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REVIEW OF DERMAL ABSORPTION

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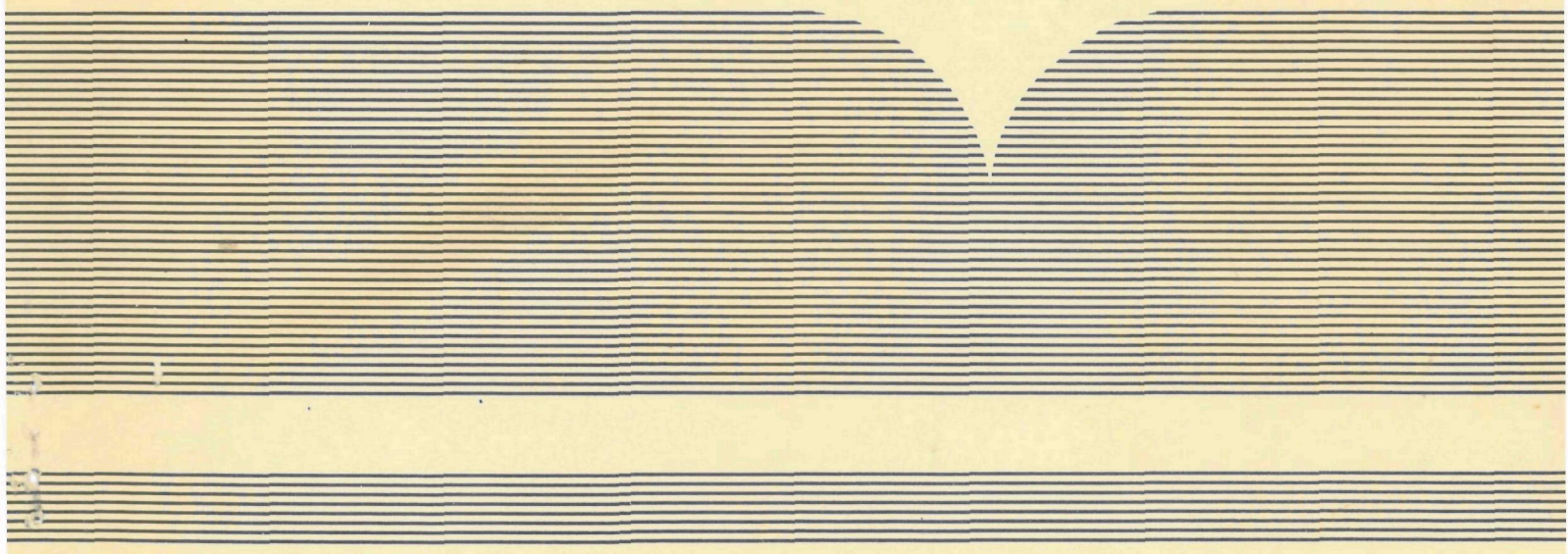

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REVIEW OF DERMAL ABSORPTION

by

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Office of Health and Environmental Assessment

Office of Research and Development

U.S. Environmental Protection Agency

Washington, DC 20460

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FOREWORD

The Exposure Assessment Group of EPA's Office of Research and Development has three main functions: 1) to conduct exposure assessments; 2) to review assessments and related documents; and 3) to develop guidelines for Agency exposure assessments. These exposure assessments are critical to the evaluation of the public health risks that are presented by toxic substances. The activities under each of these three functions are supported by and respond to the needs of the various EPA program offices. In relation to the third function, the Exposure Assessment Group conducts projects for the purpose of developing or refining techniques used in exposure assessments.

The current project was initiated to provide a review of human dermal absorption since this is a mechanism by which toxicants can enter the body. To properly conduct an exposure assessment, all routes of exposure should be determined. Besides reviewing current dermal absorption studies, future research needs are addressed which could further elucidate the methods needed for dermal exposure calculations. The Exposure Assessment Group eventually hopes to provide similar reviews for all routes of exposure.

James W. Falco, Ph.D., P.E.
Director
Exposure Assessment Group

ABSTRACT

Dermal absorption is one of the three main routes of human exposure. This report is a review of the current dermal absorption literature with an emphasis on applications for exposure assessments. The structure of the skin is described in the first technical chapter as well as factors such as occlusion or abrasion which affect absorption rates. The next chapter presents comprehensive tables of *in vivo* human dermal studies, *in vivo* animal studies, and *in vitro* results using either excised animal or human skin. Theoretical treatments of dermal absorption are discussed next, including kinetic expressions for various dermal exposure situations and ways to predict absorption using partition coefficients. Following this theoretical review some calculations of typical dermal exposure scenarios are presented such as chemicals in swimming pools or reentry into pesticide treated fields. Lastly, an analysis of some data gaps in dermal absorption knowledge is coupled with suggestions for future research.

1. INTRODUCTION

Three main routes of human exposure to toxic compounds have been identified, namely inhalation, ingestion, and dermal penetration. It is recognized that for certain scenarios, such as farm worker reentry following pesticide application or occupational handling of pure liquids or solids, exposure via the dermal route is the most critical exposure pathway. In the past, when the Agency was faced with estimating the dermal absorption of a compound without the support of scientific studies, a value of 10 percent absorption was assumed. Recently, the Office of Pesticide Program's Scientific Advisory Panel stated that if no literature was available on a compound to show otherwise, then a value of 100 percent of the penetrant on the skin should be used to represent the dermal absorption. This value of 100 percent is a conservative approach and leads to the overestimation of actual dermal absorption for most compounds. The intent of this document is to review the current state of knowledge of dermal exposure focusing on ways to assign more realistic values for absorption (i.e., <100 percent).

2. CONCLUSIONS

The review of the literature on dermal absorption presented in this report has brought out a number of factors which help to explain how dermal absorption of a penetrant occurs. It has been shown that the thin outermost layer of skin, the stratum corneum, is the rate limiting membrane for diffusion. For most penetrants, absorption through the general skin surface is the preferred route over "holes" in the skin caused by hair follicles and sweat ducts. The site of dermal exposure is important too as studies have shown up to a ten-fold difference in absorption rate depending on where on the body a penetrant is applied. There are a number of other factors which can affect the amount of penetrant absorption such as condition of the skin, occlusion or covering of the applied dose, frequency of application, metabolism of a penetrant by the skin, and the solvent or formulation used to deliver a penetrant to the skin.

In vivo studies in man have been done for approximately 50 chemicals, mostly pesticides and steroids. Studies have shown up to a 5-fold variation in skin absorption between subjects. Toxicity concerns prohibit further testing in man, thus in vivo studies are done on animals. There is a large variation in penetrant absorption in the animal species tested with the rhesus monkey and miniature swine appearing to be closest to man. In vitro studies using either excised human or animals skin give qualitative agreement with in vivo results and show the potential for quantitative use.

Attempts have been made to predict dermal absorption from partition coefficients, particularly with octanol/water or olive oil/water. It has been found, however, that a compound has to be both lipid soluble and water soluble to be a good penetrant. The solubility of the penetrant within the stratum

corneum relative to its solubility in the vehicle (i.e., the membrane partition coefficient, K_m) is a better indicator of permeability than octanol/water or olive oil/water partition coefficients. Values for K_m have been used to predict the permeability of the linear primary alcohol series with good success.

The absorption rate is just one factor needed to calculate dermal intake for an exposure assessment. In addition, one needs to know the duration of the exposure per each event, the frequency of events expressed in number of exposures per some unit time, the ambient concentration of the penetrant as a function of time plus the medium or vehicle in which it is applied to the skin, and the area of exposed skin. The uncertainty contained in these factors is large and may necessitate the use of approximations.

3. RECOMMENDATIONS

One stated goal of this review is to work towards assigning a more realistic value for the percent absorption of an untested penetrant rather than the current conservative approach of using 100 percent. Since so few compounds have been tested *in vivo* in man and toxicity concerns prevent the testing of many compounds, it is necessary to study penetrants by *in vivo* animal tests, *in vitro* animal or human tissue tests, or by calculating potential absorption from partition coefficients or other physical parameters. In order to work towards the goal of being able to assign a value of less than 100 percent for the absorption rate of an untested penetrant, it is recommended that:

- A data base be established of K_m and diffusivity values for a number of chemicals representing the various chemical classes.

- Quantitate the factors affecting absorption such as vehicle, site of application, condition of the skin, occlusion, frequency of application, and metabolism. In particular, the effect of the carrier solvent (vehicle) on the rate of absorption should be determined by measuring the rate for a pure penetrant and then dissolving the penetrant in a number of solvents (such as those commonly found in industry).

- Develop kinetic expressions to represent the various dermal exposure situations that occur such as finite amount of a fast penetrant, finite amount of a slow penetrant, excess of a penetrant where the diffusion through the stratum corneum is not rate limiting, buildup of penetrant in blood such that the concentration (C_0) is $\neq 0$, etc. Also, it is necessary to develop procedures for identifying which kinetic expression is appropriate for use with a given penetrant.

- Determine the most suitable animals species (probably the rhesus monkey or miniature swine, or rat because of ease of handling and economy) and do all animal *in vivo* work only on this species. The relationship between *in vivo* in man versus *in vivo* in animals should be established by comprehensive testing of penetrants that have already been studied in man.

- For *in vitro* work, decide from what part of the body tissue should be taken (forearm, back, stomach). Investigate problem of low water

solubility penetrants by using solvent/water or straight solvent for the receptor cell (the bottom part of the diffusion cell apparatus). Work on making this method more quantitative so that in vitro results can substitute for in vivo results. Also, the relative permeability of all body tissue that could potentially be exposed should be measured and correlated to the body tissue area selected for the in vitro studies.

- Establish the relationship between dermal absorption and the various partition coefficients, particularly K_m and $K_{octanol}$, and see if the methods used to calculate partition coefficients such as structure-activity, number and kind of functional groups, etc., can be used to calculate dermal absorption directly.

- Determine the feasibility of grouping penetrants into a numerical system like 100-10-1-.1% absorption depending on in vivo or in vitro studies, or physical parameters like K_m or other partition coefficients.

It may be possible to develop a model which includes all possible parameters affecting dermal intake based on the results of the recommended studies. One could store in the model such factors as: skin area for total body and for each body region depending on age, sex, and body weight; thickness of skin for different body regions; differences in percent or rate of absorption (based on some unity scale) for various body sites; kinetic expressions to determine the absorption for a range of scenarios; representative K_m and diffusion constants for chemical classes and/or functional groups, etc. Then one could enter data specific for a particular exposure, such as penetrant partition coefficients, volatility, concentration, and number and kind of functional groups, vehicle (including any binding to soil, or other substrates), contact time, skin area plus where on the body, etc., and have the model calculate the dermal intake.

4. SKIN AS A BARRIER TO ABSORPTION

4.1 STRUCTURE OF SKIN

Skin is one of the largest organs of the body comprising approximately 10 percent of the normal body weight. The skin consists of two different layers with the thinner outer layer known as epidermis and the thicker, inner layer as dermis (Fig. 1). Although skin thickness varies with location in humans, the epidermis is approximately 0.1 mm and the dermis 2-4 mm thick (Rongone, 1983). The outermost layer of the epidermis is called the stratum corneum and is from 10 to 50 um thick. In this document we are mainly concerned about the stratum corneum as it has been shown that the stratum corneum is at least three, and frequently as much as five, orders of magnitude less permeable to most substances as the dermis (Michaels et al., 1975). Also, the permeability of the entire epidermis is indistinguishable from that of the stratum corneum alone. Thus, the skin can be thought of as a three layer laminate of stratum corneum, remainder of the epidermis, and dermis with permeation occurring by diffusion through the three layers in series.

The stratum corneum is a heterogeneous structure containing about 40% water, about 40% protein (primarily keratin), and about 15 to 20% lipids (mainly triglycerides, free fatty acids, cholesterol, and phospholipids) (Michaels et al., 1975 and Anderson and Cassidy, 1973). The stratum corneum can absorb up to six times its own weight in water, and in its fully hydrated state, its permeability to water and other low molecular weight penetrants is increased (Scheuplein, 1967). The lipid components of the stratum corneum may be the chief reason for its uniquely low permeability as when this tissue is extracted

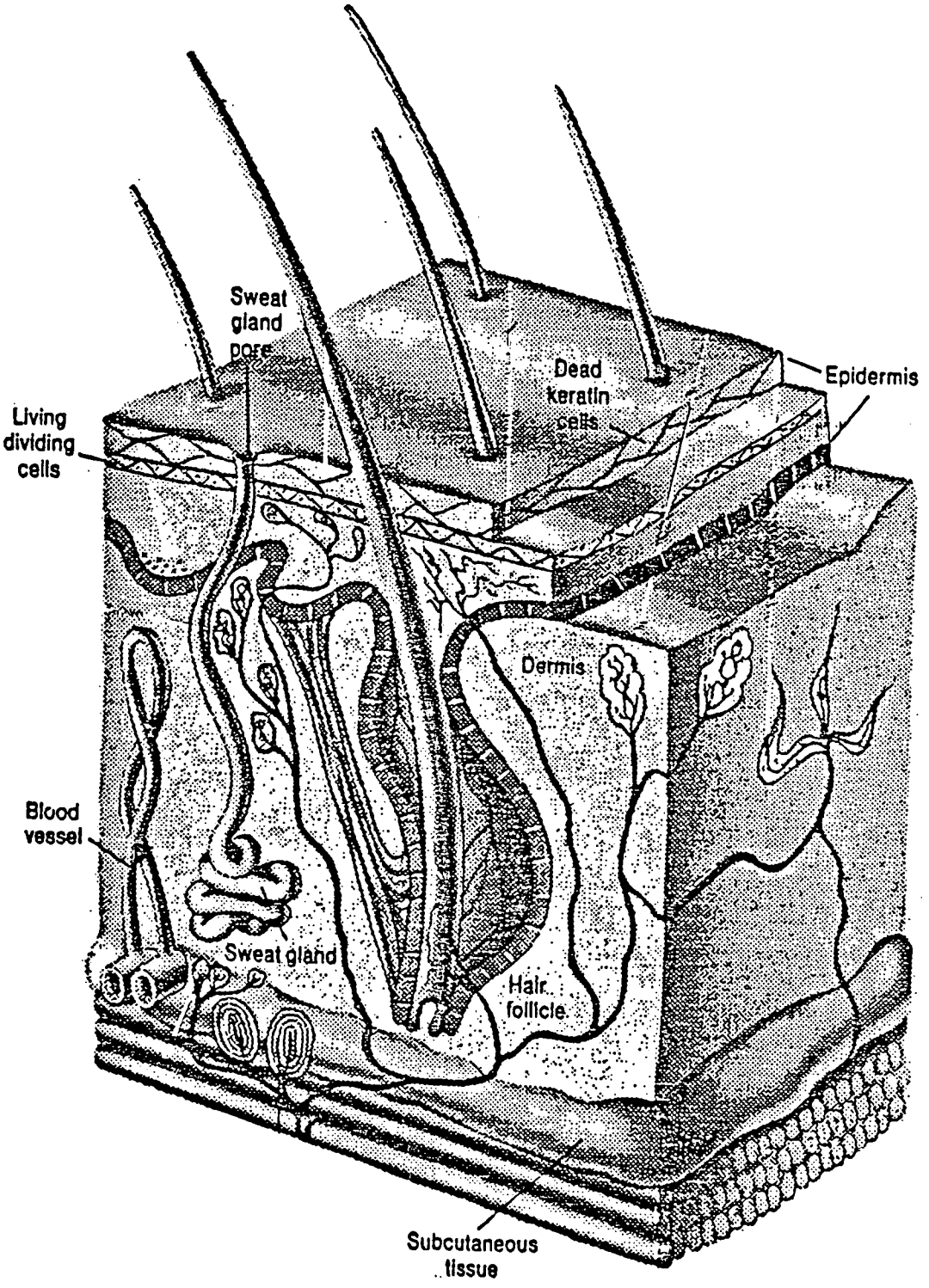

Figure 1. Skin Cross Section
(By Permission from the New York Times)

with a fat solvent and then rehydrated, the water permeability of the stratum corneum and its permeability to larger molecules is greatly enhanced (Scheuplein, 1967).

