Dermal Toxicology of Developmental products

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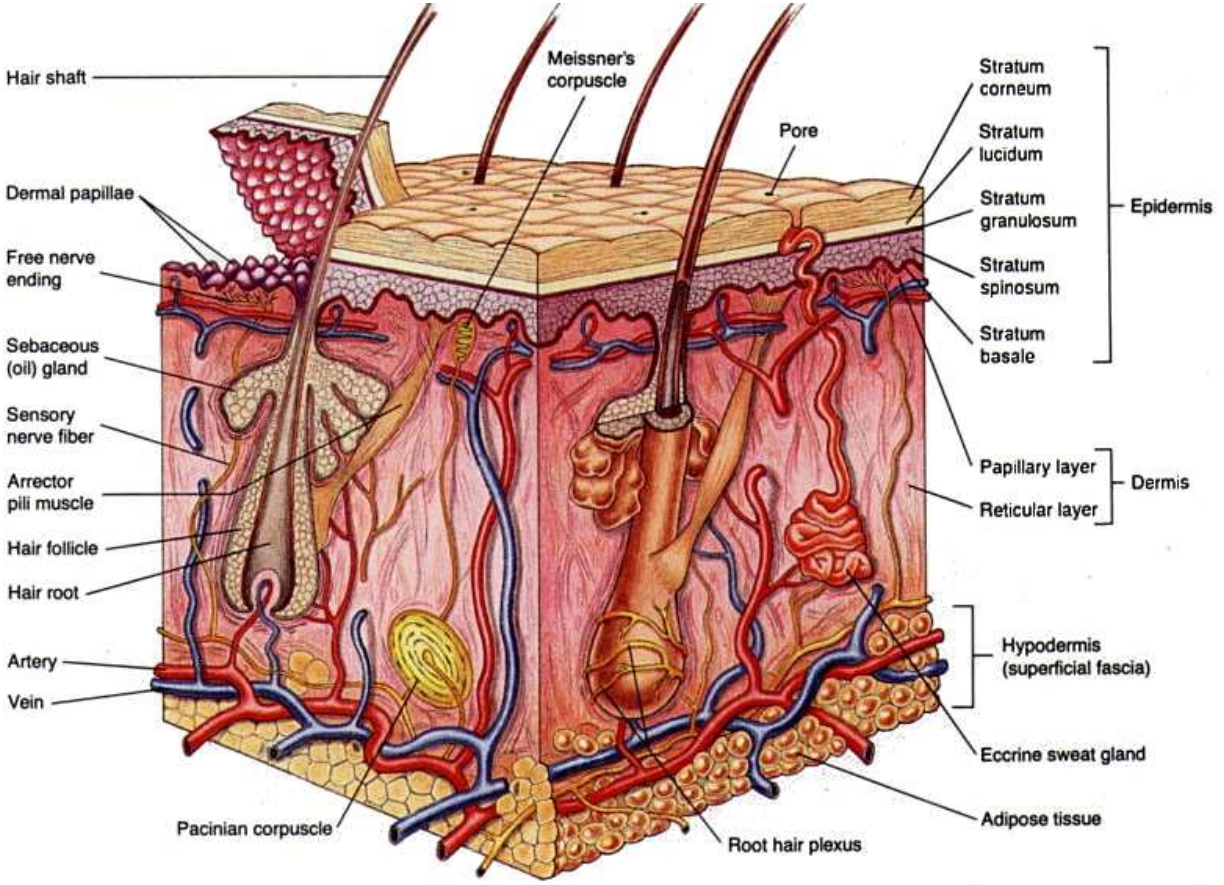
Skin - Histology



Skin

- Skin of an average adult body covers a surface of approximately 2 m^2
- Skin receives about one-third of the blood circulating through the body
- Human skin surface contains, 10 - 70 hair follicles and 200-250 sweat ducts on every square centimeters of the skin area
- Skin accounts for about 16 percent of a person's total body weight.
- Average weight of skin is 20 Pounds, which is the second heaviest organ following skeletal muscle
- Entire superficial layer of the skin is replaced every two weeks

Skin

- Skin is the most easily abused organ due to its external exposure
- There is considerable interest in the skin as a site of drug application both for local and systemic effects
- However, the stratum corneum, poses a formidable barrier to drug penetration thereby limiting topical and transdermal bioavailability
- In spite of technological advancements, there is no magic answer to increase bioavailability of drugs through skin

