FRANCQUI CHAIR
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VUB VRIJE UNIVERSITEIT BRUSSEL

DERMAL ABSORPTION TESTING *IN VITRO* OF DERMATO-COSMETIC INGREDIENTS

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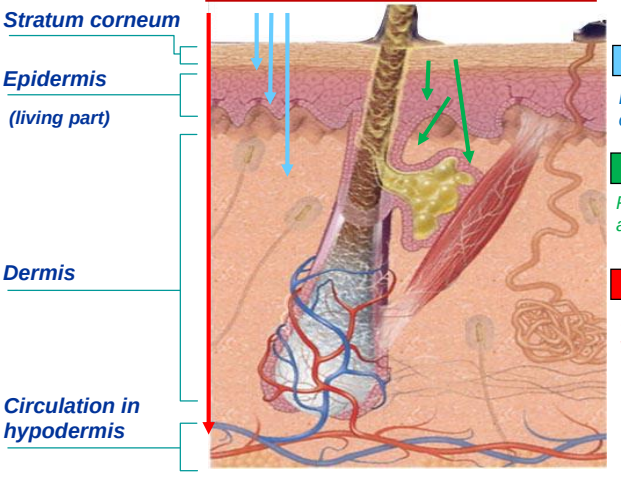
- SPECIAL CASES
  - ① NO DERMAL ABSORPTION DATA AVAILABLE
  - ② LOW DERMAL ABSORPTION SUSPECTED
- CONCLUSIONS

WHAT IS DERMAL ABSORPTION ?

DERMAL ABSORPTION: - TRANSPORT OF SUBSTANCES FROM THE OUTHER SURFACE INTO THE SKIN AND IN THE SYSTEMIC CIRCULATION

- DIFFERENT TERMINOLOGY IS BEING USED

PASSAGE THROUGH THE SKIN

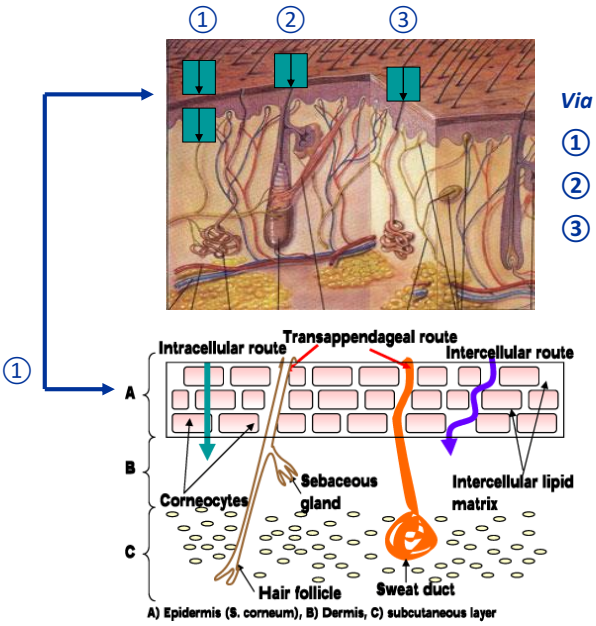


Penetration
Entry into a particular layer or stucture

Permeation
From one layer into another layer

Resorption
Uptake into the vascular system

WHAT ROUTES OF ENTRY FOR DERMAL ABSORPTION ?



Via :

① *skin*

② *hair follicles*

③ *eccrine sweat glands*

DERMAL ABSORPTION

➢ **MOSTLY PASSIVE DIFFUSION OF ACTIVES FROM TOPICAL PREPARATIONS**

→ **FICKs LAW**

$dQ/dt = (K \times Cv \times Ds \times A)/h$

K : partition coëfficient vehicle and SC
Cv : C solved in vehicle
A : skin surface
h : thickness skin layer
Ds : diffusion coëfficient in SC
dQ/dt: penetration rate

→ **LAW OF HIGUCHI** (Skin layers are considered to be homogeneous)

$dQ/dt \sim K \times Cv$ Thermodynamic activity

➡ **MANY VARIABLES, FACTORS AFFECTING DERMAL ABSORPTION**

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WHY TO MEASURE DERMAL ABSORPTION ?