A recent study by Elias, et al. (1981) related dermal absorption to stratum corneum structure and lipid composition. The extreme sensitivity of the permeability barrier to damage by lipid solvents suggest that lipids are important determinants of skin penetration. In addition to lipids, several other stratum corneum structural parameters, including thickness, number of cell layers, and geometric organization could determine stratum corneum permeability. The authors correlated the in vitro penetration of water and salicylic acid across leg and abdominal stratum corneum with both the lipid composition and the structure of the same samples. One finding from this study is that there is an apparent noncorrelation of penetration of both substances with either stratum corneum thickness or number of cell layers. The results suggest that dermal absorption of both substances correlate with the lipid content by weight of the sample. Also, the study suggests that relatively small inherent variations in lipid concentration may explain observed differences in permeation across different topographic regions. The authors predict that regions of high permeability such as palms and soles would have a low lipid weight percent, whereas those of low permeability such as facial or perineal stratum corneum, would have a relatively high lipid weight percent.

The role of skin appendages such as hair follicles and sweat ducts on skin permeability has been studied extensively. One would think that these "holes" in the skin would facilitate the passage of a penetrant; however, their total area is relatively small and, for most penetrants, absorption through the general skin surface is the preferred route (Dugard, 1983). In the case of some

molecules that penetrate the bulk of the stratum corneum slowly, such as electrolytes and polar molecules with three or more labile polar groups, the route through follicles and ducts may predominate (Scheuplein, 1980).

4.2 FACTORS AFFECTING ABSORPTION

A general definition of percutaneous absorption can be given as the penetration of substances from the outside into the skin and through the skin into the blood stream. Schaefer and Schalla (1980) have broken this process into individual parts as: (1) penetration is considered to be the process of entrance into 1 layer; (2) permeation is the migration through 1 or several skin layers; (3) resorption is the uptake by the cutaneous microcirculation; and (4) absorption is the sum of all these processes.

There are a number of parameters which can affect the amount of penetrant absorption. The concentration of applied dose and surface area are the two most important factors, with the greatest potential for absorption occurring when a high concentration of a penetrant is spread over a large surface area of the body (Wester and Noonan, 1980a). The relationship between the concentration of applied dose and the efficiency of absorption is not necessarily a linear one as studies have shown the efficiency to decrease with increasing dose (Table 1). The site of application was the forearm for both man and rhesus monkey in Table 1; note the similarity in percent absorbed. The absolute dose absorbed increases, of course, as the dose applied is increased.

The solvent or formulation used to deliver a penetrant to the skin (vehicle) can have a decided impact on the efficiency of absorption. The ability of a compound to penetrate the skin and exert its effect is dependent on two consecutive physical events. The compound must first diffuse out of the vehicle

TABLE 1. RELATIONSHIP BETWEEN DOSE AND EFFICIENCY OF ABSORPTION

Penetrant	Dose (ug/cm^2)	Percent of Dose Absorbed	
		Rhesus	Man
Hydrocortisone	4	2.9	1.9
	40	2.1	0.6
Benzoic Acid	4	59.2	42.6
	40	33.6	25.7
	2000	17.4	14.4
Testosterone	4	18.4	13.2
	400	2.2	2.8

from Wester and Noonan 1980a

to the skin surface, and then must penetrate the skin in route to the site of action (Ostreng et al., 1971). If the membrane diffusion constant for the penetrant and the thickness and solvent properties of the membrane are unchanged by the nature of the external vehicle, then the rate of absorption is proportional to the chemical potential in the vehicle. When the chemical potential and penetrant concentration are linearly related, Fick's law of diffusion (see Chap. 6) is obeyed for the vehicle (Dugard, 1983). Some solvents, such as DMSO, actually dissolve lipids, destroying the barrier function and carrying the penetrant along with it into the body. The effect of a solvent on the rate of absorption of a penetrant should be studied further.

The site of application of a penetrant can also be an important fact in the efficiency of absorption. Maibach and Feldmann (1971) have measured the absorption of several compounds at various sites on the body. Their results show a wide range of values with the palm allowing approximately the same penetration as the forearm, the abdomen and dorsum of the hand having twice the penetration of the forearm, the scalp, angle of the jaw, postauriacular area, and forehead having four-fold greater penetration, and the scrotum allowing almost total absorption (Table 2a).

Guy and Maibach (1984) divided the body into five regions for the purpose of calculating correlation factors for use with forearm penetration data: genitals, arms, legs, trunk, and head. Table 2b gives "penetration indices" or the ratio of skin penetration for one of the five anatomic sites divided by skin penetration for the forearm. These penetration indices are derived from hydrocortisone skin penetration data and from absorption results using the pesticides malathion and parathion. The authors show the relative proportion of body area and actual skin area for the five anatomic sites in Table 2c.

TABLE 2a. RATIO COMPARING ABSORPTION OF VARIOUS SITES TO FOREARM

Site	Parathion	Malathion	Hydrocortisone
Forearm	1.0	1.0	1.0
Palm	1.3	0.9	0.8
Foot, ball	1.6	1.0	---
Abdomen	2.1	1.4	---
Hand dorsum	2.4	1.8	---
Scalp	3.7	---	3.5
Jaw angle	3.9	---	13.0
Forehead	4.2	3.4	6.0
Scrotum	11.8	---	42.0

From Maibach and Feldmann, 1971.

TABLE 2b. PENETRATION INDICES FOR FIVE ANATOMIC SITES

Site	Penetration index based on hydrocortisone	Penetration index based on pesticide data
Genitals	40	12
Arms	1	1
Legs	0.5	1
Trunk	2.5	3
Head	5	4

From Guy and Maibach, 1984 (see original article for the number of footnotes describing how these indices were calculated)

TABLE 2c. BODY SURFACE AREAS FOR FIVE ANATOMIC SITES

	Adult[a]		Child[b]		Neonate[c]	
	Body area (%)	Area (cm^2)	Body area (%)	Area (cm^2)	Body area (%)	Area (cm^2)
Genitals	1	190	1	75	1	19
Arms	18	3420	19	1425	19	365
Legs	36	6840	34	2550	30	576
Trunk	36	6840	33	2475	31	595
Head	9	1710	13	975	19	365
Total		19000		7500		1920

From Guy and Maibach, 1984

[a]Adult: weight = 70 kg; height = 1.83m
[b]Child: weight = 19 kg; height = 1.10m
[c]Neonate: weight = 3 kg; height - 0.49m

The condition of the skin, such as loss of barrier function of the stratum corneum through disease or damage, will also affect absorption. Absorption can be virtually 100% if all barrier function is removed. Occulusion or covering of the applied dose as with bandaging or putting on clothing after a dermal application will increase absorption. Occlusion changes the hydration and temperature of the skin and also prevents the accidental wiping off or evaporation of an applied dose (Wester and Noonan, 1980a).

The frequency of dermal applications is also a factor which could affect absorption. In one study the absorption of a single application of a high concentration of penetrant was greater than when the equivalent concentration was applied in equally divided doses (Wester et al., 1977).

In another study the effect of repeated applications of a penetrant was tested. The absorption on the 8th day of application of the same penetrant dose was found to be significantly higher than on the first day. The authors suggest that the initial applications of penetrant altered the barrier function of the stratum corneum, resulting in increased absorption for subsequent applications (Wester et al., 1980b).

The skin contains many of the same enzymes as the liver, thus penetrant metabolism by the skin could affect absorption. The metabolizing potential of skin has been estimated to be about 2% of the liver with most of the enzyme activity localized in the epidermal layer (Pannatier et al., 1978). The slower a penetrant is absorbed through the skin the greater the opportunity for some metabolism to occur. In vitro penetrant absorption studies using excised human skin will not show the results of any potential metabolism and, thus, may not reflect the actual in vivo absorption for some penetrants. The metabolism potential of skin should be studied further.

CHAPTER 5 - TECHNIQUES FOR MEASURING ABSORPTION

5.1 IN VIVO

Percutaneous absorption in vivo is usually determined by measuring radioactivity in excreta following the topical application of a labeled compound. The penetrant under study is labeled with carbon 14 or tritium and following application, the total amount of radioactivity excreted in urine or urine plus feces is determined. The radioactivity in the excreta can be a mixture of the parent compound and any metabolites. The amount of radioactivity retained in the body or excreted through expiration or sweat can be determined by measuring the amount of radioactivity excreted following an intravenous (i.v.) injection; the fraction determined by i.v. dose is then used to correct the amount of radioactivity found after topical administration (Wester and Maibach, 1983).

Another in vivo method of measuring absorption is the detection of plasma radioactivity following topical administration. This method may be difficult to apply because penetrant concentrations in blood after topical administration are often very low. Determining the loss of material from the surface as it penetrates the skin is another way to measure in vivo absorption. It is assumed that the difference between applied dose and residual dose is the amount of penetrant absorbed. The difficulties inherent in skin recovery, volatility of penetrant, and errors associated with using the difference between amount applied and amount left make this method less quantitative. Biological or pharmacological responses, such as vasodialation, have also been used to estimate absorption for a limited number of compounds (Stoughton et al., 1960)

The main source of human in vivo data is the work of Feldmann and Maibach

(1969 and 1970). These authors studied the percutaneous absorption of some steroids, pesticides, and organic compounds using radiolabeled (C^{14}) doses administered intravenously and topically to human volunteers. The intravenous dose was used to correct for the amount of radiolabeled penetrant that is percutaneously absorbed but not recovered in urine samples (i.e., excreted in feces or retained in the body). The topical dose of 4 ug/cm^2 was dissolved in acetone and applied to a 13 cm^2 circular area of the ventral forearm. All urine was collected for five days divided into suitable time periods so that either the absorption rate (%/hr) or total absorption (% of dose) could be calculated. The skin sites were not protected and not washed for 24 hours. Tables 3-5 are a compilation of the 49 penetrants studied.

Table 3 shows the percutaneous penetration of some steroids in man. For the intravenous dose, one microcurie (^{14}C) of the steroid was dissolved in 5 to 20 ml of saline (or saline plus ethanol) and injected. The half life, which is an indicator of the relative speed of elimination of the injected dose, was obtained from plotting the amount of ^{14}C excreted divided by the time period expressed as the percent of dose administered versus the collection time. The values for percent recovery are given in the first part of Table 3 with testosterone showing almost complete elimination from the body and fluocinolone yielding only one-third of the injected dose. Cortisone, corticosterone, 17-OH desoxycorticosterone, desoxycorticosterone, and 17-OH progesterone are assumed to be excreted similarly as hydrocortisone. Likewise, the value for testosterone was used for the acetate and propionate, plus dehydrocortisone and androstenedione. The second part of Table 3 shows absorption after topical administration. The

TABLE 3. PERCUTANEOUS ABSORPTION OF STEROIDS IN MAN

I.V. Administration --

Steroid	% Recovered	Half Life (hrs)
Hydrocortisone	65.4	5
Hydrocortisone acetate	68.9	5
Estradiol	51.6	8
Testosterone	92.1	4
Progesterone	68.7	4
Fluocinolone acetonide	37.0	5.5
Dexamethasone	47.4	4

Topical Administration*--

Steroid	Absorption Rate (%/hr)						Total Absorption		No. Subjects
	Time (hrs) 0-12	12-24	24-48	48-72	72-96	96-120	% of dose	S.D.	
Hydrocortisone	.005	.023	.019	.018	.016	.010	1.87	1.59	15
Hydrocortisone acetate	.020	.089	.032	.024	.015	.008	2.65	1.80	6
Cortisone	.015	.037	.039	.036	.032	.024	3.38	1.64	7
Corticosterone	.013	.065	.139	.070	.050	.039	8.78	5.35	6
17-OH DOC	.041	.101	.084	.076	.062	.055	8.41	4.28	5
Desoxycorticosterone	.197	.313	.143	.069	.035	.020	12.55	8.53	6
17-OH Progesterone	.042	.120	.213	.211	.078	.031	14.76	11.35	7

Continued on following page

Table 3 Continued -

Topical Administration*--

Steroid	Absorption Rate (%/hr)						Total Absorption		No. Subjects
	Time (hrs) 0-12	12-24	24-48	48-72	72-96	96-120	% of dose	S.D.	
Progesterone	.208	.264	.135	.045	.024	.011	10.81	5.78	6
Flucinolone acetonide	.002	.011	.012	.016	.008	.005	1.34	1.05	9
Dexamethasone	.005	.003	.004	.003	.002	.002	.40	.23	3
Estradiol	.008	.056	.099	.101	.107	.103	10.62	4.86	3
Testosterone	.147	.364	.156	.066	.036	.018	13.24	3.04	17
Testosterone acetate	.103	.133	.048	.015	.007	.004	4.62	2.28	6
Testosterone propionate	.061	.096	.035	.015	.009	.005	3.44	1.03	9
Dehydroepian-drosterone	.265	.446	.249	.091	.046	.028	18.45	7.71	6
Androstenedione	.183	.334	.155	.076	.043	.028	13.47	5.56	11

From Feldmann and Maibach, 1969

*Corrected for i.v. recovery

average amount of absorption for this series of steroids is roughly 10 percent with a fifty-fold difference between the least and the most absorbed. However, inspection of the standard deviation column (S.D.) shows that there is a great deal of uncertainty in this methodology with penetrants like hydrocortisone having a standard deviation of ± 85 percent. The authors point out a physical relationship between the number of hydroxyl groups on the steroids and their total absorption; corticosterone and 17-OH desoxycorticosterone each have two while desoxycorticosterone and 17-OH progesterone each have one. For each pair, the absorptions are reasonably close. Also, the series of steroids shows a stepwise increase in absorption for each hydroxyl removed (Feldmann and Maibach, 1969).

