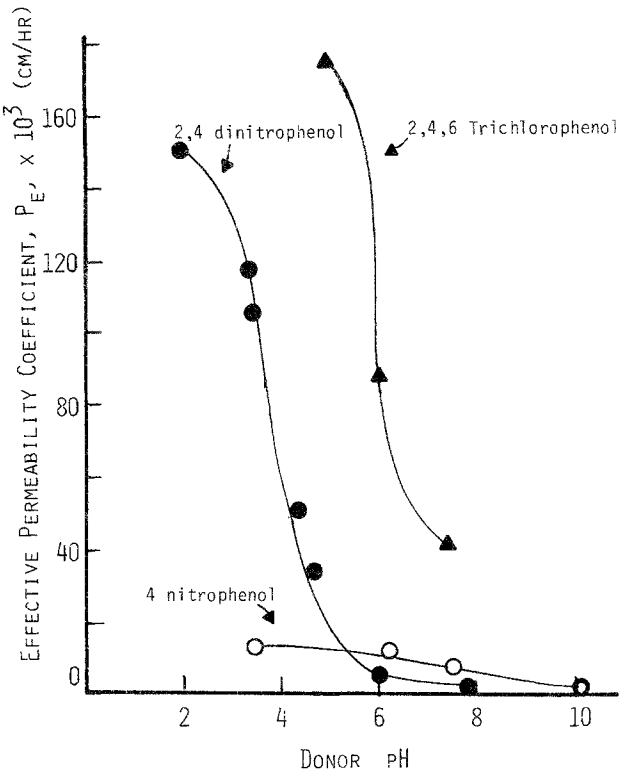

Fig. 4. Effective permeability coefficient of whole skin vs donor pH. Receiver side pH is alkaline. Intrinsic partition coefficients were determined in n-octanol/aqueous solution (pH ≫ pK_a), *i.e.*, 1.96 for 4-nitrophenol (○); 1.52 for 2,4 dinitrophenol (●); and 3.69 for 2,4,6-trichlorophenol (▲)

through the horny layer which does not involve the equivalent of oil/water partitioning.

Comparison of Mouse Data With Human Data and Risk Projections

The principal purpose of this study was to gather basic data for estimation of human risk of absorption of USEPA identified phenolic pollutants. It was pointed out that lag times are short and therefore steady states of permeation will likely be obtained by the compounds during typical periods of water exposure. It is reassuring that the obtained intrinsic (free acid) permeability coefficients for the hairless mouse (model) are in accord with similar data obtained by others (Roberts *et al.* (1978)) with human epidermis (Table 5). While the overlap between studies involves only four compounds, it is readily seen that all values are of similar magnitude with no mouse parameter being more than a factor of two different from its human epidermal counterpart. Therefore, it seems safe to project a limiting permeability coefficient through intact human skin

Table 5. Comparison of permeability coefficients for various phenol analogs in hairless mouse and human skin at 37°C

	$P_e \times 10^3$ cm/hr	
Permeant	Hairless mouse	Human[a]
Phenol	18.8	21.1
2-Chlorophenol	106.5	61.9
2,4,6-Trichlorophenol	174.0	108.
4-Chloro-3-cresol	118.9	108.

[a] Calculated from literature values (Behl *et al* 1984; Morrison and Boyd 1959)

for the phenols on the order of ~0.15 cm/hr. The permeability coefficients may be lower than this by a factor of ten or more if the phenol is polar or ionized. When the skin is denuded (stripped), the O/W partitioning dependency of phenolic permeability is completely lost and permeability coefficients of ~0.36 cm/hr are seen. Therefore, a value of 0.5 cm/hr can be taken as a safe, conservative upper permeability coefficient limit to use in risk analysis. This value leads one to the conclusion that after about a half hour's exposure an upper, attainable whole body absorption rate of a human adult totally immersed in water contaminated with 1 ppm phenol would be approximately 10 mg/hr or almost 250 mg/day. This estimate assumes an adult surface area of 2 m^2, which would be for a large (~200 lb) adult. Reduction of either the involved area, the contaminating concentration of the phenol, or the phenolic permeability coefficient produces exactly proportional reductions in the amounts absorbed. For example, the total body area absorption rate of a 1 ppm solution of phenol estimated from phenol's permeability coefficient determined in this research would be 0.38 mg/hr or 9 mg/day.

One last observation of possible significance to toxicity of the phenols is that, using HPLC, they were not metabolized or conjugated during their diffusive transport through the skin. They are known to be partially converted in the gastrointestinal mucosa to more easily eliminated, less toxic conjugates as glucuronides after their oral ingestion. Therefore, it is likely that amounts absorbed through the skin are more toxic than equivalent amounts by the oral route. Not only does this have bearing on the assessment of toxicity of phenols, but in a general way, it shows that the toxicologic data upon which the risk assessments are to be based must be evaluated for their appropriateness relative to the route of absorption.

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References

Barlin GB, Perrin, DB (1966) Prediction of the strengths of organic acids. Quart Rev 20:75–100

Behl CR, Flynn GL, Linn EE, Smith WM (1984) Percutaneous absorption of corticosteroids: Age, site, and skin-sectioning influences on rates of permeation of hairless mouse skin by hydrocortisone. J Pharm Sci 73:1287–1290

Behl CR, Linn EE, Flynn GL, Pierson CL, Higuchi WI, Ho NFH (1983) Permeation of skin and eschar by antiseptics I: Baseline studies with phenol. J Pharm Sci 73:391–397

Durrheim H, Flynn GL, Higuchi WI, Behl CR (1980) Permeation of hairless mouse skin I: Experimental methods and comparison with human epidermal permeation by alkanols. J Pharm Sci 69:781–785

Flynn GL, Durrheim H, Higuchi WI (1981) Permeation of hairless mouse skin II: Membrane sectioning techniques and influence on alkanol permeabilities. J Pharm Sci 70:52–56

Hansch C, Leo AJ (1979) Substituent constants for correlation analysis in chemistry and biology, John Wiley & Sons, New York, 339 pp

Morrison RT, Boyd RN (1959) Organic chemistry. Allyn and Bacon, Boston, pp 585–588

Roberts MS, Anderson RA, Swarbrick J (1977) Permeation of human epidermis to phenolic compounds. J Pharm Pharmacol 29:677–683

Roberts MS, Anderson RA, Swarbrick J. Moore DE (1978) The percutaneous absorption of phenolic compounds: The mechanism of diffusion across the stratum corneum. J Pharm Pharmacol 30:486–490

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