IMPORTANCE OF SAFETY OF COSMETICS

COSMETICS REGULATION N° 1223/2009/EC

- ✓ PRODUCT SAFE FOR CONSUMER (art. 3)
- ✓ SAFETY BASED ON SAFE INGREDIENTS (art. 10)
  (toxicological profile, chemical structure, exposure)
- ✓ DEMONSTRATION OF SAFETY

SAFETY EVALUATION

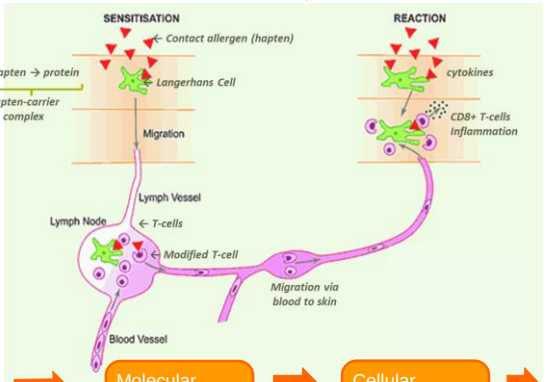
LOCAL TOXICITY — SYSTEMIC TOXICITY

CHALLENGE:
- VALIDATED NON-ANIMAL METHODOLOGY
- ~~REFINEMENT~~, ~~REDUCTION~~, REPLACEMENT

WHY TO MEASURE DERMAL ABSORPTION ?

LOCAL TOXICITY **FOR SKIN SENSITISATION**

➡ **DERMAL ABSORPTION IS FIRST STEP**



AOP

Chemical Structure & Properties	Molecular Initiating Event	Cellular Response	Organ Response
1) Skin penetration 2) Electrophilic substance: directly or via auto-oxidation or metabolism	3-4) Haptenation: covalent modification of epidermal proteins	5-6) Activation of epidermal keratinocytes & dendritic cells	7-8) Presentation of haptenated protein by dendritic cells resulting in activation & proliferation of specific T cells

WHY TO MEASURE DERMAL ABSORPTION ?

SYSTEMIC TOXICITY

➡ **FOR QUANTITATIVE RISK ASSESSMENT (TRESHOLD TOXICITY ASSUMED)**

MoS IS CALCULATED:

$$MoS = \frac{(NOAEL)_{syst}}{SED} \geq 100$$

MoS = Margin of Safety

$(NOAEL)_{syst}$ = No Observable Adverse Effect Level

SED = Systemic Exposure Dose

C = Concentration of ingredient

➡ **SED =**

$$\frac{\text{DERMAL ABSORPTION (\%) x C (\%) x dermal exposure}}{\text{body weight}}$$

$$\frac{\text{DERMAL ABSORPTION (µg/cm²) x surface x frequency}}{\text{body weight}}$$

➡ **DERMAL ABSORPTION IS CRITICAL FACTOR WHICH DETERMINES THE SYSTEMICALLY AVAILABLE AMOUNT**

➡ **$(NOAEL)_{SYST}$ < DERIVED FROM *IN VIVO* STUDIES !**

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FACTORS AFFECTING DERMAL ABSORPTION