Table 4 presents further in vivo percutaneous absorption studies by Feldmann and Maibach (1970) on some organic compounds. The values for percent recovery shown in the first part of the table indicate that several of the compounds are poorly absorbed and excreted (i.e., the correction factor for hexachlorophene is greater than 20 which means that the percent recovery for this compound in urine after topical administration has to be multiplied by this factor). Three compounds were deemed too toxic to test intravenously in man and thus were administered to guinea pigs. Nicotinic acid, nicotinamide, hippuric acid and phenol were assumed to be excreted similarly to salicylic acid, while thiourea was assumed to behave like urea. The second part of Table 4 gives the absorption after topical administration. Three compounds, caffeine, dinitrochlorobenzene, and benzoic acid, had total absorptions of about 50 percent after being corrected for incomplete absorption following i.v. administration. Three other compounds, nicotinic acid, hippuric acid, and thiourea displayed total absorptions of less

TABLE 4. PERCUTANEOUS ABSORPTION OF SOME ORGANIC COMPOUNDS IN MAN

I.V. Administration--

Compound	% Recovered	Half Life (hrs)
Caffeine	59.4	6
Chloramphenicol	67.4	6
Colchicine	27.9	4
Diethyltoluamide	52.3	4
Dinitrochlorobenzene	64.0	4
Hexachlorophene	4.4	48
Nitrobenzene	60.5	20
Potassium thiocyanate	10.2	12
Salicylic acid	89.8	4
Urea	71.7	8
Butter Yellow*	58.6	6
Malathion*	76.0	4
Methylcholanthrene*	18.2	14

*Recovery in urine after i.v. administration in guinea pigs.

Table 4 continued--

Topical Administration*--

Compound	Absorption Rate (%dose/hr)						Total Absorption		
	Time (hrs) 0-12	12-24	24-48	48-72	72-96	96-120	% of dose	S.D	No. Subjects
Acetylsalicylic acid	.141	.438	.334	.147	.076	.060	21.81	3.11	5
Benzoic acid	3.036	.340	.055	.000	.000	.000	42.62	16.45	6
Butter yellow	.215	.685	.289	.083	.054	.022	21.57	4.88	4
Caffeine	.559	1.384	.855	.109	.032	.014	47.56	20.99	12
Chloramphenicol	.007	.019	.021	.022	.015	.012	2.04	2.46	6
Colchicine	.036	.038	.033	.040	.025	.004	3.69	2.50	6
Dinitrochloro-benzene	3.450	.565	.134	.045	.018	.009	53.14	12.41	4
Diethyltoluamide	.773	.331	.084	.036	.016	.012	16.71	5.10	4
Hexachlorophene	.029	.031	.020	.028	.034	.030	3.10	1.09	7
Hippuric acid	.005	.003	.001	.001	.001	.001	.21	.09	7
Malathion	.313	.170	.044	.017	.011	.006	7.84	2.71	7
Methylcholanthrene	.062	.329	.258	.127	.064	.045	16.81	5.16	3
Nicotinic acid	.000	.002	.001	.001	.002	.007	.34	.09	3
Nicotinamide	.019	.168	.177	.088	.052	.031	11.08	6.17	7
Nitrobenzene	.022	.022	.013	.013	.011	.006	1.53	.84	6
Paraaminobenzoic-acid	.159	.648	.444	.196	.058	.044	28.37	2.43	13
Phenol	.254	.091	.010	.601	.000	.000	4.40	2.43	3
Potassium thiocyanate	.051	.060	.078	.097	.100	.093	10.15	6.60	6
Salicylic acid	.116	.535	.356	.156	.080	.033	22.78	13.25	17
Thiourea	.046	.035	.010	.008	.007	.007	.88	.22	3
Urea	.008	.021	.051	.073	.075	.034	5.99	1.91	4

From Feldmann and Maibach, 1970

*Corrected for i.v. recovery.

than 1 percent of the applied dose. The range for total absorption for these compounds is greater than 250-fold, much larger than the series of steroids in Table 3. However, as with the steroids, the standard deviations are very high for the compounds, indicating a large degree of uncertainty, with chloramphenicol showing a standard deviation of greater than 100 percent. The authors point out two examples of closely related compounds showing great differences in penetration: benzoic acid was absorbed 200 times more than its glycine conjugate, hippuric acid; nicotinic acid showed minimal penetration while 10 percent of its amide, nicotinamide, was absorbed. The authors also generally found a good correlation between the maximum penetration rate for each compound and its total absorption.

Table 5 presents similar in vivo absorption studies by Feldmann and Maibach (1974) on some pesticides and herbicides. The authors used the same experimental method as discussed previously to study 5 organophosphates, 3 chlorinated hydrocarbons, 2 carbamates, and 2 herbicides. The total excretion from the i.v. dose varied over a wide range with dieldrin, aldrin, and carbaryl at 3-7 percent and malathion, baygon, and 2,4-D being over 80 percent. For the topical administration, diquat was the only compound with only slight penetration; carbaryl, on the other hand, was nearly completely absorbed after multiplying the topical results by the large correction factor obtained from the i.v. administration. The authors discuss the rather large standard deviations of 1/3 to 1/2 of the mean value found in their studies. They claim that the difference is due to actual differences between people rather than experimental error as repeat studies on the same subject gave similar results. The authors assume a normal distribution and find that 1 person in 10 will absorb twice

TABLE 5. PERCUTANEOUS PENETRATION OF SOME PESTICIDES AND HERBICIDES IN MAN

I.V. Administration--

Compound	Absorption rate (% dose/hr)(time period in hr) Time (hrs) 0-4	4-8	8-12	12-24	24-48	48-72	72-96	96-120	Total Absorption % Dose	SD	Half-life (hr)
Azodrin	1.816	2.721	1.701	1.000	0.679	0.341	0.173	0.088	67.7	5.3	20
Ethion	0.832	1.041	1.892	0.791	0.316	0.123	0.071	0.065	38.4	3.6	14
Guthion	1.513	1.204	1.590	1.041	0.813	0.458	0.257	0.127	69.5	6.9	30
Malathion	12.949	5.571	2.420	0.368	0.052	0.017	0.008	0.004	90.2	9.7	3
Parathion	0.035	1.321	2.508	1.124	0.469	0.135	0.069	0.037	45.8	5.3	8
Baygon	10.361	7.290	1.478	0.192	0.064	0.053	0.047	0.043	83.8	7.2	8
Carbaryl	0.459	0.394	0.211	0.102	0.037	0.021	0.011	0.008	7.4	2.2	9
Aldrin	0.224	0.091	0.113	0.040	0.023	0.013	0.011	0.008	3.6	0.9	6
Dieldrin	0.038	0.067	0.074	0.046	0.046	0.013	0.015	0.008	3.3	1.0	28
Lindane	0.688	0.611	0.552	0.244	0.232	0.132	0.125	0.102	24.6	6.1	26
2,4-D	3.001	4.063	5.312	1.728	0.737	0.275	0.153	0.097	100.0	2.5	13
Diquat	9.328	1.544	1.825	0.292	0.127	0.059	0.054	0.045	61.2	16.0	4
Topical Administration--											
Azodrin	0.057	0.048	0.092	0.121	0.183	0.147	0.113	0.073	14.7	7.1	
Ethion	0.004	0.005	0.026	0.036	0.044	0.041	0.015	0.011	3.3	1.1	
Guthion	0.044	0.202	0.294	0.276	0.207	0.125	0.059	0.040	15.9	7.9	
Malathion	0.089	0.408	0.396	0.149	0.029	0.008	0.006	0.005	8.2	2.7	
Parathion	0.007	0.116	0.243	0.202	0.110	0.062	0.036	0.029	9.7	5.9	
Baygon	0.351	0.705	1.204	0.543	0.093	0.023	0.020	0.028	19.6	5.2	
Carbaryl	0.005	1.212	3.027	1.944	0.853	0.277	0.154	0.105	73.9	21.0	
Aldrin	0.086	0.074	0.078	0.079	0.066	0.053	0.060	0.061	7.8	2.9	
Dieldrin	0.143	0.137	0.135	0.093	0.066	0.060	0.043	0.034	7.7	3.2	
Lindane	0.064	0.135	0.245	0.113	0.088	0.066	0.051	0.048	9.3	3.7	
2,4-D	0.009	0.012	0.020	0.029	0.068	0.082	0.043	0.027	5.8	2.4	
Diquat	0.005	0.002	0.005	0.003	0.003	0.003	0.002	0.001	0.3	0.1	

From Feldmann and Maibach, 1974

*Corrected for i.v. recovery. There were 6 subjects for each compound for both i.v. and topical administration.

the mean value while 1 in 20 will absorb 3 times this amount. They have also found that experimental subjects differ by a factor of 5 in the amount of percutaneous absorption. The authors also comment that the experimental subjects did not sweat extensively such as field worker might when exposed to these pesticides; the effect of sweating should be studied. Also, Larry Hall (personal communication) points out that the skin penetration tables in Feldmann and Maibach's studies are accurate only if the rate of elimination is much greater than the rate of skin absorption.

5.2 IN VIVO ANIMAL STUDIES

The toxicity of many penetrants prohibits in vivo human studies, thus researchers have had to use animal models to obtain absorption data. Unfortunately there are a number of problems associated with the extrapolation of animal data to humans. Animal species variation, different sites of application, shaved skin vs. unshaved, occluding or restraining devices, and skin metabolism (or lack thereof) are some of the factors which need to be considered when using animal studies to predict human absorption.

Bartek et al. (1972) compared percutaneous absorption in rats, rabbits, miniature swine, and man; Table 6 shows the results of their study. Radiolabeled compounds were applied to the shaved skin of the back and protected by a non-occluding device. In general, the penetration through the skin of the pigs and man was similar and much slower than it was through rat and rabbit skin. For the compounds studied, haloprogin was completely absorbed by the rat and rabbit, while only 11 percent was absorbed by man. About half of the caffeine applied was absorbed by all of the animals plus man. The results of this study show that absorption in the rabbit and rat would not be predictive of that in man while the miniature swine appears closest to man.

TABLE 6. *IN VIVO* PERCUTANEOUS ABSORPTION BY RAT, RABBIT, PIG, AND MAN

Penetrant	Percent dose absorbed*			
	Rat	Rabbit	Pig	Man
Haloprogin	95.8	113.0	19.7	11.0
N-Acetylcycteine	3.50	1.98	6.00	2.43
Testosterone	47.4	69.6	29.4	13.2**
Cortisone	24.7	30.3	4.06	3.38**
Caffeine	53.1	69.2	32.4	47.6**
Butter Yellow	48.2	100.0	41.9	21.6**

From Bartek, LaBudde, and Maibach, 1972.

*Corrected for recovery following i.v. administration

**Human data taken from Feldmann and Maibach, 1969 and 1970.

Bartek and LaBudde (1975) also studied the absorption of 4 pesticides in the rabbit, pig and squirrel monkey using the same techniques as their previous study. The results were compared to man where the site of application was the forearm (Table 7). The in vivo absorption of pesticides in the rabbit was greater than in man, while absorption in the pig and squirrel monkey was closer to that in man.

Shah et al. (1981) investigated the dermal penetration of some insecticides in mice. Acetone solutions of the radiolabeled test penetrants were applied to shaved backs of female mice. Mice were placed in metabolism cages equipped with CO_2 trapping devices and urine collection jars. Urine, blood, specific tissues, and organs were sampled at various time intervals. The remaining portion of the body was termed carcass. Table 8 shows the percent of radiolabeled penetrant recovered in various fractions at 5, 15, and 60 minutes following topical administration. Recovery of radioactivity was 90% or more in all cases. The authors also tried to relate physical properties such as molecular weight, solubility, and partition coefficients to dermal absorption. Partition coefficients for chloroform-water, olive oil-water, and benzene-water were determined using radiolabeled compounds and a high-speed centrifuge. Table 9 presents a comparison between physical parameters and rates of penetration. The authors did not draw any conclusions from their study other than that there is a lack of correlation among partition systems themselves. One interesting aspect of this study is that an aqueous wettable powder formulation of carbaryl penetrated more rapidly than did the acetone formulation.

Researchers have attempted to bridge in vivo animal and human dermal absorption studies by transplanting human skin to the athymic (nude) mouse.

TABLE 7. IN VIVO PERCUTANEOUS ABSORPTION OF SEVERAL PESTICIDES BY RABBIT, PIG, SQUIRREL MONKEY, AND MAN

Pesticide	Percent Dose absorbed*			
	Rabbit	Pig	Monkey	Man**
DDT	46.3	43.4	1.5	10.4
Lindane	51.2	37.6	16.0	9.3
Parathion	97.5	14.5	30.3	9.7
Malathion	64.6	15.5	19.3	8.2

From Bartek and LaBudde, 1975

*Corrected for recovery following i.v. administration (except the monkey data).
**Human data from Feldmann and Maibach, 1974

TABLE 8. GEOMETRIC MEANS OF PERCENTAGE ^{14}C RECOVERED IN VARIOUS FRACTIONS AT 5, 15, AND 60 MIN POST APPLICATION

Toxicant	Blood			Liver			Fat			Excretory prod.[a]			Carcass		
	5 min	15 min	60 min	5 min	15 min	60 min	5 min	15 min	60 min	5 min	15 min	60 min	5 min	15 min	60 min
Carbamates															
Carbaryl	0.1	0.3	1.5	0.2	1.1	3.9	<0.1	<0.1	<0.1	<0.1	0.2	5.0	31.2	54.2	60.8
Methomyl	0.1	1.8	2.9	0.1	3.9	5.0	0.1	0.1	0.2	<0.1	0.2	12.9	24.4	49.2	55.9
Carbofuran	<0.1	1.7	0.5	0.1	2.4	0.6	<0.1	0.2	<0.1	<0.1	0.4	8.3	32.4	67.3	66.4
Organophosphates															
Parathion	<0.1	0.2	0.7	0.1	0.3	2.4	0	0	<0.1	<0.1	0.1	2.5	9.6	7.7	25.5
Malathion	0.3	0.9	0.5	0.1	0.1	0.5	<0.1	<0.1	<0.1	<0.1	1.5	2.2	12.9	18.3	21.2
Chlorpyrifos (methyl)	0.3	0.7	2.9	0.2	0.4	1.5	<0.1	0.2	0.9	<0.1	0.1	2.1	13.9	31.2	47.5
Chlorpyrifos	<0.1	0.4	0.9	<0.1	0.4	0.7	<0.1	0.1	0.3	<0.1	0.1	0.7	22.8	63.5	66.6
Botanical type															
Nicotine	0.6	2.1	3.0	1.3	4.0	3.3	0.1	0.3	0.3	0.2	3.6	14.2	25.4	49.7	49.8
Permethrin	<0.1	1.0	5.8	0.1	0.7	5.0	<0.1	0.1	0.6	<0.1	0.2	7.4	40.8	60.4	60.9
Chlorinated hydrocarbons															
DDT	<0.1	<0.1	0.1	<0.1	0.3	2.8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	12.2	21.3	30.9
Hexachlorobiphenyl[b]	<0.1	<0.1	0.2	<0.1	<0.1	1.2	<0.1	<0.1	0.1	<0.1	<0.1	0.3	28.7	44.6	52.4
4-Chlorobiphenyl	0.1	0.5	2.4	0.4	2.5	8.0	0.1	0.3	0.5	0.1	0.3	7.6	12.9	48.5	56.8
Chlordecone	<0.1	<0.1	<0.1	0.1	0.2	1.2	<0.1	<0.1		<0.1	0.1	0.1	34.2	42.2	52.4
Dieldrin	<0.1	<0.1	0.2	<0.1	0.2	2.2	<0.1	<0.1	0.1	<0.1	<0.1	0.1	3.0	25.9	30.9