Functions of Skin

1. Environmental Barrier
    a. Diffusion Barrier
    b. Metabolic Barrier
2. Mechanical Support
3. Neurosensory reception
4. Physiologically, skin participates directly in
    a. Thermal regulation
    b. Regulation of blood flow, hair, fur growth and sweating
    c. Metabolism
    d. Keratin, melanin, lipids, vitamin D synthesis, respiration and biotransformation
    e. Immune regulation
    f. Sebacious glandular secretion

Dermal Exposures

Accidental or deliberate exposure

- ✓ commercial and home and garden pesticides
- ✓ polymer and paint chemicals
- ✓ detergents and cleaning chemicals
- ✓ a broad range of heavy industrial chemicals
- ✓ unscheduled exposures to environmental accidents and
- ✓ mishandling of toxic waste disposal
- ✓ Cosmetics
- ✓ Pharmaceuticals

Primary Irritant Chemicals

- Ammonia
- Acids
- Alkaline agents
- Hydrogen peroxide
- Phenol
- Chlorine
- Antiseptic or germicidal agents (cresol, iodine, boric acids, hexachlorophene, thimerosal)
- Thimerosal is the mercury vaccine preservative.

Coconut Coir

No Chemical Irritation in olden days and even today in villages

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Manifestation

- Contact Dermatitis
- Allergic Contact Dermatitis
- Ulcers
- Urticaria
- Toxic Epidermal Necrolysis
- Acneiform Dermatoses
- Pigment Disturbances
- Photosensitivity
- Skin Cancer