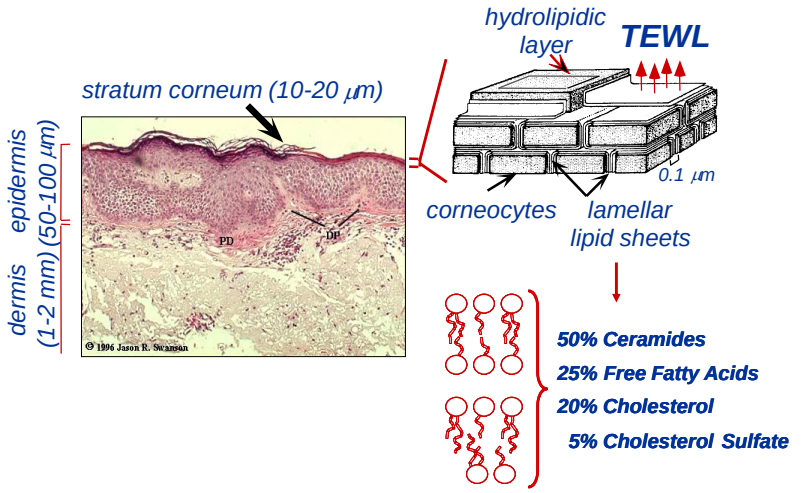
① BIOLOGICAL FACTORS

- BARRIER FUNCTION AND SKIN INTEGRITY
- SKIN SOURCE
- SKIN SURFACE AREA
- AGE
- OCCLUSION

FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS

BARRIER FUNCTION AND SKIN INTEGRITY

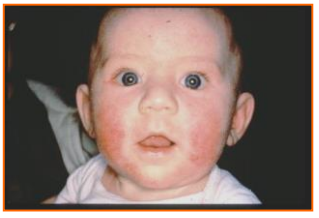


➡ *BARRIER FUNCTION IS LIMITING FACTOR FOR DERMAL ABSORPTION*

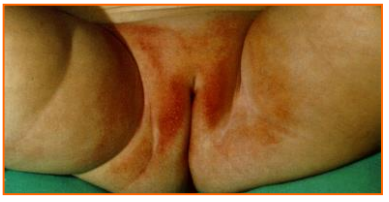
FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS

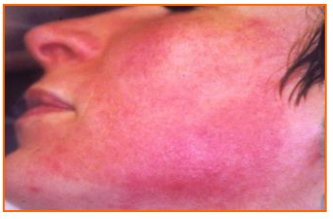
BARRIER FUNCTION AND SKIN INTEGRITY



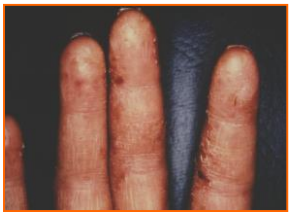
Atopic skin



Napkin dermatitis



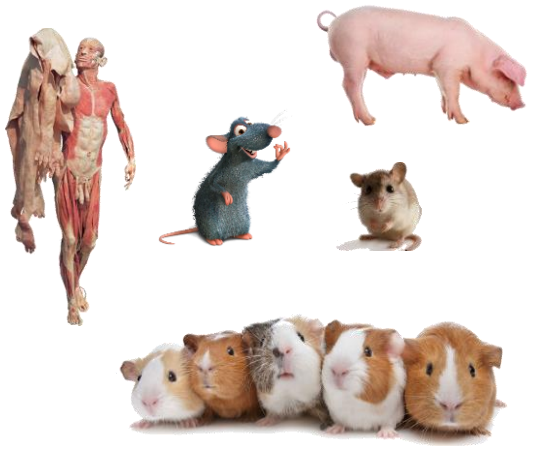
Peeling with α-OH-acids



House wife dermatitis

FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS — SKIN SOURCE



In ranking order:

- **Human**
- **Pig**
- **Rat**
- **Mouse**
- **Guinea Pig**

➡ HUMAN SKIN AND PIG SKIN ARE SOURCES USED FOR COSMETIC INGREDIENTS

FACTORS AFFECTING DERMAL ABSORPTION

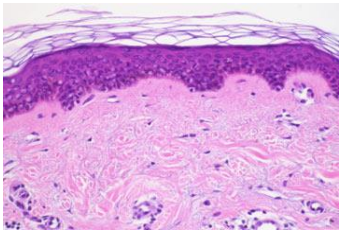
① BIOLOGICAL FACTORS — SKIN SOURCE

	Human	Pig	Rat
Characteristics	-Golden standard -Not easily available	-Easily available -Large surface	*-In vitro/In vivo* relationship
Hair follicles/cm²	11	11	289
Stratum corneum	10-20µm	≈	↓
Dermal absorption	Relevant	Relevant	Overestimated

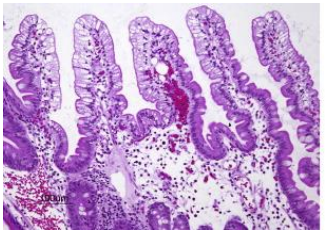
➡ HUMAN SKIN AND PIG SKIN HAVE COMPARABLE PROPERTIES

FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS — SKIN SURFACE



Human skin
±2m²



Human gastro intestinal tract
±200m²

➡ ***DERMAL ABSORPTION SURFACE AREA IS LIMITED IN COMPARISON WITH ABSORPTION ORGAN***

FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS — SKIN SURFACE

Cube side	2	4
Surface area	24	96
Volume	8	64
Surface area/volume	3.0	1.5

http://www.mommyguru.com/wp-content/uploads/2013/04/BB20EE2732B542A4B4846A21D04EED7B.ashx_.jpg

DIFFERENCE WITH ADULT PERSON:
SURFACE AREA/BODY WEIGHT (VOLUME)

- at birth 2.3 fold
- 6 months 1.8/ fold
- 12 months 1.6 fold
- 5 years 1.5 fold
- 10 years 1.3 fold

➡ **SURFACE/VOLUME (BODY WEIGHT) MAY AFFECT THE SAFETY EVALUATION OF COSMETIC INGREDIENTS (EXTRA DEFAULT VALUE)**

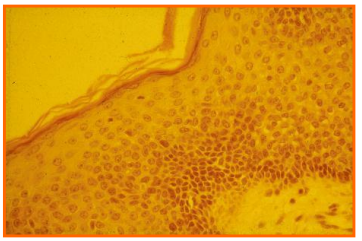
FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS

ANATOMICAL SITE

THICKNESS OF STRATUM CORNEUM DIFFERS WITH BODY REGION

Skin of handpalm



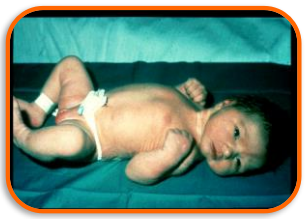
Skin around eyes

➡ **MUCOSAE HAVE NO STRATUM CORNEUM AND SHOW HIGH DERMAL ABSORPTION**

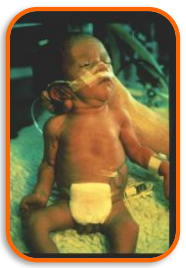
FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS

AGE



Full-term baby



Premature baby

- **IN FULL-TERM BABIES: FULLY FUNCTIONAL BARRIER PRESENT ALREADY 1 DAY AFTER BIRTH**
- **IN PREMATURE BABIES: BARRIER FUNCTION DEPENDS ON GESTATIONAL AGE AND MAY BE EVEN ABSENT**
- **NAPPY ZONE IN BABIES SUFFERS FROM OCCLUSION**

FACTORS AFFECTING DERMAL ABSORPTION

① BIOLOGICAL FACTORS

② ENVIRONMENTAL FACTORS

- TEMPERATURE
- RELATIVE HUMIDITY
- OCCLUSION

③ COMPOUND/PRODUCT-RELATED FACTORS

FACTORS AFFECTING DERMAL ABSORPTION

② ENVIRONMENTAL FACTORS

- Physico-chemical properties: MW, structure, pH, solubility, lipophilicity, ionisation, log Pow, partition coefficient K
- Duration of exposure, frequency
- Dilution, applied amount
- Solvents, penetration enhancers, surfactants, ….

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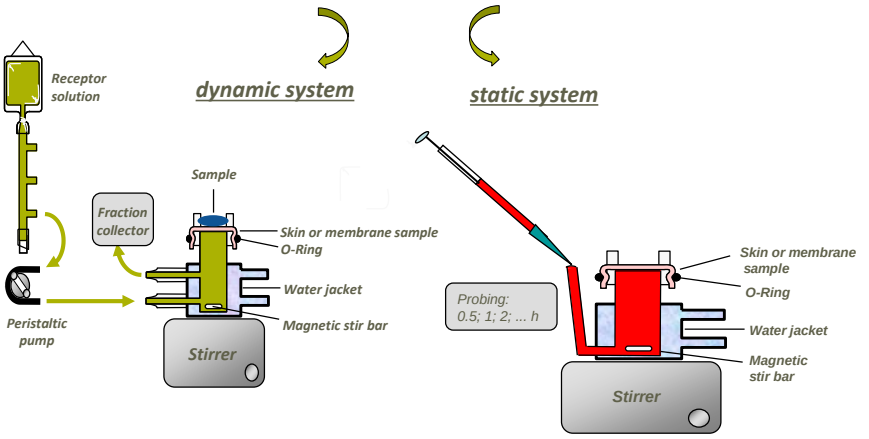
HOW TO MEASURE DERMAL ABSORPTION ?