From Shah, et al. 1981

[a]Urine, CO_2, and feces

[b]2,4,5,2',4',5'-Hexachlorobiphenyl

TABLE 9. COMPARISON BETWEEN PHYSICAL PARAMETERS AND GEOMETRIC MEANS OF RATE OF PENETRATIONS

Toxicant	Molecular Weight	H_2O Solubility	Partition Coefficients			T0.8 (min)	Geometric means of percentage penetration					
			$CHCl_3$ Water	Olive Oil-Water	Benzene-Water		1 min	5 min	15 min	60 min	480 min	2880 min
Carbamates												
Carbaryl	203	40 ppm	277	46	139	12.8±4.1	22.2	31.5	56.0	71.7	88.5	98.9
Methomyl	162	58,000 ppm	15	0.1	---	13.3±2.8	18.8	24.7	55.5	84.5	88.3	----
Carbofuran	221	700 ppm	32	5	40	7.7±1.5	28.5	32.6	71.7	76.1	94.7	----
Organophosphates												
Parathion	292	24 ppm	433	1738	659	66.0±21.9	4.4	9.8	8.3	31.9	85.4	99.6
Malathion	330	145 ppm	112	56	91	129.7±47.7	5.5	13.4	22.7	24.6	66.7	97.8
Chlorpyrifos (methyl)	318	4.7 ppm	92	245	116	51.6±6.5	8.3	14.3	32.2	54.4	78.2	----
Chlorpyrifos	350	2 ppm	374	1044	480	20.6±5.9	15.7	28.8	64.4	69.0	73.9	----
Botanical type												
Nicotine	162	Miscible	2	0.02	0.4	18.2±2.4	5.2	27.9	59.5	71.5	90.7	----
Permethrin	390	0.07 ppm	269	360	80	5.9±1.3	36.2	40.9	63.1	79.7	88.1	----
Chlorinated hydrocarbons												
DDT	355	1.2 ppb	532	1775	170	105.4±25.6	3.9	12.3	21.7	34.1	71.1	91.3
Hexachlorobiphenyl[a]	361	0.953 ppb	2465	887	1398	43.8±7.0	17.3	28.7	44.7	55.3	66.8	----
4-Chlorobiphenyl	188	<0.6 ppm	684	482	---	16.8±2.3	6.5	13.5	53.5	84.5	97.5	----
Chlordecone	490	0.4 ppm	---	185	---	41.3±8.8	18.8	34.4	42.5	54.0	65.9	----
Dieldrin	384	0.18 ppm	190	282	144	71.7±17.3	1.2	3.1	26.1	33.7	82.6	94.0

From Shah, et al. 1981 (and references therein)
[a]2,4,5,2',4',5'-hexachlorobiphenyl

Krueger and Shelby (1981) used human skin obtained from skin grafts or organ donors to graft to nude mice. The authors found that the human skin grafts undergo a proliferative response when 10 ng of the tumor promoter 12-O-tetradecanoyl phorbol 13-acetate is applied while nude mice do not respond to this dose. The authors state that their studies show that the unit function of human skin after transplant is similar to that prior to transplant.

5.3 IN VITRO

In vitro studies have also been used extensively to estimate absorption. For these studies, a piece of excised skin is attached to a diffusion apparatus which usually consists of a top chamber to hold the applied dose of the penetrant plus any solvent, an O-ring to hold the skin in place, and a temperature controlled bottom chamber containing saline or other solvents plus a sampling port to withdraw fractions for analysis (Fig. 2). Human forearm skin is difficult to obtain, thus it is common practice to use abdominal skin collected at autopsy. For most studies, the stratum corneum is heat separated from the epidermis and dermis, then studied by itself.

Franz (1975) studied the in vitro permeability of 12 organic compounds which had been previously studied in vivo in man. Special emphasis was given to duplicate the in vivo conditions, such as amount of dose applied, in order to show how accurately in vitro absorption studies can reflect the living state. Each piece of skin was mounted in a diffusion cell (diffusion area of either 1 cm^2 or 2.5 cm^2) with the epidermal side of the skin exposed to ambient air while the dermal side was bathed in a saline solution containing an antibacterial/antifungal plus buffers. The temperature was maintained at 37^{o}C by a water

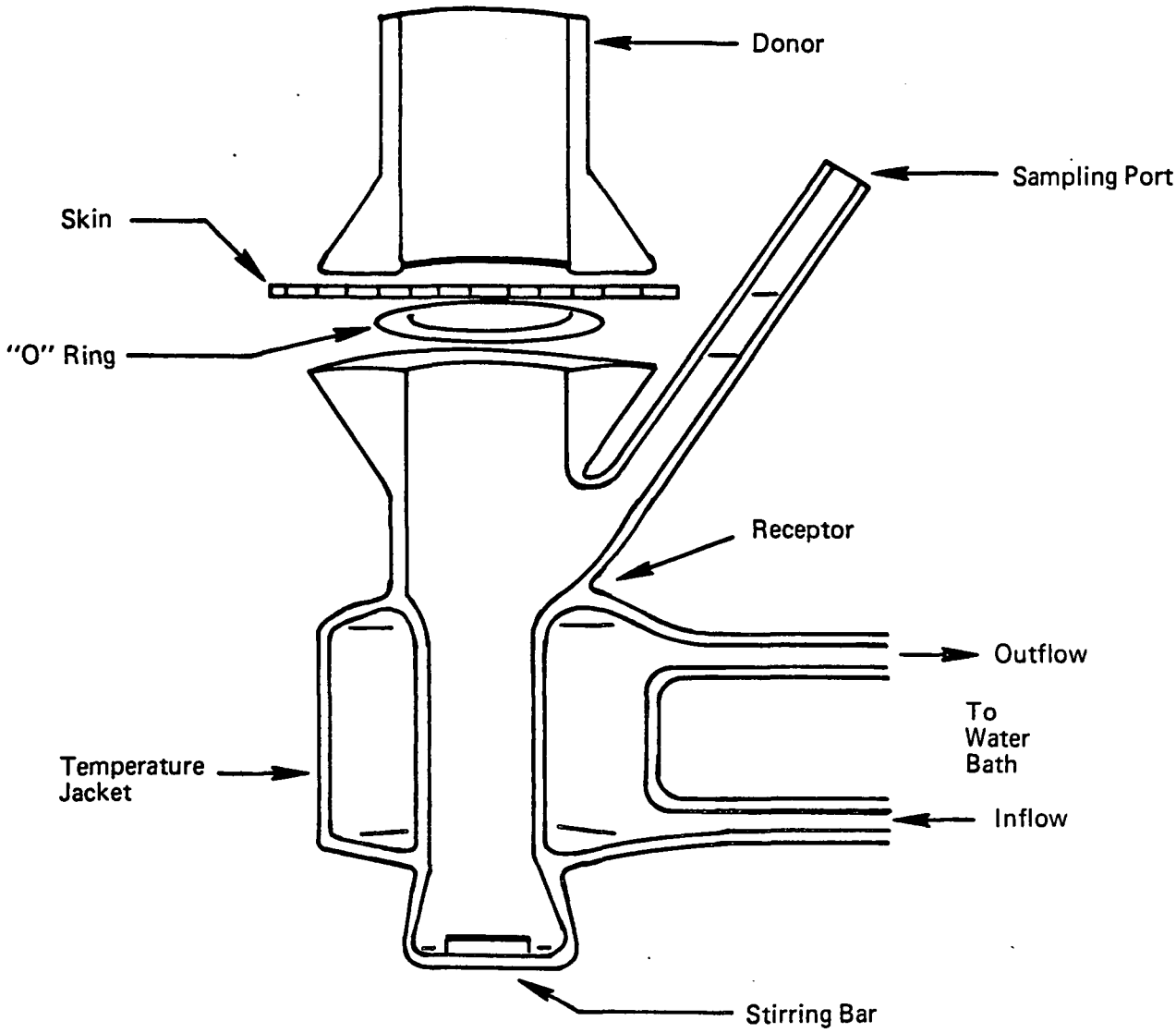

Figure 2. Schematic representation of a diffusion cell with top open to the ambient environment.(Franz, 1975)

jacket which surrounded the chamber. A small amount of the radiolabeled penetrant in the same dose range as the in vivo studies was dissolved in acetone and spread across the entire exposed surface with the acetone evaporating in less than one minute. At selected intervals after the addition of penetrant to the epidermis the dermal bathing solution was removed in its entirety, gelled and analyzed in a liquid scintillation spectometer. Either the total absorption was determined by one sample taken at 24-hr intervals or the kinetics of the absorption process were determined by taking frequent samples throughout the day.

Table 10 shows the total absorption of 12 organic chemicals that have been studied both in vivo and in vitro. Highly water-insoluble compounds were not selected for in vitro study since their permeability might be limited due to insolubility in the dermal bathing (saline) solution. In viewing the data from Table 10 only two compounds, chloramphenicol and benzoic acid appear to be in quantitative agreement. The in vivo studies are from Feldmann and Maibach and, as discussed previously, are from 5-day urine collection of an applied dose to the forearm. The in vitro studies, on the other hand, use human adominal tissue, last for two days, and have a much smaller receptor volume when compared to the entire body (i.e., you might see a build-up of penetrant concentration in the receptor cell that you would not see when the dose is applied in vivo).

Franz (1978) further investigated the apparent differences between the in vivo and in vitro approaches. Since in only two of the twelve compounds studied was there no radiolabeled compound in the urine on day 5, the author thought that this collection period might be inadequate and lead to underestimation of

TABLE 10. COMPARISON OF HUMAN *IN VIVO* AND *IN VITRO* ABSORPTION

Compound	Total Absorption (expressed as percent of applied dose)	
	In Vivo[a]	In Vitro[b]
1. Hippuric acid	0.2 ± 0.1 [7]	1.2 (0.8, 2.7) [15]
2. Nicotinic acid	0.3 ± 0.1 [3]	3.3 (0.7, 8.3) [19]
3. Thiourea	0.9 ± 0.2 [3]	3.4 (2.4, 5.5) [52]
4. Chloramphenicol	2.0 ± 2.5 [6]	2.9 (1.0, 5.7) [12]
5. Phenol	4.4 ± 2.4 [3]	10.9 (7.7, 26) [7]
6. Urea	6.0 ± 1.9 [4]	11.1 (5.2, 29) [22]
7. Nicotinamide	11.1 ± 6.2 [7]	28.8 (16, 65) [21]
8. Acetylsalicylic acid	21.8 ± 3.1 [3]	40.5 (17, 49) [14]
9. Salicylic acid	22.8 ± 13.2 [17]	12.0 (2.3, 23) [10]
10. Benzoic acid	42.6 ± 16.5 [6]	44.9 (29, 53) [18]
11. Caffeine	47.6 ± 21.0 [12]	9.0 (5.5, 20) [17]
12. Dinitrochlorobenzene	53.1 ± 12.4 [4]	27.5 (19, 33) [18]

From Franz, 1975 (*In vivo* data from Feldmann and Maibach, 1970)

[a]Mean ± standard deviation. The figure in brackets is the number of subjects studied.
[b]Median with 95% confidence interval given in parentheses.

the actual amount absorbed. Also, there are differences in the permeability of skin from different sites of the body. Franz restudied four compounds (hippuric acid, nicotinic acid, thiourea, and caffeine) for which there was poor agreement between the in vivo and in vitro results. This time adominal skin was used for both the in vivo and in vitro tests plus urine was collected in the in vivo tests until background levels of radioactivity were reached. Also, to minimize the differences that might be caused by desquamation of the epidermis in vivo, the surface of the skin was washed with acetone and water 24 hours after application of the test compounds in both sets of experiments.

Table 11 shows the comparison between human in vivo and in vitro absorption, using Franz's refined techniques, for the four compounds originally showing poor agreement. Hippuric acid, thiourea, and caffeine gave excellent quantitative agreement, but nicotinic acid displayed the lack of agreement found in the previous study (see Table 10). Franz found that less than 15 percent of an intravenously administered dose of ^{14}C-nicotinic acid was excreted in the urine; Feldmann and Maibach (1970) did not directly measure the excretion of nicotinic acid following an i.v. dose, but assumed on the basis of similar chemical structures that it would behave like salicylic acid, a compound in which 90 percent of the i.v. dose is excreted. Thus, when the value of 0.32 percent absorption observed in vivo in humans is corrected for incomplete urinary excretion, the value of 2.1 percent is in better agreement with the value observed in vitro. Franz (1978) states that based on the data obtained from the compounds studied to date, it appears that in vitro studies accurately portray the phenomenon of absorption as it occurs in living man.

TABLE 11. COMPARISON OF HUMAN IN VIVO AND IN VITRO ABSORPTION USING REFINED TECHNIQUES

Compound	Total Absorption (expressed as percent of applied dose) In Vivo[a]	T[b]	In Vitro[a]
1. Nicotinic Acid	0.32 ± 0.10 [3]	21	2.3 ± 0.9 [4]
2. Hippuric Acid	1.0 ± 0.4 [6]	3	1.25 ± 0.5 [4]
3. Thiourea	3.7 ± 1.3 [4]	21	4.6 ± 2.3 [5]
4. Caffeine	22.1 ± 15.8 [4]	7	24.1 ± 7.8 [4]

From Franz, 1978

[a]Mean ± SD. The figure in brackets is the number of subjects studied.

[b]Number of days urine was collected.

Bronaugh et al. (1982) compared *in vivo* and *in vitro* absorption through female rat skin using benzoic acid, acetylsalicyclic acid, urea, and caffeine. For the *in vivo* studies, petrolatum was used as a nonvolatile vehicle because of its ability to adhere to the skin; in this manner the concentration was known and, therefore, permeability constants could be calculated. Full thickness, lightly shaved skin (with the subcutaneous fat removed) was used in a standard diffusion cell for the *in vitro* experiments. Since the permeability constant (k_p) is defined as the steady-state rate of absorption (amount/cm^2/hr) divided by the concentration of solute applied to the skin (amount/cm^3), a k_p value (cm/hr) is obtained by the diffusion cell procedure. The determination of a k_p value from *in vivo* data is based on the measurement of absorption rate. This rate is estimated by considering the absorbed compound to accumulate in both the body of the animal and the excreted waste. The k_p was calculated from the rate of body accumulation plus the rate of excretion divided by the concentration of solute in vehicle. The quantitative agreement between the *in vivo* and *in vitro* work of Bronaugh et al. (1982) appears good as shown in Tables 12 a, b, and c.