Highest Incidence of Chronic Irritants Dermatitis of Hands

Food handler

Janitorial workers

Construction worker

Mechanics

Metal worker

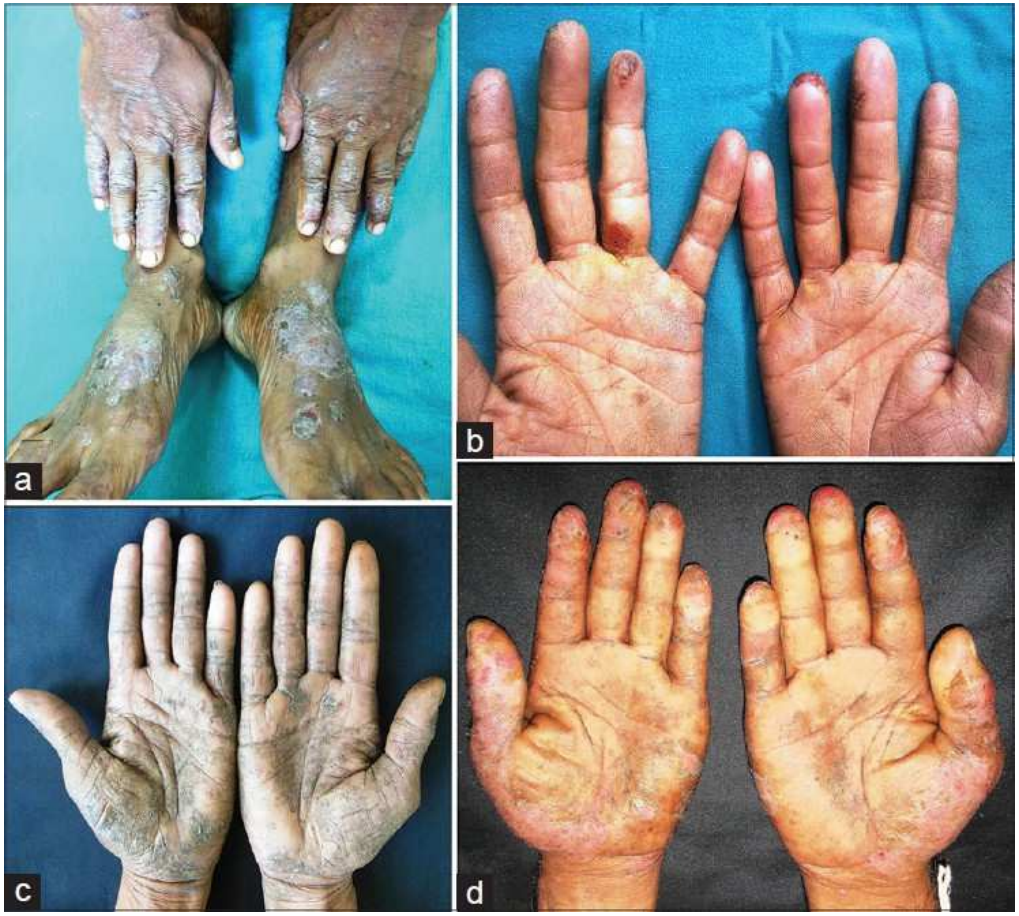
Horticulture

Hairdresser

Nurses

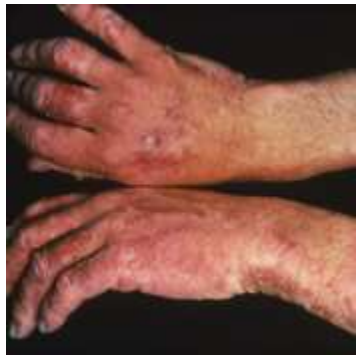
Leather industry

Carpentry workers



Allergic Contact Dermatitis

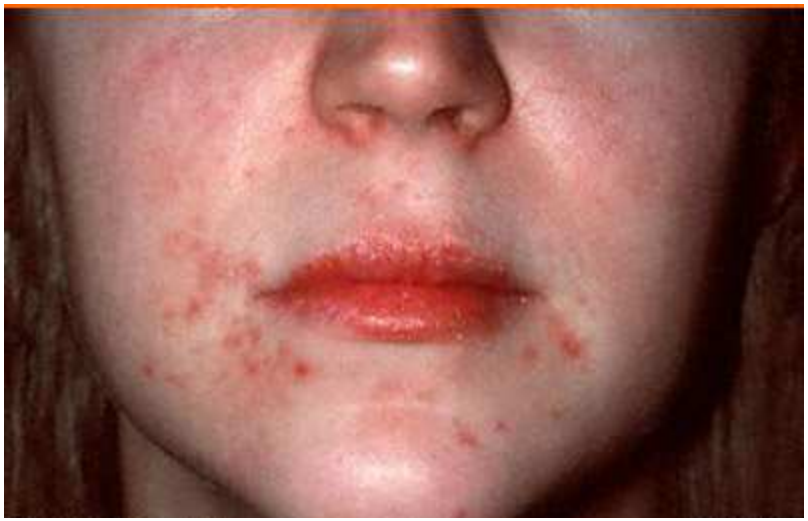
- ✓ **Acute and Delayed type IV hypersensitivity reaction that is mediated by a triggered immune response.**
- ✓ **Similar to irritant contact clinically but more severe and not restricted to the site of exposure.**
- ✓ **First exposure: little to no response**
- ✓ **Second exposure: sensitization**
- ✓ **Genetics play a role in it. (e.g. hay fever)**



Acneiform Dermatosis

The most common causes of acne in work places is petroleum, coal tar, and cutting oil products.

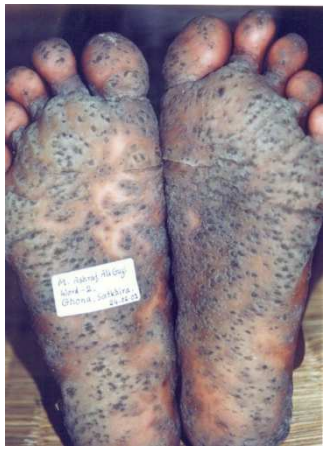
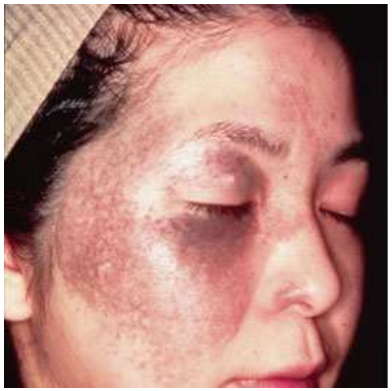
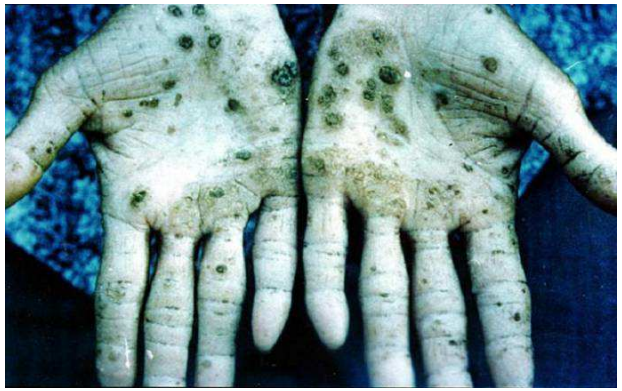
Halogenated chemicals – polyhalogenated naphthalene



Pigments Disturbances

Hyperpigmented inducers: coal tar compounds, mercury, lead, arsenic, petroleum oils.