VALIDATED IN VITRO METHODOLOGY: OECD 428, ECB. 45



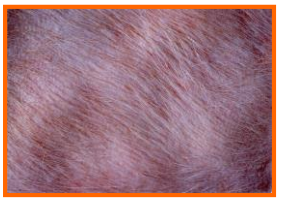
➡ **MOSTLY USED FOR COSMETIC INGREDIENTS IS DYNAMIC SYSTEM, MIMICKING *IN VIVO* EXPOSURE**

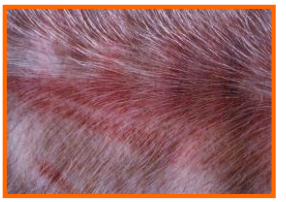
Courtesy W. Steiling

HOW TO MEASURE DERMAL ABSORPTION ?

HOW TO MEASURE DERMAL ABSORPTION ?

Pig skin from slaughter house animal (max. 8hrs. post mortem)





Quality check for damage

- PREFERABLY HUMAN SKIN IS USED (MOST RELEVANT)
- MOSTLY, PIG SKIN IS USED: HUGE QUANTITIES (REPRODUCIBILITY ↑)

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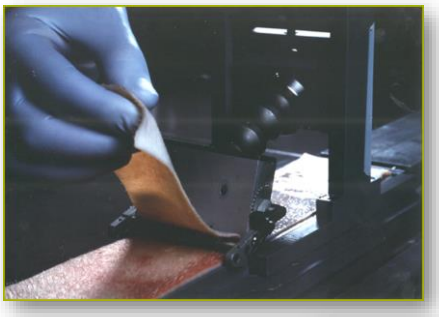
HOW TO MEASURE DERMAL ABSORPTION ?



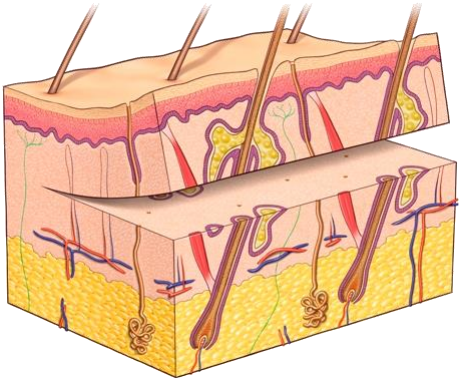
Dermatomed skin
(subcutaneous fat removed)
(600 µm)

Cutting according to diffusion cell surface

Courtesy W. Steiling

HOW TO MEASURE DERMAL ABSORPTION ?

➡ ***THICKNESS OF THE SKIN***



Split thickness
(dermatomed skin: 200-600 µm)

Full thickness (±1000 µm)

Problems with full thickness
- Longer diffusion path
- Underestimated values for lipophilic compounds

➡ **MOSTLY DERMATOMED SKIN IS USED FOR COSMETIC INGREDIENTS**

HOW TO MEASURE DERMAL ABSORPTION ?

Diffusion cell (Franz cell)

➡ **MOUNTING OF SKIN IN FRANZ CELL**

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HOW TO MEASURE DERMAL ABSORPTION ?

- SAMPLES:

USUALLY 2-3 REPLICATES OF 4 TO 6 DIFFERENT SKIN DONORS (HUMAN OR PIG)

- INTEGRITY CHECK:

WITH TRITIATED WATER

OR BENCHMARK CHEMICAL e.g. CAFFEINE

OR MEASURING TRANS ELECTRICAL RESISTANCE OF SKIN (TER)

OR MEASURING OF TRANSEPIDERMAL WATER LOSS (TEWL)

HOW TO MEASURE DERMAL ABSORPTION ?