TABLE 12a. PERCUTANEOUS ABSORPTION OF ACETYLSALICYLIC ACID IN RATS

Days	Absorption (% of applied dose)[a] In Vivo	In Vitro
1	8.5 ± 1.6	8.8 ± 1.2
2	7.9 ± 2.0	8.5 ± 1.2
3	4.0 ± 0.9	4.6 ± 0.5
4	2.8 ± 0.5	4.3 ± 0.4
5	1.9 ± 0.5	2.9 ± 0.1
Total	24.8 ± 4.4	29.0 ± 0.1

From Bronaugh et al. 1982

[a]Results are expressed as the $\bar{X}$ ± SE of four or five determinations.

TABLE 12b. IN VIVO VS. IN VITRO PERCUTANEOUS ABSORPTION THROUGH RAT SKIN[a]

Test Compound	Rate (ng/hr/cm^2) Body	Urine	Permeability constant In Vivo	In Vitro
Caffeine	16.3	27.8	2.1×10^{-4}(7)	3.1×10^{-4}(6)
Acetylsalicylic acid	0	11.4	5.2×10^{-5}(7)	6.5×10^{-5}(5)

From Bronaugh et al. 1982

[a]Compounds in a petrolatum vehicle were applied to a 2.0-cm^2 area of skin on the living animals and in diffusion cells. Results are the means of the number of determinations in parenthesis.

TABLE 12c. COMPARISON OF PERMEATION VALUES WITH THOSE OF OTHER STUDIES

Test Compound	In Vivo Rat (Petrolatum)	Human[a] (Acetone)	In Vitro Rat (Petrolatum)	Human[b] (Acetone)
Benzoic acid	37.1	42.6	49.1	44.9
Acetylsalicylic acid	24.8	21.8	29.0	40.5
Urea	8.1	6.0	7.2	11.1

From Bronaugh et al. 1982

[a]Values from Feldmann and Maibach (1970)
[b]Values from Franz (1975)

CHAPTER 6 - THEORETICAL TREATMENT OF DERMAL ABSORPTION

6.1 FICK'S LAW APPLIED TO DERMAL ABSORPTION

For those unfamiliar with Fick's Laws of Diffusion, it may be helpful to reference a basic physical chemistry text such as Moore, 1962. Scheuplein and Blank (1971) developed an integrated form of Fick's law to express diffusion through the skin barrier. The flow across the membrane is called the flux, J_S, and the expression for the steady-state flux of solute across an inert membrane is given by:

$$J_S = \frac{D(C_1 - C_2)}{\delta}$$

where J_S = steady-state flux of solute (moles cm^{-2} hr^{-1})

D = Average membrane diffusion coefficient for solute ($cm^2 sec^{-1}$) (sometimes interchanged with D_m to represent diffusion coefficient through skin membrane)

C = Concentration of solute

δ = Membrane thickness (cm)

For skin, the stratum corneum is not an inert structure but one with an affinity for the applied solute; thus, the concentrations at the surfaces of the membrane are not usually equal to the concentrations in the external solutions. The correlation between external and surface concentrations can be stated in terms of the solvent-membrane distribution coefficient, K_m. The intergrated form of Fick's law then becomes $J_S = \frac{K_m D \Delta C_S}{\delta}$

and $$k_p = \frac{K_m D}{\delta}$$

where ΔC_S = Concentration difference of solute across membrane (moles cm^{-3})

k_p = Permeability constant for solute (cm hr^{-1})

$$\therefore \quad J_S = k_p \Delta C_S$$

In the application of the expanded form of Fick's law, i.e., $J_S = k_p \Delta C_S = \frac{K_m D \Delta C_S}{\delta}$, only the skin membrane thickness, diffusivity, and the partition coefficient for the solute-solvent membrane (K_m) are considered. This equation describes reasonably well the permeability of the skin to non-electrolytes, but this simplified expression applies only to steady-state permeability. The time it takes for the permeability to reach a steady-state is called the lag time (τ) for diffusion. For an simple, ideal membrane, the lag time is related to the diffusion constant by $\tau = \frac{\delta^2}{6D_m}$ (Dugard, 1983). Another factor of interest is the amount of penetrant remaining dissolved within the stratum corneum at the end of a short period of contact. The penetrant that is within the stratum corneum cannot be quickly washed away and eventually will enter the body (unless volatile or lost through desquamation), leading to the term "reservoir effect." A diagram showing the concentration profile across stratum corneum during steady-state absorption, assuming uniform properties across the membrane, is shown in Figure 3.

A corollary of Fick's law is that the chemical potential of a penetrant is maximal in a saturated solution and therefore a maximum absorption rate occurs from a saturated solution. The chemical potential of a particular chemical is the same in all saturated solutions (i.e., neat liquid or solid), regardless of solvent. This means that there is a single maximum absorption rate definable for any penetrant. The absorption rate for an ideal system is proportional to the degree of saturation, and, thus, all half-saturated solutions of a penetrant have equal chemical potential and all give half the maximum possible absorption

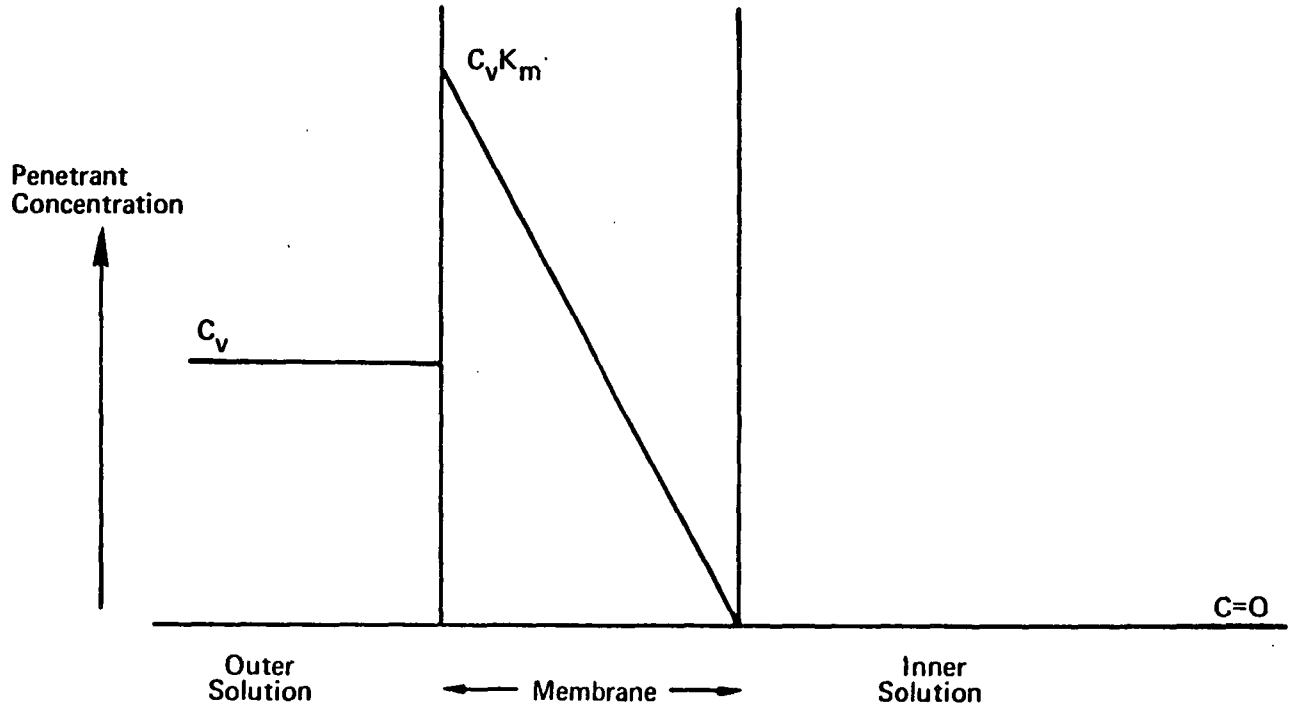

Figure 3. Diagram showing the concentration profile across stratum corneum during steady-state absorption (From Dugard, 1983)

rate (Dugard, 1983 and ref. therein).

Knowledge of any physical equilibrium condition may be used to relate the chemical potential in one vehicle or physical state to that in another. Predictive treatments based on solubilities or on equilibria are largely untested and depend on several systems behaving close to the ideal, thus any predictions should be regarded as qualitative. Reasons for the breakdown of solubility-derived predictions include solvent damage to stratum corneum, deviations from ideality near penetrant saturation in vehicles, variations in stratum corneum hydration in contact with different solvents, and entry of vehicle components into the stratum corneum to alter its solvent properties (Dugard, 1983).

6.2 NON-STEADY STATE DIFFUSION

The diffusion of the penetrant across the stratum corneum is usually the rate determining step in the dermal absorption process; however, there are instances where this is not the case. Higuchi (1962) has considered the condition where the penetrant is entirely dissolved in the vehicle and its diffusion in this medium is slow. Assuming that any penetrant reaching the stratum corneum is immediately absorbed and that not more than 30% of the original amount of penetrant is absorbed, then the total amount of penetrant (Q_t) released from the vehicle by time t is approximately

$$Q_t = 2C_v\left(\frac{D_v t}{\pi}\right)^{1/2}$$

where D_v is the diffusion constant of the penetrant and C_v is the concentration of the penetrant in the vehicle.

A second situation where diffusion across the stratum corneum is not rate limiting occurs when the vehicle contains suspended penetrant whose dissolution is rate limiting. Higuchi (1960) described the release and absorption of the

penetrant when it is in small particles evenly dispersed in the vehicle as

$$Q_t = (2\ A_v - S_v) \left[\frac{D_v t}{1 + 2\ (A_v - S_v)/S_v} \right]^{1/2}$$

where A_v is the total amount of penetrant, dissolved and suspended, in the vehicle per unit volume, and S_v is its solubility in the vehicle. The rate of absorption at a given time t is obtained by differentiating Q_t (Dugard 1983, and references therein) to yield

$$\frac{dQ}{d_t} = 1/2 \left[\frac{D_v(2A_v - S_v)S_v}{t} \right]^{1/2}$$

If A_v is much greater than S_v, these equations reduce to

$$Q_t = (2A_v D_v S_v t)^{1/2} \text{ and } \frac{dQ}{dt} = \left(\frac{A_v\ D_v\ S_v}{2t} \right)^{1/2}$$

Another type of non-steady state absorption can occur when a small amount of penetrant is applied. "Small" means that the source of the penetrant is significantly reduced in quantity by the absorption process. Figure 4 shows the manner in which the absorption rate changes with time for a fast penetrant, moderate penetrant, and a very slow penetrant, when depletion of the external source of penetrant occurs (Dugard, 1983).

Looking at Figure 4 we see that for curves A and B a specific maximum rate is achieved. The time taken to reach the maximum rate, τ_{max}, is obtained from the relationship $\tau_{max} = \frac{\delta^2 - h^2}{6D_m}$ (Scheuplein and Ross, 1974). The thickness of the penetrant layer, h, is usually small in comparison with δ, the stratum corneum thickness, thus

$$\tau_{max} = \frac{\delta^2}{6D_m}$$

This expression is the same as given earlier for the relationship between the lag time for steady-state permeability and the diffusion constant. Scheuplein and

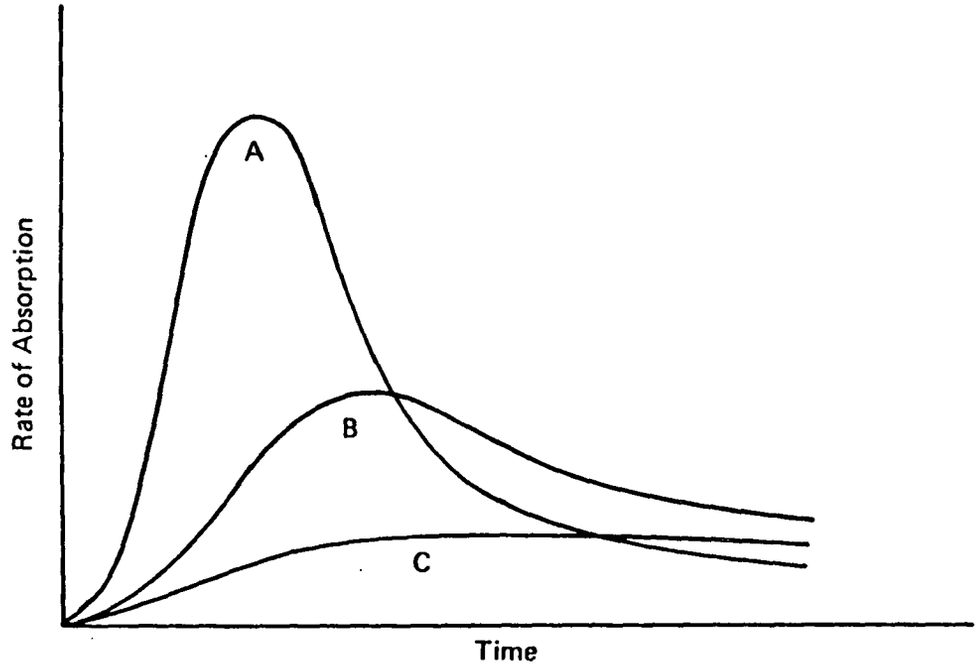

Figure 4. The pattern of changing absorption rate for small amounts of penetrant per unit area of skin. Curve A, moderately fast penetrant; curve B, slower penetrant; curve C, very slow penetrant. Curves A and B show the effect of the depletion of penetrant source. (Dugard, 1983).

Ross (1974) also state that the peak rate of absorption is directly proportional to the amount of penetrant applied per unit area, which they designate as the specific dose A.

For very slow penetrants such as cortisone, the authors found the rate of absorption to be constant for a relatively long period of time (such as curve C in Figure 4). This rate is approximately proportional to the specific dose, thus the flux can be expressed as:

$$J = k_t A \text{ (slow penetrant, } \delta > h)$$

where k_t is a transfer coefficient. Instead of permeability constant (k_p) defined in terms of flux and concentration, the transfer coefficient (k_t) is defined in terms of flux and specific dose (A). Unlike the permeability constant, as A increases k_t gradually decreases because the source of penetrant comes to contain excess material where additions cause no further increase in absorption rate (Dugard, 1983).