Hypopigmentation agents: phenols and catechols

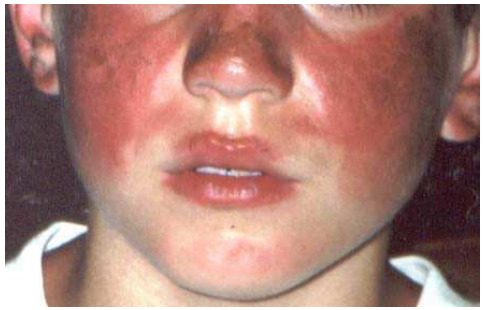

Photosensitivity

UVB is 100-fold more potent than UVA.

UVB causes erythema

Chronic exposure to UV light causes:

- **freckling,**
- **wrinkling,**
- **precancerous and malignant skin lesions**
- **UV light is the primary cause of skin cancer**

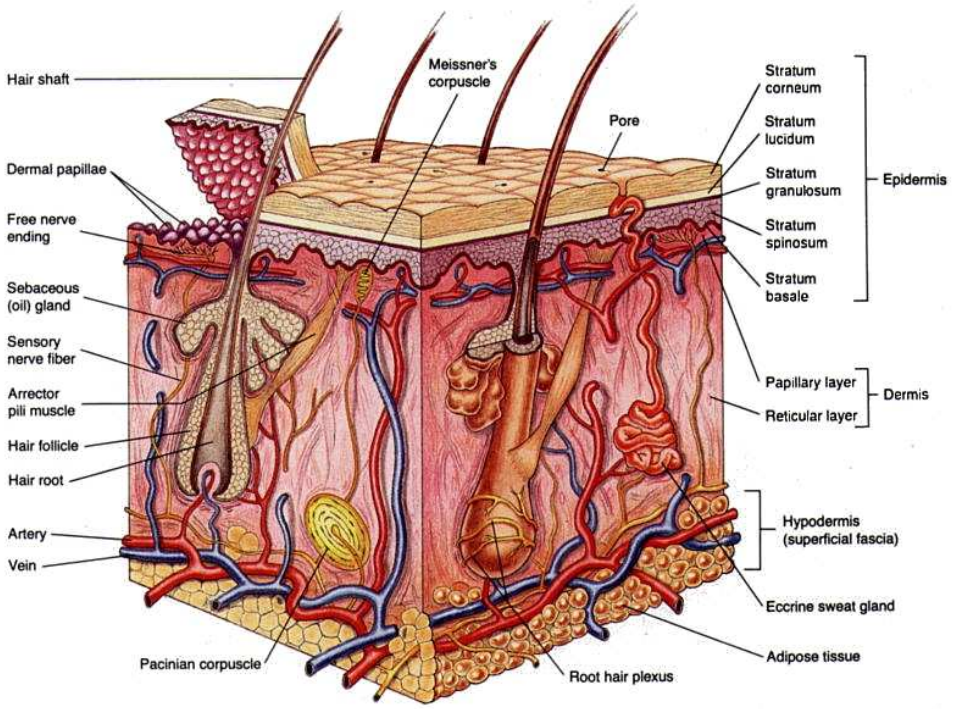


Percutaneous Absorption

1. Dermal absorption is prevalent for any compounds, with the exemption of highly volatile chemicals and polymers.

2. Research is directed towards understanding transdermal flux rates and the toxicological consequences of penetration.

3. At the practical end, such data contribute to risk assessment.



1. **The rate-determining barrier in the dermal absorption of chemicals is the epidermis—especially the stratum corneum (horny layer), the upper most layer of the epidermis.**

2. **The cell walls are chemically resistant with much lower permeability for toxicants by diffusion—the stratum corneum cells have lost their nuclei and are biologically inactive (dead)-Keratin.**

3. **Once a toxicant is absorbed through the stratum corneum, absorption through the other epidermal layers is rapid.**