- *Application of sample in µg/cm²*
- *Use of *labelled substance*
- *Realistic use conditions: occlusion, open application*

Measurement of all relevant compartments

- *Excess on skin surface*
- *Stratum corneum (strips!)*
- *Living epidermis (-SC)*
  Dermis
  Receptor fluid

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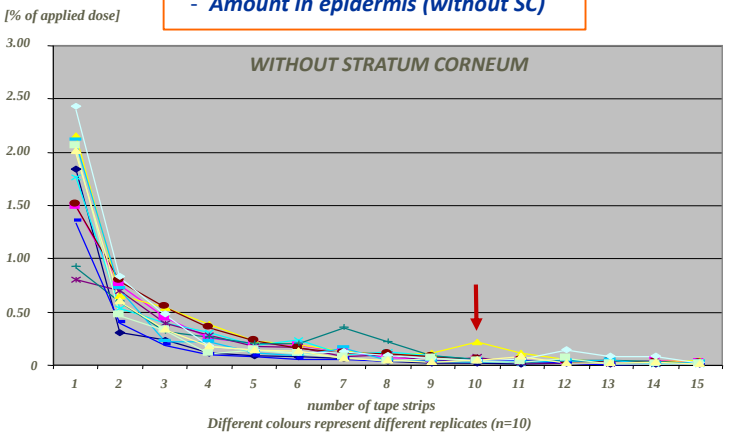
➡ ***Recovery: 85-115%***

HOW TO MEASURE DERMAL ABSORPTION ?

➡ ***CALCULATION***

Amount dermally absorbed :

- ***Amount in receptor fluid***
- ***Amount in dermis***
- ***Amount in epidermis (without SC)***



➡ **NUMBER OF STRIPS: ACTUALLY 10 to maximal 20 strips**

Courtesy W. Steiling

HOW TO MEASURE DERMAL ABSORPTION ?

		HAIR DYE I		HAIR DYE II	
		aqueous solution	standard formulation	aqueous solution	standard formulation
rinse off	[%] (SD)	93.517	91.130	87.749	90.899
adsorbed to the stratum corneum	[%] (SD)	3.71 (1.94)	0.957 (0.524)	<0.094 (0.000)	<0.094 (0.000)
absorbed by the remaining skin (epidermis/dermis)	[%] (SD)	2.99 (1.91)	1.96 (1.03)	0.106 (0.106)	0.066 (0.024)
penetrated through the skin	[%] (SD)	1.2 (0.805)	0.477 (0.257)	<0.094 (0.000)	<0.094 (0.00)
recovery rate	[%] (SD)	101 (4.43)	84.3 (3.79)	88 (4.53)	91.3 (5.22)
taken as **bio-available**	[%] (SD)	**4.190**	**2.437**	**<0.11**	**<0.16**

HOW TO MEASURE DERMAL ABSORPTION ?

INFLUENCES ON DERMAL ABSORPTION

- **MW > 1000 → penetration unlikely**
- **IONISATION → highly ionised chemicals penetrate poorly**
- **LIPOPHILICITY → log Pow = 1-3, best penetration**
- **COMPATIBILITY TO THE SKIN**
- **VOLATILITY**
- **STABILITY OF COMPOUND IN VEHICLE**
- **SOLUBILITY IN RECEPTOR FLUID**

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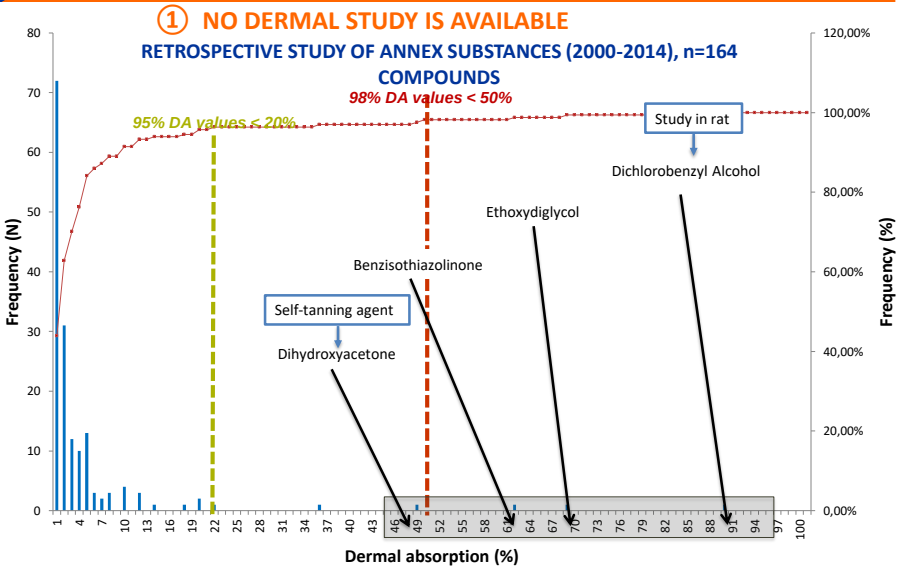
SPECIAL CASE