6.3 CALCULATION OF k_p and K_m

Scheuplein (1965) calculated k_p values for the homologous series of normal primary alcohols C_1 - C_8 by measuring the <u>in vitro</u> permeability of the alcohols through human abdominal skin in a diffusion cell. The solvent-membrane distribution coefficients K_m, or partition coefficients, were computed from the loss in concentration of the original solution after equilibrium with the tissue, i.e.:

$$K_m = \frac{\text{moles alcohol absorbed per unit mass of dry tissue}}{\text{moles alcohol in solution per unit mass of water}}$$

Penetration rates (J_s) were obtained from the rate of increase of concentration on the receptor (bottom half of the diffusion cell). Permeability constants k_p were computed directly from the linear portion of the accumulation curve or by graphical procedures.

Table 13 gives a summary of the membrane permeability and partition coefficients for the normal primary alcohol series. The molecular volume increases by a factor of 4 within the series but its effect on the rate of diffusion is less than a factor of 1.6. The Table also shows that the permeability constant increases as the molecular weight increases instead of decreasing; this is a result of the increasing alcohol-membrane solubility as a consequence of the decreasing polar character of the alcohols.

It has been known for some time that the permeability of nonelectrolytes through membranes increases as the membrane solubility of the penetrating molecule increases. More precisely it is the solubility of the penetrating molecule within the membrane relative to the solubility in the solvent (i.e., the membrane partition coefficient, K_m) which directly influences the permeability. The actual partition coefficients of lipophilic membranes are usually estimated by approximating their lipophilic solubility with olive oil-water partition coefficients. Aqueous membrane partition coefficients K_m have been measured for the alcohols and are compared with the olive-oil water values K_o in Table 13. It is apparent that there are large differences between the two partition coefficients. The olive oil-water coefficient is a poor approximation to K_m except in a narrow range near a value of $K_o = K_m = 10.0$. The deviation near the origin stems from the fact that K_m must be greater than 0.6, the lowest conceivable weight fraction of water in the tissue, while K_o values for water and the very polar alcohols are in the order of 10^{-3} owing to their low solubility in olive oil. From the deviation at higher K values it is clear that stratum corneum is a less potent absorbent for strongly nonpolar molecules than is olive oil (Scheuplein, 1965).

TABLE 13. MEMBRANE PERMEABILITY AND PARTITION COEFFICIENTS*

	$\frac{k_p}{K_m}$	$\frac{k_p V^{1/3}}{K_m}$	K_m	k_p	M	V	K_o
Water	3.3	8.65	0.3	1.0	18.02	18.02	0.000
Methanol	1.7	5.85	0.6	1.0	32.04	40.05	0.008
Ethanol	1.7	6.64	0.6	1.0	46.07	59.3	0.03
Propanol	0.7	2.95	2.0	1.4	60.09	74.9	0.17
Butanol	1.0	4.51	2.5	2.5	74.12	91.5	0.5
Pentanol	1.2	5.72	5.0	6.0	88.15	107.9	5.0
Hexanol	1.3	6.50	10.0	13.0	102.2	124.8	11.5
Heptanol	1.07	5.55	30.0	32.0	116.2	141.3	62.0
Octanol	1.04	5.60	50.0	52.0	130.2	157.4	220.0

* from Scheuplein, 1965

k_p = Permeability constant

K_m = Solvent-membrane distribution coefficient

K_o = olive oil-partition coefficient

V = Molecular volume

M = Molecular weight

Scheuplein et al. (1969) also studied a series of steroids to see how permeable these larger molecules, some with polyfunctional character, are through skin. Since steroid molecules have considerably larger molecular volumes than the linear primary alcohols and usually have several polar groups, lower diffusion rates are expected. The diffusion constants for the linear primary alcohols are approximately the same with $D = 10^{-9}$ cm^2 sec^{-1}, thus the diffusion constants for the steroids are expected to be lower. Similar experimental procedures (as for the linear alcohol series) were used to obtain permeability constants for 14 common steroids, except that partition coefficients with amyl caproate and hexadecane were also measured.

The data in Table 14 were computed from the steady state portions of flux vs. time curves; lag times (T) were extrapolated from the steady state portions of these "penetrations curves" (see Fig. 5). The concentration gradients used in the experiments were extremely small as the aqueous donor solutions were not saturated with steroid. The observed fluxes listed in column 2, Table 15, therefore do not represent maximum obtainable fluxes from water solutions of the steroids. Since Fick's law is obeyed at the very dilute concentrations characteristic of saturated aqueous solutions of steroids, maximum obtainable fluxes may be computed. These are listed in column 3 of Table 15 (Scheuplein et al. 1969).

Scheuplein et al. (1969) chose the steroids for this study to include as wide a range in polarity as possible within a certain range of molecular weight. Only a 25% difference in molecular weight exists between the lowest steroid (estrone, MW = 270.3) and the highest (hydrocortisone, MW = 360.4). However, there is approximately a 1000-fold difference in permeability between these two compounds. The difference in k_p must lie in the respective values for K_m and

TABLE 14. PERMEABILITY CONSTANTS AND PARTITION COEFFICIENTS FOR SOME STEROIDS

Steroid	ΔC_s	k_p	D	K_m	K_{ac}	K_{hex}
Progesterone	2.0	1500	160	104	56	17.0
Pregnenolone	5.1	1500	220	50	52	4.2
Hydroxypregnenolone	2.9	600	155	43	49	1.6
Hydroxyprogesterone	10.0	600	166	40	46	2.5
Cortexone	2.4	450	135	37	30	3.0
Testosterone	10	400	195	23	16	2.6
Cortexolone	10	75	36.1	23	11.2	0.1
Corticosterone	1.7	60	39.2	17	6.8	0.024
Cortisone	0.97	10	13.1	8.5	1.52	0.28
Hydrocortisone	1.8	3	4.8	7	1.30	0.009
Aldosterone	0.74	3	4.9	6.8	----	----
Estrone	2.5	3600	870	46	80	3.0
Estradiol	2.5	300	72.4	46	66	0.63
Estriol	7.0	40	19.3	23	1.64	0.23

From Scheuplein et al. (1969)

ΔC = Initial donor concentration in moles/cc x 10^{-9}

k_p = Permeability constant in cm hr^{-1} x 10^{-6} (e.g. estrone k_p = 3600 cm hr^{-1} x 10^{-6})

D = Diffusion constant in cm^2 sec^{-1} x 10^{-13}

K_m = Stratum corneum/water partition coefficient

K_{ac} = Amyl caproate/water partition coefficient

K_{hex} = Hexadecane/water partition coefficient

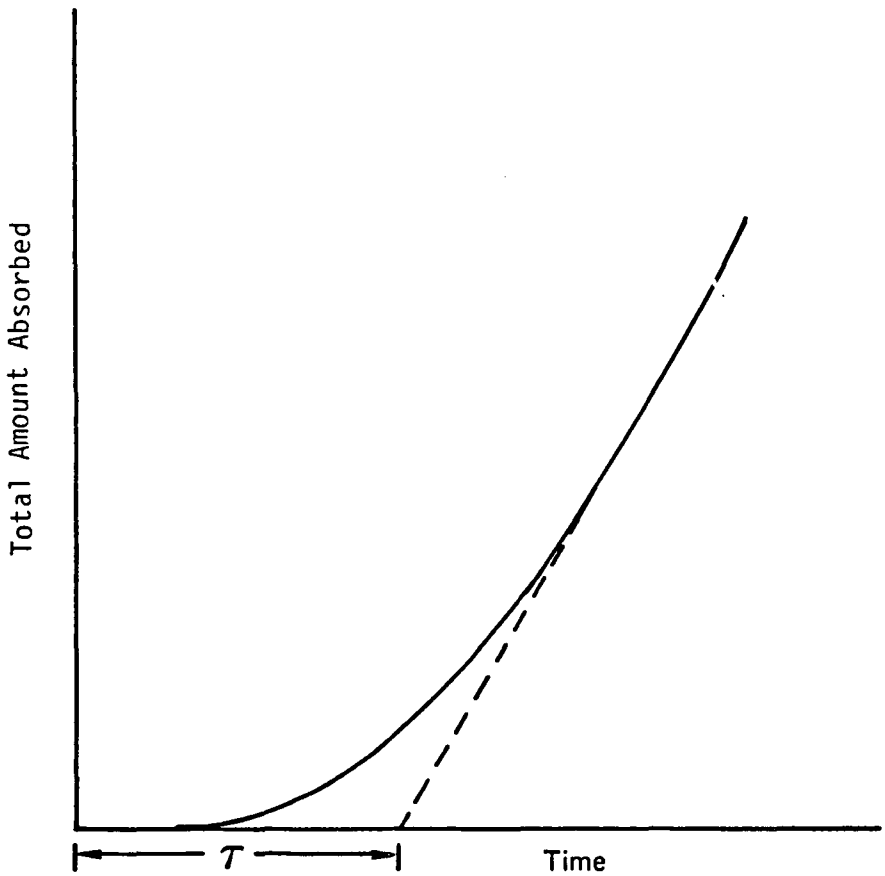

Figure 5. The extrapolation to zero absorption of the steady-state, linear region of the graph of total amount absorbed versus time yields τ, the lag time.

TABLE 15. STEROID FLUXES

The second and third columns are the observed (J.exp) and the maximum obtainable fluxes (J.max) with aqueous steroid solutions. In the last column are calculated values from *in vivo* penetration measurements where aqueous solutions were not used.

Steroid	J_s (exp)	J_s (max)	J_s (*in vivo*)
Progesterone	30	430	
Pregnenolone	51.3	810	
Hydroxypregnanolone	17	555	
Hydroxyprogesterone	60	171	
Cortexone	10.9	1350	
Testosterone	40	349	150
Cortexolone	7.5	[433]	
Corticosterone	1.04	287	
Cortisone	0.097	65	
Hydrocortisone	0.055	23	7.0 - 20.0
Aldosterone	0.022	[75]	
Estrone		173	
Estradiol		13.2	
Estriol		58.4	

From Scheuplein et al. (1969), and references therein.

J_s (exp) = Flux observed experimentally moles $cm^{-2}hr^{-1} \times 10^{-13}$.

J_s (max) = Predicted flux for saturated aqueous steroid solutions.

J_s (*in vivo*) = Values obtained from the literature.

[ ] = indicates estimates of solubility used for calculation.

D_m. As shown in Table 14 the membrane-water partition coefficient K_m changes only 15-fold through the group of steroids in comparison to the approximately 1000-fold change in k_p. Thus, it is apparent that the principal determining factor which decreases the permeability of the steroids is the decrease in the diffusion constant D_m. Since the molecular volume of the steroids is about 3-4 times that of an average small molecule such as pentanol, we expect a decrease in D_m from this factor alone arising from the increased degree of chemical interaction between the larger steroid molecule and the lipid-protein-H_2O matrix within the stratum corneum membrane. Introducing additional polar groups into the molecule lowers the diffusion constant still further. Because of this change in D_m within the series of steroids one cannot expect a proportionality between permeability constant and partition coefficient. Reference to Table 14 shows that there is no systematic relationship between k_p and K_m values.

Partition coefficients for the steroids between an ester, amyl caproate, and water and between an alkane, hexadecane, and water were also measured. Comparison of these data shows that the stratum corneum is much more similar in its solvating properties to partially polar amyl caproate than to non-polar hexadecane. Although there doesn't appear to be any direct proportionality between K_m and k_p within this group of steroids, there does exist the possibility of an useful correlation between the solubility of a steroid in a particular solvent and its permeability. From the data in Table 14, Scheuplein et al. (1969) showed that one may expect a steroid with an amyl caproate-water distribution coefficient from 20-50 to have a permeability from 400-1000 x 10^{-6} cm hr^{-1}. The rest of the steroids may be broadly grouped as:

K_{ac}	$k_p \times 10^{-6}$ cm hr^{-1}
1 - 2	1 - 10
2 - 10	10 - 70
10 - 20	70 - 400
20 - 50	400 - 1000

A similar tabulation could be made for K_{hex} and for K_m but the latter values cannot be obtained as accurately and hexadecane does not appear to serve well as an approximation for the solvating property of hydrated stratum corneum.

6.4 PREDICTION OF PERMEABILITY FOR SOME ALCOHOLS AND STEROIDS

Lien and Tong (1973) have tried to correlate percutaneous absorption data for different types of drugs or organic compounds with a number of physiochemical constants by using a computerized multiple regression analysis program. By comparing the equations of different sets of data the authors sought to obtain useful quantitative guidelines for predicting percutaneous absorption.

The authors took the absorption data and most of the chemical constants from the literature while octanol-water partition coefficients were either experimentally determined or calculated. The equation used in the computerized regression analysis program is $\log BR = - k_1 (\log P)^2 + k_2 \log P + k_3$ (electronic term) + k_4 (steric) + k_5 where BR is the biological response expressed as the molar concentration (C) of the drug absorbed, 1/C for a standard response (such as erthema or vasoconstriction), or the permeability constant (k_p). The coefficients k_1 through k_5 are obtained using the method of least squares. For some cases the solubility in water appeared to be important; therefore a log S term was also included in the analysis. The addition of other steric or electronic terms, such as molar refraction, Taft's polar sub-

stitute constant, and molecular weight, significantly improved the correlation in some cases.

For the alcohols and steroids, the variation in permeability constants is primarily due to the difference in lipophilic character. By comparing an equation derived from the permeability experiment on aliphatic alcohols through human epidermis with an equation from similar experiments on steroids, i.e.,

(alcohols): $\log k_p = 0.934 \log K_m - 2.891$ ($n = 8$, $r = 0.986$, $s = 0.121$)

(steroids): $\log k_p = 2.626 \log K_m - 7.537$ ($n = 14$, $r = 0.931$, $s = 0.377$)

we see that the permeability of steroids through the epidermis is much more dependent on the partition coefficient into stratum corneum (K_m) as compared with the alcohols. The lower intercept of the equation for steroids as compared to the equation for alcohols shows the stronger hydrophobic interactions between the steroids and the epidermis than between the alcohols and the epidermis (see Fig. 6). Tables 16 and 17 display the good correlations found for both alcohols and steroids indicating that this approach enables a chemist to predict the relative degree of penetrant absorption through skin from the physicochemical constants of a series of compounds plus the absorption data of a few parent molecules (Lien and Tong, 1973).