Percutaneous Transport

1. **Molecules traverse membranes either by**
   a. **Passive diffusion**
      - **Solute flux is linearly dependent on the solute concentration gradient**
   b. **Active transport**
      - **Typically involves a saturable mechanism**

2. **Percutaneous flux is directly proportional to the concentration gradient and, therefore, transport across the skin occurs primarily by passive diffusion**

Factors that Affect Stratum Corneum Absorption of Toxicants

1. **Hydration of the *stratum corneum***
   - **The stratum corneum is normally 7% hydrated which greatly increases permeability of toxicants. (10-fold better than completely dry skin).**
   - **On additional contact with water, toxicant absorption can increase by 2- to 3-fold.**
2. **Damage to the *stratum corneum***
   - **Acids, alkalis and mustard gases injure the epidermis and increase absorption of toxicants.**
   - **Burns and skin diseases can increase permeability to toxicants.**
3. **Solvent Administration**
   - **Carrier solvents and creams can aid in increased absorption of toxicants and drugs {e.g. dimethylsulfoxide (DMSO)}.**
5. **The skin has reservoir capacity for the chemicals.**

All toxicants Move Across the Stratum Corneum by Passive Diffusion

1. Polar substances diffuse through the outer surface of protein filaments of the hydrated stratum corneum.

2. Non-polar molecules dissolve and diffuse through the lipid matrix between protein filaments.

3. The rate of diffusion is proportional to lipid solubility and inversely proportional to molecular weight.

4. Once absorbed, the toxicant enters the systemic circulation by-passing first-pass metabolism.
5. Regional variations in skin permeability are correlated with quantitative differences in lipid content rather than SC thickness or cell number

Factors Affecting Percutaneous Absorption

Biological

- Skin age
- Skin condition
- Anatomical site
- Skin metabolism
- Circulatory effects

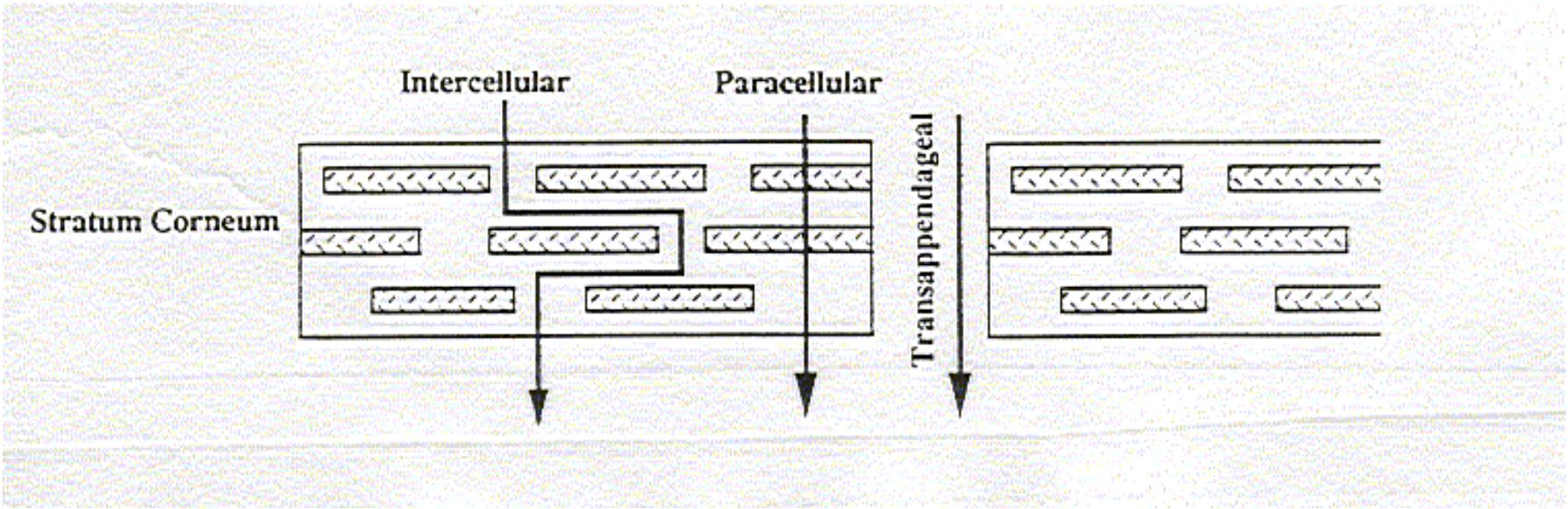
Physical

- Drug concentration
- Surface area
- exposure time
- Occlusion
- Vehicle

Physicochemical

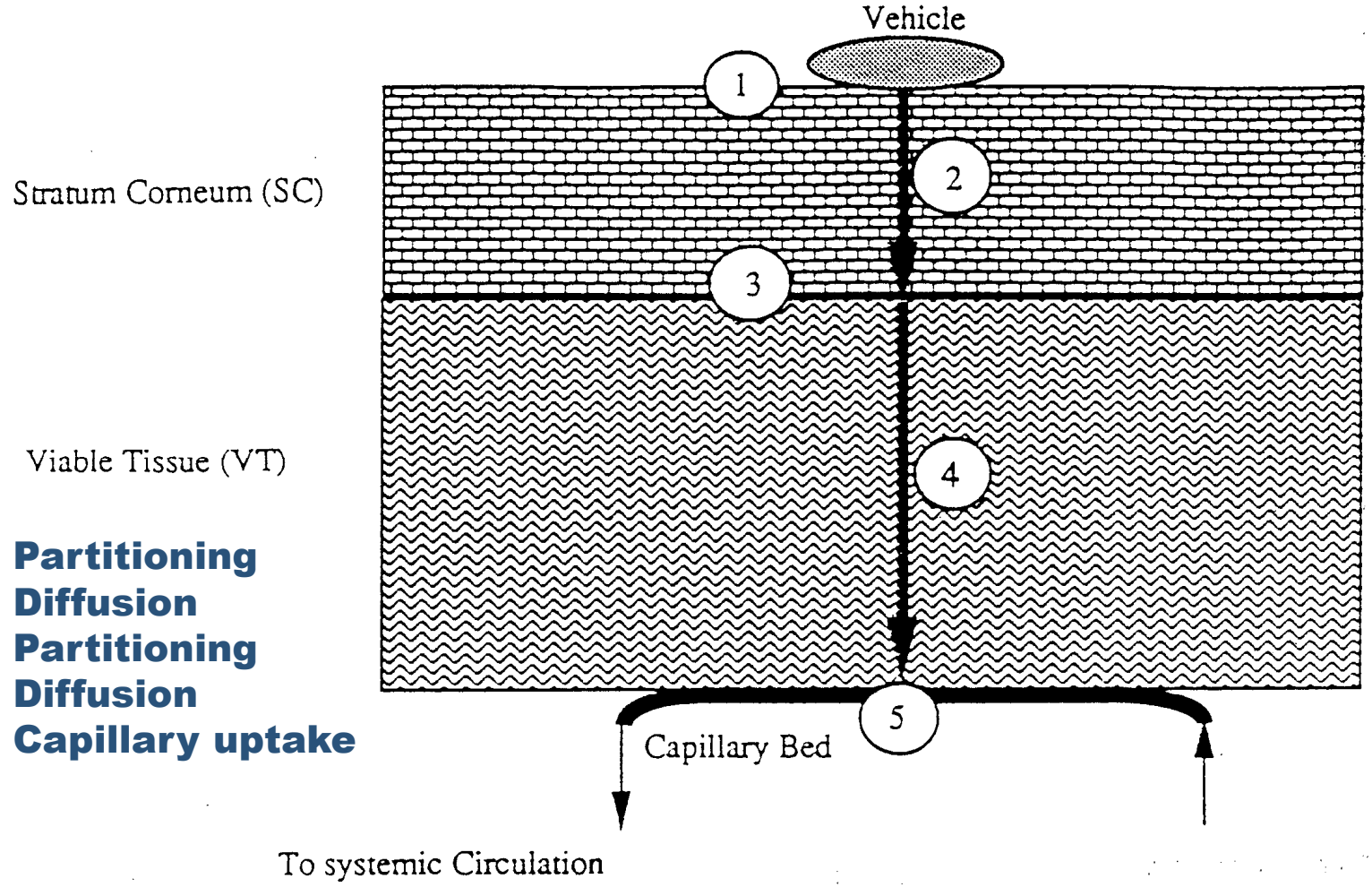
- Hydration
- Drug-skin binding
- Temperature

The Putative Pathways of Penetration Across the Stratum Corneum



Mukhtar, H., 1992. Pharmacology of the Skin. CRC Press, Inc., Boca Raton, FL.