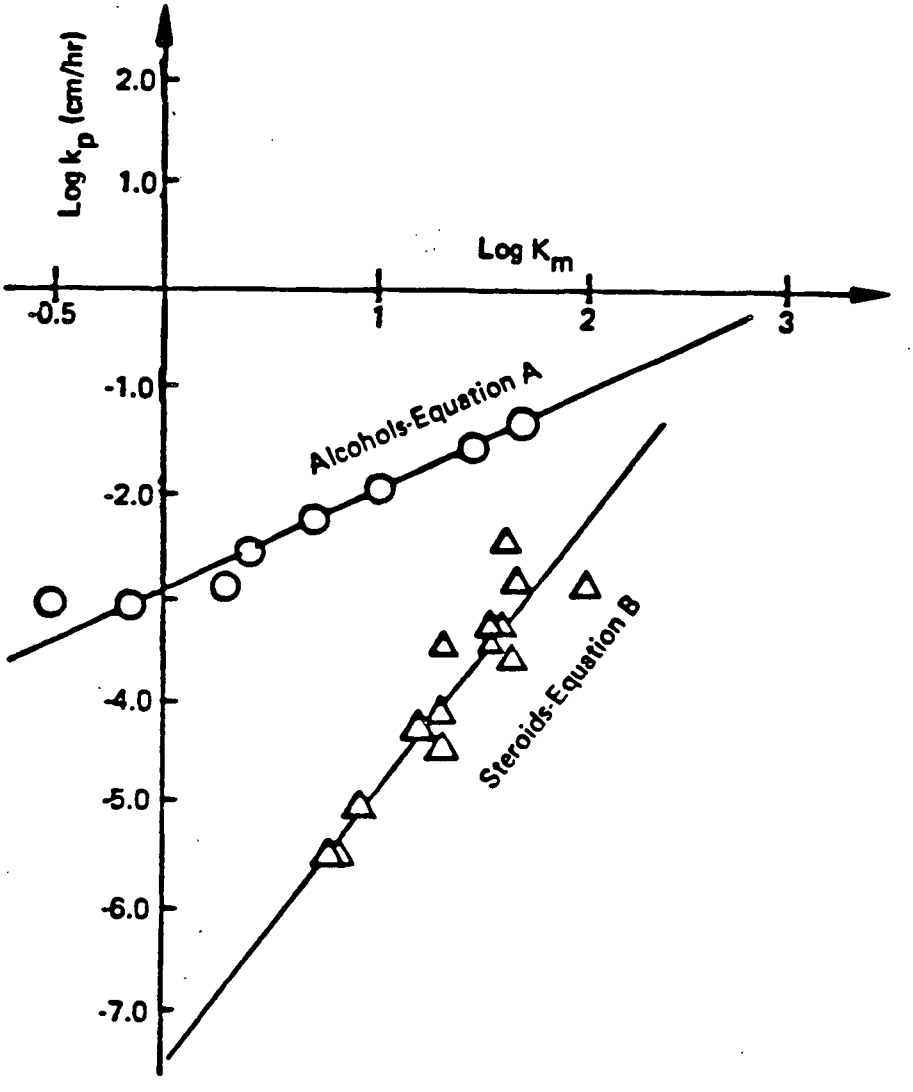

Figure 6. Dependence of the permeability constant (log k_p) on the stratum corneum/water partition coefficient (log K_m) Equation A, log k_p=0,934 log K_m-2.891 is derived from the data of a alcohols absorbed through human epidermis; Equation B, log k_p=2.626 log K_m-7.537 is derived from the data of alcohols absorbed through human epidermis (From Lien and Tong, 1973).

TABLE 16. IN VITRO PERMEABILITY OF ALIPHATIC ALCOHOLS THROUGH HUMAN EPIDERMIS*

Compound	Log K_o	Log P_o	Log K_m	Log k_p (cm/hr) Obsd.	Calcd.	Diff.
Water	. . .	. . .	-0.52	-3.00	. . .	. . .
Methanol	-2.10	-0.66[a]	-0.22	-3.00	-3.24	0.24
Ethanol	-1.52	-0.16[a]	-0.22	-3.00	-2.97	-0.03
n-Propanol	-0.77	0.34[b]	0.30	-2.85	-2.70	-0.15
n-Butanol	-0.30	0.84[b]	0.40	-2.60	-2.43	-0.17
n-Pentanol	0.70	1.34[b]	0.70	-2.22	-2.16	-0.06
n-Hexanol	1.06	1.84[b]	1.00	-1.89	-1.88	-0.01
n-Heptanol	1.79	2.34[b]	1.48	-1.49	-1.61	0.12
n-Octanol	2.34	2.84[b]	1.70	-1.28	-1.34	0.06

*From Lien and Tong 1973

[a]Experimental determined value from Leo et al., 1971.
[b]Calculated value

P_o = Octanol/water partition coefficient.

K_o = Olive oil/water partition coefficient from Scheuplein (1965)

K_m = Stratum corneum/water partition coefficient from Scheuplein (1965)

k_p = Permeability constant: observed from Scheuplein (1965), calculated from

$\log k_p = 0.544 \log P - 2.884 \quad n = 8, r = .979, s = 0.150$

TABLE 17a. IN VITRO PERMEABILITY OF STEROIDS THROUGH HUMAN EPIDERMIS*

Steroid	Log K_{hex}	Log K_{ac}	Log K_m	Log P_e
Progesterone	1.23	1.75	2.02	2.78
Pregnenolone	0.62	1.72	1.70	(2.82)
Hydroxypregnenolone	0.20	1.69	1.63	(2.24)
Hydroxyprogesterone	0.40	1.66	1.60	(2.17)
Cortexone	0.48	1.48	1.59	1.72
Testosterone	0.42	1.20	1.36	1.94
Cortexolone	-1.00	1.05	1.36	(1.11)
Corticosterone	-1.62	0.83	1.23	0.66
Cortisone	-0.55	0.18	0.93	0.15
Hydrocortisone	-2.05	0.11	0.85	0.20
Aldosterone	. . .	. . .	0.83	
Estrone	0.48	1.90	1.66	
Estradiol	-0.20	1.82	1.66	
Estriol	-0.64	0.21	1.36	

*From Lien and Tong, 1973

K_{hex} = Hexadecane/water partition coefficient from Scheuplein et al., 1969

K_{ac} = amyl caproate/water partition coefficient from Scheuplein et al., 1969

K_m = stratum corneum/water partition coefficient from Scheuplein et al., 1969

P_e = ether/water partition coefficient: experimental values without parentheses; values in parentheses were calculated from Flynn, 1971.

TABLE 17b. IN VITRO PERMEABILITY OF STEROIDS THROUGH HUMAN EPIDERMIS*

Steroid	Log K_p (cm/hr)			Log D (cm^2/sec x 10^{-13})		
	Obsd.[a]	Calcd.[b]	Diff.	Obsd.[a]	Calcd.[c]	Diff.
Progesterone	-2.82	-2.23	-0.59	2.20	2.47	-0.27
Pregnenolone	-2.82	-3.07	0.25	2.34	2.49	-0.15
Hydroxypregnenolone	-3.22	-3.26	0.04	2.19	2.18	0.01
Hydroxyprogesterone	-3.22	-3.34	0.12	2.22	2.14	0.08
Cortexone	-3.35	-3.36	0.01	2.13	1.91	0.22
Testosterone	-3.40	-3.97	0.57	2.29	2.02	0.27
Cortexolone	-4.12	-3.97	-0.15	1.56	1.59	-0.03
Corticosterone	-4.22	-4.31	0.09	1.59	1.35	0.24
Cortisone	-5.00	-5.10	0.10	1.12	1.08	0.04
Hydrocortisone	-5.52	-5.31	-0.21	0.68	1.11	-0.43
Aldosterone	-5.52	-5.36	-0.16			
Estrone	-2.44	-3.18	0.74			
Estradiol	-3.52	-3.18	-0.34			
Estriol	-4.40	-3.97	-0.43			

[a] From Scheuplein et al. 1969

[b] Calculated from log $k_p = 2.626\ K_m - 7.537$ ($n = 14$, $r = 0.931$, $s = 0.377$)

[c] Calculated from log D (cm^2/sec x 10^{-13}) $= -0.221\ (\log P_e)^2 + 1.170 \log P_e + 0.734$ ($n = 10$, $r = 0.961$, $s = 0.180$)

CHAPTER 7 - DERMAL ABSORPTION IN EXPOSURE ASSESSMENTS

7.1 CURRENT AGENCY PRACTICE

As stated in the introduction of this report the current Agency policy is to use a value of 100 percent to represent the dermal absorption of a penetrant unless a lower value can be supported by scientific studies. For most compounds this is a large overestimation of the actual dermal absorption rate or percent fraction of penetration. In Table 4 there are three compounds, hippuric acid, nicotinic acid, and thiourea, that have a total dermal absorption of less than 1 percent of the applied dose after 5 days; the predicted absorbed dose of these three compounds would be two orders of magnitude high if their dermal absorption was assumed to be 100 percent. On the other hand, compounds like carbaryl, caffeine, dinitrochlorobenzene, and DMSO are much more thorough penetrants and their absorption could be fairly well approximated by using 100 percent. From Table 5 it appears that the majority of the pesticides are close to 10 percent for their penetration value.

There are many other parameters that go into calculating dermal exposure and intake besides the dermal absorption factor. In addition, one needs to know the area of exposed skin, the concentration of the penetrant, the duration of the exposure per each event, and the frequency of events expressed in number of exposures per some unit time (usually per year). The Exposure Evaluation Division of OTS in conjuncion with Versar, Inc., developed a general scheme for calculating the dermal intake of humans (Freed, et al. 1983). One scenario is that of a thin film of penetrant on the skin. For this finite mass situation the exposure is calculated by multiplying (concentration) x (skin area) x

(frequency) times the thickness of the film layer. The dermal intake is then calculated by multiplying the exposure times the dermal absorption factor expressed as a percent or as %/hr times the duration. Another scenario is that of an excess amount of penetrant on the skin. In this case, the thickness of the penetrant layer is not calculated; steady-state kinetics are assumed and the dermal intake is calculated by multiplying (skin area) x (concentration) x (frequency) x (duration) x (permeability constant, kp).

Freed et al. (1983) give a sample calculation in their document showing how the parameters above are derived for the case of ambient human exposure to penetrants in water from swimming. Information on the frequency and duration of outdoor swimming was found in a report by the Bureau of Outdoor Recreation which was based on a survey of 11,000 people. For this group, 34 percent swam in rivers, lakes, and oceans, the average frequency of swimming was 7 days per year, and the average duration was 2.6 hours per day yielding a periodicity of 18.2 hours/year. The availability for dermal exposure in this example was assumed to equal the total amount of human skin surface area which is 17,000 cm^2 for the average adult and 7,700 cm^2 for the average child (1-10 yrs). The ambient aqueous concentration of a penetrant can be determined by using monitoring data and/or by modeling. Multiplying (skin area) x (conc.) x (frequency) x (duration) times the absorption rate for the specific penetrant will give the dermal intake.

In another study, Brown et al. (1984) looked at the role of skin absorption as a route of exposure for volatile organic compounds (VOCs) in drinking water. They estimated absorption levels for an adult taking a 15-minute bath and

drinking two liters of water, an infant bathed 15 minutes and fed one liter of water, and a 48-pound child swimming one hour and drinking a liter of water. Concentrations of each VOC were varied from 0.005 mg/l of water to 0.5 mg/l. The authors found that for the swimmer, a minimum of 83 percent and possibly 91 percent of the chemicals entering the body came through the skin. An interesting result is that the highest doses resulted from water with the smallest concentration of the penetrant. Scheuplein and Blank (1973) have also shown the permeation rates are actually increased with dilute solutions as compared to pure liquids. They illustrate this with the example of hexanol: liquid hexanol (8.2 M) is approximately 150 times more concentrated than saturated aqueous hexanol (0.055 M), yet the permeation rate of aqueous hexanol, far from being 150 times less than the pure liquid, is almost twice as great. This apparently anomalous behavior is attributed to the compaction and dehydration of the stratum corneum when in contact with pure liquids making it less porous, as well as to the distribution factors of changing gradients and partition coefficients understood in Fick's Law.

7.2 EXAMPLES OF DERMAL INTAKE CALCULATIONS

7.2.1 Dermal Exposure from Swimming

There are several specific penetrant examples of how to calculate dermal absorption of a penetrant in swimming water. Scow et al. (1979) prepared a document for the Office of Water Planning and Standards in which the exposure levels for heptachlor and chlordane were calculated. The water flux (J) through the skin is taken to be between 0.2 to 0.5 mg/cm^2-hr while the flux of the solute (penetrant) is estimated by multiplying the water flux by the weight

fraction of penetrant in the water. At a concentration of 1 ppb, the flux of penetrant would be 0.2×10^{-9} to 0.5×10^{-9} mg/cm^2-hr. Using a representative body surface area of 1.8×10^4 cm^2, if one swam for 4 hours in any given day in water containing 1 ppb chlordane, then from 0.013 to 0.036 ug chlordane might be adsorbed.

Beech (1980) calculated the amount of chloroform absorbed over a 3 hour period by a 6 year-old boy swimming in water containing 500 ug chloroform per liter. The average 6 year-old is assumed to weigh 21.9 kg and have a surface area of 0.88×10^4 cm^2. The permeability constant (kp) for chloroform in aqueous solution was assigned the value of 125×10^{-3} cm/hour. The flux through the skin per hour is: 125×10^{-3} cm/hour x 500 ug/liter x 1 liter/1000 cm^3 x 1000 mg/1 ug = 62.5×10^{-6} mg/cm^2 hour.
The total dermal intake is then: 3 hr x 62.5×10^{-6} mg/cm^2 hr x 0.88×10^4 cm^2 = 1.65 mg chloroform.

7.2.2 Pesticide Application

Another area of potentially significant dermal exposure is that of pesticide application and worker reentry to areas where crops have been sprayed. Maddy et al. (1983) developed exposure monitoring techniques designed to investigate the factors influencing pesticide exposure to workers during the application process. The authors conducted dermal exposure monitoring of workers involved in the application of parathion, meinphos, nitrofen, DEF/Folex, and chlorobenzilate. Exposures of mixers/loaders, ground applicators, mixer/loader/ground applicators (workers performing all three activities during a single application), aerial applicators, and flaggers were determined in a total of 102 individual

exposure situations. California regulations require use of clean outer clothing to reduce the potential dermal exposure of workers to pesticides by decreasing the area of bare skin available for contact with the chemicals. Rubber or some other type of waterproof gloves were worn by all workers except the aerial applicators and flaggers.

Hand exposure was determined by rinsing the hands in a predetermined solvent containing either water, soap and water, ethanol, or a combination of the three. Hands were rinsed prior to and upon completion of the applications. Exposures to the hands, face, and neck were estimated by placing small patches on the upper collar of the coveralls in the front and back, or, in some cases, placing patches directly on the face. Values were extrapolated to the entire surface areas of these body parts. Potential exposure to skin protected by coveralls was also measured with patches.

Table 18 summarizes the average percentage of total dermal exposure found on various regions by individual chemical and job activity. The results show that estimated exposure to protected body areas represented only 23.3 percent of the total dermal exposure. Hand exposure exceeded exposure to all other areas; workers who wore waterproof gloves still experienced hand exposure representing 40.9 percent of their total dermal exposure. This result indicates why the hands are not considered to be a protected area even though waterproof gloves were usually worn. The author's possible explanations for the relative ineffectiveness of the gloves include (1) contamination of the inside material of the gloves, (2) removal of gloves during mechanical adjustments to the application equipment, and (3) the handling of the outside of contaminated

gloves while putting them on or taking them off.