Steps Involved in Percutaneous Absorption



1. **Partitioning**
2. **Diffusion**
3. **Partitioning**
4. **Diffusion**
5. **Capillary uptake**

Mukhtar, H., 1992. Pharmacology of the Skin. CRC Press, Inc., Boca Raton, FL.

Developmental Sequence for Dermal Products

1. Efficacy in appropriate model(s)
2. Pharmacokinetics of API – Dermal, Oral and IV comparison
   a. Assess first pass effect, if any
   b. Compare the ratio of kinetic parameters (AUC, C_{max}, T_{max} etc) by three routes
3. Formulation of the API
4. Pharmacokinetics of the Formulated API – by dermal route only
5. Tissue Distribution – in particular, study deposition of API at the site of application on the skin
6. Lastly safety studies

GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS

M3(R2)

5. REPEATED-DOSE TOXICITY STUDIES

The recommended duration of the repeated-dose toxicity studies is usually related to the duration, therapeutic indication and scope of the proposed clinical trial. In principle, the duration of the animal toxicity studies conducted in two mammalian species (one non-rodent) should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated-dose toxicity studies (Table 1). Limit doses/exposures that are considered appropriate in repeated-dose toxicity studies are described in Section 1.5.

Regulatory Acceptance

1. Regulatory guidelines indicate the requirement for a rodent and a non-rodent species during the safety assessment of new pharmaceutical products

2. The minipig is fully accepted as an alternative non-rodent species by the Japanese, European and USA regulatory authorities

3. The minipig is specifically mentioned as a potential non-rodent species in Japanese and Canadian guidelines and would generally be considered superior to dogs or rabbits as a dermal model

4. OECD 409 Guideline lists swine and minipigs as optional species

5. Evidence should be provided that the minipig is a suitable species – favorable metabolic or pharmacokinetic profile for the test substance or close resemblance between a main target organ in the minipig and man e.g. the skin

Margins of Safety Exposure in Animal and Human Data

1. Major considerations

a. Dose & route administered

b. Extent and duration of systemic exposure (AUC, $t_{1/2}$)

c. Daily systemic exposure, steady state (dosing regimen)

2. Some other factors…

a. Exposure & identity of metabolites between species

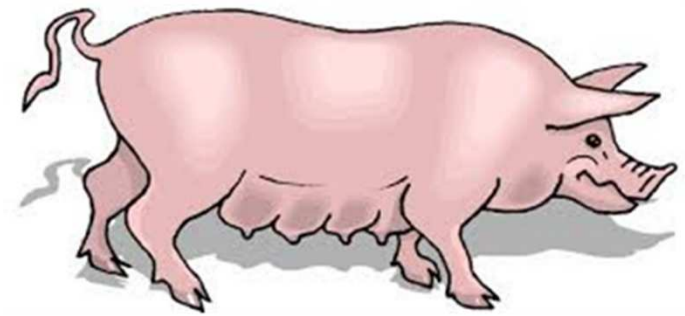
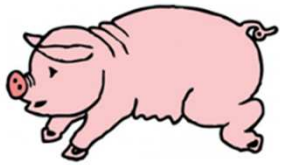
b. Exposure in target organs (accumulation?)-delayed toxicity

Minipigs in Dermal Product Development

Minipig species of choice

- **Similarities between minipig and human skin**
  - **Sparse hair coat**
  - **Thickness of epidermis: 70-140 mm in minipigs, 50-120 in humans, 10-20 mm in rats**
- **Differences Man: eccrine sweat gland**
- **Minipig: apocrine glands**
- **pH on skin surface: man 5, minipig 6-7**

Pigs in Research, and why Göttingen Minipigs?

Landrace		Göttingen Minipigs
		
90 kg	**Size** (6mts)	12 kg
⇩	**Price**	⇧
Mixed – not defined	**Quality**	Genetically and microbiologically defined

Dermal Repeat Dose Toxicity Study

1. **Maximum feasible treatment area**
   - **Back and sites ≈ 10% of body surface area**
2. **Daily treatment time and regimen should mimic clinical use**

➢ **Consider: protection of application site**

- **Protection (semi-occlusion): several layers of cotton gauze fixed Prevents spreading of formulation in pens**

- **Pro**
  - **Prevents cross-contamination**
  - **In most cases, protection is not in line with clinical application (mostly open)**
- **Con**
  - **Daily application time is limited**