Maddy et al. (1983) conclude that more attention should be given to the monitoring of the hands, head, face, and neck, and less attention to monitoring protected areas. They also suggest that since hand exposure was responsible for more than 42 percent of the total dermal exposure, estimates of total dermal exposure could be derived by multiplying the hand exposure by a factor of 2.5.

7.2.3 Treated Field Reentry

A second example of dermal intake resulting from pesticide application is the worker reentry study of Popendorf et al. (1979). Five peach orchards were harvested each for three days at decreasing post-application intervals. Both aerosol and dermal exposure estimates were made for the organophosphate pesticide Zolone plus its metabolite Zoloxon. Low levels of Guthion (also an organophosphate) were present in some of the orchards from prior applications. Aerosol samples were collected at 1.7 lpm for one to two hours during the workday from near the breathing zone of each worker. Respiratory doses were calculated from the airborne concentrations and estimated respiratory volumes of 24 m^3 during each three day work sequence. Dermal doses to pickers were estimated using 4 x 4 inch gauze patches taped to the skin. A knit nylon glove backed by a pad was used to monitor hand exposure. At the end of each week, the patches from each location were pooled for extraction and analysis. The resulting value was adjusted for exposure time and patch area at each location and then multiplied by its proportionate body surface area; these were summed to estimate the whole body dermal dose of each compound for each week (see Table 19). Approximately

TABLE 18A. RELATIVE CONTRIBUTIONS TO TOTAL DERMAL EXPOSURE OF BODY AREAS TO PESTICIDES AS STUDIED BY THE CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE

Chemical	Number of Exposures Monitored	Average Percentage of Total Dermal Exposure Found on Hands	Average Percentage of Total Dermal Exposure Found on Head, Face and Neck	Average Percentage of Total Dermal Exposure Found on Hands and Head, Face and Neck	Average Percentage of Total Dermal Exposure Found on Protected Area
Parathion	3	23.4	67.4	90.8	9.2
Mevinphos	22	48.0	34.6	82.6	17.4
TOK	24	49.2	22.7	71.9	28.1
DEF/Folex	32	46.8	23.7	70.5	29.5
Chlorobenzilate	21	27.1	59.4	86.5	13.5
Total	102	42.9	33.8	76.7	23.3

TABLE 18B. RELATIVE CONTRIBUTIONS TO TOTAL DERMAL EXPOSURE OF BODY AREAS BY JOB ACTIVITY

Job Activity	Number of Exposures Monitored	Average Percentage of Total Dermal Exposure Found on Hands	Average Percentage of Total Dermal Exposure Found on Head, Face and Neck	Average Percentage of Total Dermal Exposure Found on Hands and Head, Face and Neck	Average Percentage of Total Dermal Exposure Found on Protected Areas
Mixer/Loader, Ground Applicator	4	18.1	57.5	75.6	24.4
Mixer/Loader	36	50.7	22.0	72.7	27.3
Aerial Applicator	18	54.6	27.4	82.0	18.0
Ground Applicator	25	30.4	47.9	78.3	21.7
Flagger	19	38.7	38.6	77.3	22.7
Total	102	42.9	33.8	76.7	23.3

From Maddy et al. 1983

98-99% of the total doses thus calculated were attributed to the dermal rather than respiratory route. These calculations do not account for possible variations in the rate of chemical absorption at different skin locations.

7.2.4 Hypothetical Dermal Intake of PCBs

Versar, Inc. (1983a) in conjunction with the Exposure Evaluation Division of OTS has estimated maximum probable dermal intake to PCBs for several scenarios. The first example deals with dermal exposures in the occupational environment. Annual dermal intake of PCBs resulting from use of a specific chemical or product (assuming that no protective clothing or equipment is worn) can be estimated from

Amount PCBs absorbed (mg/yr)	=	PCBs available for absorption (mg/event)	x	Frequency of exposure (events/yr)	x	Absorption (%)

where, for liquids, PCBs available for absorption = T x L x C x S

- T (liquid film thickness) is assumed to be 0.0018 cm. This is the average of the measured film thicknesses of five solutions on the skin after immersion of hands into the solution followed by a partial wipe with a rag: mineral oil, cooking oil, bath oil, 50% bath oil/50% water, and water (Versar, 1983b)
- L (density of liquid) is assumed to be 1.6×10^3 mg/cm^3.
- C is the PCB concentration in the liquid (kg/kg)
- S (skin area exposed) is assumed to be the entire surface area of both hands which is taken to be 870 cm^2.

and, for dusts, PCBs available for absorption = M x C x S

- M is the maximum mass of a dust that can adhere to one cm^2 of skin which is 2.77 mg/cm^2 (Versar, 1982)

TABLE 19. WORKER REENTRY EXPOSURE

INTEGRATED DERMAL DOSE TO EACH LOCATION, ALL VALUES IN mg

	Week			
Zolone	1	2	3	4
Hands	95.0	116.1	84.2	158.0
Forearms	9.8	17.6	12.2	12.3
Upper arms	4.3	14.5	13.4	13.1
Head	7.3	11.6	23.0	24.1
Neck	1.6	2.4	2.4	2.6
Shoulders	0.4	1.7	1.4	0.7
Chest	1.0	1.8	1.1	2.5
Back	0.6	1.7	2.0	3.8
Hips	0.2	0.2	0.6	1.3
Thighs	0.7	1.0	2.3	5.1
Calves	1.4	0.3	0.5	7.9
Feet	0.2	0.1	0.1	0.9
Total without hands	27.3	52.9	58.9	74.3
Overall	122.3	169.0	143.1	232.1
	Guthion†	Zoloxon	Zoloxon	Zoloxon
Hands	20.8	2.17	1.82	1.39
Forearms	2.82	0.28	0.29	0.08
Upper arms	0.57	0.17	0.35	0.11
Head	1.38	0.17	0.68	0.22
Neck	0.30	0.03	0.05	0.04
Shoulders	0.06	0.04	0.04	0.01
Chest	0.24	< 0.01	0.03	0.03
Back	0.06	< 0.01	0.05	0.10
Hips	0.04	< 0.01	0.02	0.02
Thighs	0.14	< 0.01	0.06	0.07
Calves	< 0.02	< 0.01		0.01
Feet		< 0.01		0.01
Total without hands	5.62	0.69	1.56	0.74
Overall	26.4	2.86	3.38	2.13

†A total dose of 2.6 mg Zoloxon (2.5 to the hands) in week 1 is not listed.

From Popendorf et al. 1979

- C is the PCB concentration in dust (kg/kg)
- S is the skin area exposed per event (cm^2/event)

A sample calculation for the exposure scenario of loading/unloading a liquid containing 50 mg/kg PCB with a frequency of 96 times/yr (with absorption assumed to be 100%) is:

Amount PCBs absorbed (mg/yr) = T x L x C x S x 96 events/yr. x 100%

= 0.0018cm x 1.6 x 10^3 mg/cm^3 x 50 mg/kg x 870cm^2 x 96 events/yr

= 12 mg/yr

The second example is the calculation of the maximum probable individual dermal intake to PCBs resulting from routine use of general household cleaners containing incidental PCBs. The dermal intake calculations apply to typical use of a solid household detergent designed to be mixed with water. The following assumptions were made:

- 96 gm of detergent is used per cleaning job and detergent is mixed with 1 gallon (3.785 l) of water.
- Detergent contains 25 percent by weight of the PCB - contaminated chemical.
- A 0.0024cm thick film of the cleaning solution remains on the hand and part of the forearm, covering a skin surface area of 500 cm^2, after each immersion. This is the measured film thickness for a solution of 50% water/50% bath oil retained on the skin after immersion of hands into the solution followed by a partial wipe with a rag (Versar, 1983b). Therefore, 0.0024 cm x 500 cm^2 = 1.2 cm^3 (ml) of solution remain on the skin.

- Detergent is used once a week for a total annual frequency of 52 times a year.
- One hand is immersed 20 times in the bucket during weekly cleaning sessions and no gloves are worn.
- All PCBs in the solution remaining on the skin after each immersion are available for dermal absorption.

The PCBs available for absorption (mg/event) = A x B x C x D

A = PCB in constituents (mg/kg)

B = Aqueous dilution of detergent; quantity detergent per volume of water (kg/ml)

C = Weight fraction of PCB - contaminated constituent

D = Volume of solution on skin per immersion (mg/event)

Frequency = 20 immersions per week x 52 weeks per year = 1,040 events/yr

Absorption = 100 percent

A sample calculation for PCB concentration of 50 mg/kg is :

$$\text{Amount PCBs absorbed} = A \times B \times C \times D \times \text{Frequency} \times \text{Absorption}$$

$$= \frac{50\ \text{mg}}{\text{kg}} \times \frac{.096\ \text{kg}}{3.785 \times 10^3\ \text{ml}} \times .25 \times 1.2\ \frac{\text{ml}}{\text{event}} \times 1{,}040\ \frac{\text{events}}{\text{yr}}$$

$$= .4\ \text{mg/yr}$$

CHAPTER 8 - ANALYSIS/FUTURE RESEARCH NEEDS

The most direct method of obtaining percutaneous absorption data for a penetrant is to conduct a human *in vivo* study. We have seen, however, that there are many problems with this direct approach such as toxicity concerns, having to monitor urine output for 21 days for slow penetrants, and the 5-fold variation possible between subjects. Kligman (1983), in a recent review of percutaneous absorption, states in comparing *in vivo* to *in vitro* results that *in vitro* data are more credible on technical grounds alone as the methods are more precise, involve less experimental error, and the variables are under much greater control. From the limited number of *in vitro* results presented in this review, it appears that the *in vitro* studies are more reproducible than *in vivo* studies. Because of the problems associated with *in vivo* studies, it is suggested that research be focused on compiling absorption data with *in vitro* studies using 1 type of skin which then can be compared to the permeability of skin from all other parts of the body (i.e., adominal skin could be tested and ratios such as found in Table 2 could be used to determine absorption at other sites like the forearm or palm). The *in vitro* results could then represent actual penetration *in vivo*; however, it may be prudent to include a "safety factor" of 5 (or other number derived from more extensive studies) to account for the variation between subjects as noted in the *in vivo* studies.

The previous chapters have shown the importance of the stratum corneum/water partition coefficient, K_m. Unfortunately, there is only a limited data set of measured K_m values, thus its utility as a predictor of dermal absorption is largely unknown. Furthermore, the accuracy of measured K_m values is questioned

by Scheuplein et al. (1969) who state that partition coefficients like $K_{amyl\ caproate}$ (K_{ac}) or K_{hexane} can be obtained more accurately than K_m.

It is suggested that a program to measure K_m for a large number of chemicals and covering a variety of functional groups be established. In this manner we could determine how well *in vitro* K_m measurements represent the actual *in vivo* partition between the penetrant and skin. Also, we would like to know if K_m can be calculated from other partition expressions like K_{ac} or $K_{olive\ oil}$. For example, a regression analysis was done for the linear alcohol series giving $K_m = aK_{olive\ oil}^{b}$ with $r = .97$, $a = 3.748$, and $b = .448$ showing a good correlation for this series. However, a similar regression analysis for the steroids (the only other chemicals with measured K_m values) had a poorer fit with $r = .78$, $a = 8.742$, and $b = .4288$, possibly due to inaccuracies in the measurement of K_m.

The second parameter of importance needed to calculate permeability is the diffusivity (D). We have see that for the linear primary alcohols D is relatively constant ($D = 10^{-9}\ cm^2\ sec^{-1}$), while for the series of steriods, D is not constant, varying by approximately 200-fold. This variation can be explained in part by changes in molecular volume and polar functional groups. As with K_m, it is suggested that D values be tabulated for a number of chemicals covering a variety of functional groups so that the dynamic range of D can be determined. It may be possible to approximate D for an unknown chemical by comparison to chemicals with measured D values.

The compilation of K_m and D values for a number of representative chemicals will facilitate the estimation of k_p for unknown chemicals. In

order to promote consistency in the compilation of these values, it would be prudent to decide what tissue (man or animal) to use in diffusion cells and partition measurements of K_m plus from what body site. If animals are to be used either for *in vivo* or *in vitro* studies, it would be most helpful for comparative purposes to decide what animal species is closest to man and use only the species chose. The rhesus monkey and miniature swine are closest in absorption properties to man, but the rat may be the species of choice due to economy and ease of handling. Also, it is suggested that clearcut procedures be developed to measure K_m and D values for penetrants of low water solubility. *In vitro* diffusion cell measurements are currently restricted to penetrants that are at least partially water solubile, although some effort is underway to use solvent/water mixtures for the receptor bath.

Values for K_m and D are required to calculate the permeability when there is an excess of penetrant and steady-state kinetics are followed. However, there are other scenarios, such as when a finite amount of penetrant (i.e., thickness of penetrant layer is smaller than the thickness of the stratum corneum) is applied and the penetrant is fast; Fickian diffusion kinetics may not be followed for this case. It would be useful to establish expressions for kinetic flux for all possible scenarios such as: finite amount of a fast penetrant, finite amount of a slow penetrant, excess of a penetrant where the diffusion through the stratum corneum is not rate limiting, buildup of penetrant in blood so that $C_0 \neq 0$, etc. In this manner, once the appropriate scenario is determined from the exposure

situation, the proper kinetic expression can be used to determine the flux.

The use of partition systems to predict dermal absorption has been discussed in the previous chapters. Partition coefficients themselves have been predicted from structure-activity relationships, number and kind of functional groups, and by other techniques (Sato and Nakajima 1979, Hansch et al. 1972 and 1973, Fujita et al. 1964, Leo and Hansch 1971a and 1971b, and Katz and Shaikh 1965). Thus, it may be possible to use the methods developed to predict partition coefficients to calculate dermal absorption once the relationship between a partition coefficient and permeability is established.

We have seen in the previous chapters that there are two approaches used to represent the amount of penetrant diffusing through the stratum corneum. The first and most common method is to use the total percent adsorption calculated or estimated (such as 100% absorption); this method does not take into account the contact time or the particular kinetics that may apply depending on the speed of the penetrant, its thickness on the skin, or if it is bound to the vehicle in some manner. The second method is to use an absorption rate which then has to be coupled with a contact time. It would be valuable to review the advantages/disadvantages of these approaches, particularly in terms of their relative uncertainty.

Since the conservative approach when no data is available for a penetrant is to use 100 percent absorption, it may be possible to group penetrants into a numerical system such as 100% - 10% - 1% - .1% absorption depending on physical parameters such as K_m and D, on partition coefficients,

or *in vitro* tests. This grouping of penetrants into a numerical system based on physical parameters is similar in intent to the grouping of steroids by their K_{ac} value as is done by Scheuplein et al. (1969) (see Chapter 6). The use of a numerical system is an "order of magnitude" approach which may be justified when the uncertainty of all the factors leading to dermal intake are taken into consideration.

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