Dermal Toxicology Studies Methodologic Issues

1. Clipping of hairs and its frequency – avoid abrasion
2. Tape stripping in case of minipigs
3. Use of depilation
4. Wrapping procedures
    a. Semi-occluded
    b. Full occlusive dressing
    c. Application of stretchable bandage around the middle torso
    d. Need for naïve group without wrapping
5. Duration of Application – varies up to 20-hours per day
6. Consider washing (with soap and water) application area following daily application period
7. Lack of correlation between Draize Score Vs histology at the end of the study

Dermal Repeat Dose Toxicity Study

Dermal Application of Formulation

- Final clinical formulation, if possible
- Vehicle (formulation without drug substance)
- Low dose = clinical strength
- Mid dose
- High dose = increased concentration of active maximum feasible concentration without changing formulation composition

- Maximum feasible dose volume depends on formulation properties - Aqueous gel – cream - ointment

Specific Matters for Dermal Products

- **local and systemic exposure**

➢ Local toxicity

- Skin irritation and other effects
- Skin sensitization
- Phototoxicity

➢ Systemic toxicity

- Target organs
- Dose dependence
- Relationship to exposure
- Reversibility of effects
- Systemic NOAEL

OECD Guidelines for Dermal Toxicity Studies

Tier I tests

1. OECD 439 In vitro skin Irritation and Corrosion Test with Reconstructed Human Skin
2. OECD 432 In Vitro 3T3 NRU Phototoxicity Test
3. ICH in vivo Phototoxicity Test
4. OECD 402 Acute Dermal Systemic Toxicity - Limit Dose of 2 g/Kg
5. OECD 406 Guinea Pig Skin Sensitization (Buehler or Maximization Test)

Tier II Studies: Safety Pharm and Mutagenicity

Studies		Species	Support
Safety Pharmacology, single dose			
Cardiovascular		Minipig	IND
CNS		Rat	IND
Respiratory		Rat	IND
Mutagenicity Studies			
In vitro	Ames Point Mutation	Salmonella	IND
	Clastogenicity	Human Peripheral Blood Lymphocytes (HPBL)	IND
In vivo	Clastogenicity	In Vivo Micronucleus Rat	NDA

OECD Guidelines for Dermal Toxicity Studies

Tier III Tests

1. OECD 410 Repeated Dose Dermal Toxicity (21/28 Days Study) – Rats and Minipigs
2. OECD 411 Sub-chronic Dermal Toxicity (90-Days Study) – Rats and Minipigs
3. OECD 452 Chronic Dermal Toxicity Usually for 6-month in Rats and 9-month in Minipigs
4. OECD 414 Developmental Toxicity in Rats and Rabbits

Toxicology Studies for Topical Drug Development

Type of Study	Rodent	Non-Rodent	Phase
General Toxicology			
7 or 14-DRF study	Rat	7-Day Dermal DRF study	Pre-IND
1 Month +TK+Rec	Rat Dermal and IV	Minipig Dermal	IND
3 Month +TK+Rec	Rat Dermal	Minipig Dermal	Phase II
Chronic+TK+Rec	6-Month Rat Dermal	9-Month Minipig Dermal	Phase III
Reproductive Toxicity			
Developmental Tox. (Embryo-fetal toxicity – Segment II)	Rat	Rabbit	Phase II or Phase III
Fertility and Early Developmental Toxicity (Segment I)	Rat	NA	Phase II and III
Peri & Postnatal (Segment III)	Rat	NA	Phase II and III
Carcinogenicity	2-Year Rat (Last Resort) – **Request for an exemption**	NA	CAC NDA

How to Address Safety Concerns? Scenario

1. **Scenario 1: Plasma exposure of metabolites in humans not covered by animal studies**

2. **Scenario 2: Impurities found in clinical batch were not qualified in pre-clinical studies**

Summary of Findings

1. In vivo studies may be used to make reasonable predictions of dermal absorption and penetration of components in humans.
2. Rodent dermal studies, in general, overpredict the toxicity to humans due to high permeability
3. Minipig offers a reasonable alternative large animal species which mimics human bioavailability and toxicity
4. Dermal delivery of a large number of drugs under development which is an effective way to deliver compound systemically, where GI tract prevents absorption due to gastric stability, transporter mediated membrane transfer or first pass effect
5. For compounds delivered by the dermal route, one IV study in rodents is in order to assess true systemic toxicity, due to lower dermal penetration which may not necessarily assess potential systemic toxicity

Thank you !

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