① NO DERMAL STUDY IS AVAILABLE



➡ *IF NO DERMAL STUDY IS AVAILABLE, A DEFAULT VALUE OF 50% IS USED FOR NORMAL SKIN*

Taken up in SCCS NoG, 9th revision 2015

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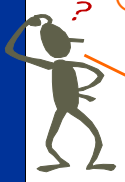
SPECIAL CASE

② VERY LOW DERMAL ABSORPTION SUSPECTED



COSMETIC INGREDIENTS WITH SUSPECTED
LOW DERMAL ABSORPTION.....
LOW ORAL BIOAVAILABILITY????

MAJOR QUESTIONS:

→ DO WE NEED ORAL STUDIES IN ANIMALS (NOAELsyst-VALUES) FOR SUBSTANCES FOR WHICH INTERNAL EXPOSURE IS MINIMAL AND SYSTEMIC TOXICITY MIGHT NOT BE AN ISSUE ?

→ CAN WE LEARN FROM PREVIOUS RISK ASSESSMENTS OF ANNEX SUBSTANCES (preservatives, colorants, UV-filters, hair dyes) ?

→ IS IT POSSIBLE TO USE PHYSICO-CHEMICAL DESCRIPTORS TO IDENTIFY COMPOUNDS WITH LOW DERMAL ABSORPTION AND PROBABLY VERY LOW BIOAVAILABILITY ?

↓

RETROSPECTIVE STUDY OF SCCS OPINIONS 2000-2014

Ates et al., Reg. Toxicol. Pharmacol. 76(2016)74

SPECIAL CASE

② LOW DERMAL ABSORPTION SUSPECTED

RETROSPECTIVE STUDY OF ANNEX SUBSTANCES (2000-2014)

APPROACH USED (n=70)

➡ *IN ANALOGY WITH LIPINSKI RULES → 4 ALERTS*

➡ *CUTT-OFF VALUE FOR DA: pragmatically chosen*

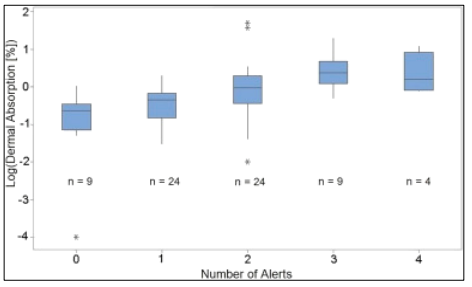
MW < 180 Da
logP ≥ 0.3
MP < 100°C
TPSA < 40 Ä²

KEY DESCRIPTORS FOR DERMAL ABSORPTION

HIGH DA ≥ 1.3% | ***LOW DA < 1.3%***

SKEWED → LOG_{10} VALUES

BOXPLOT log% DA vs. number alerts



➡ *WHEN NUMBER OF ALERTS ↑ → DA ↑*

➡ *WHEN NO ALERTS → DA VERY LOW*

Ates et al., Reg. Toxicol. Pharmacol. 76(2016)74

MW = Molecules Weight; logP = octanol/water partition coefficient;, MP = Melting Point; TPSA = Topological Polar Surface Area

SPECIAL CASE

② LOW DERMAL ABSORPTION SUSPECTED

➡ ***CUT-OFF VALUE FOR DA: 1.3%***

PERFORMANCE OF THE RULE SET ON THE DATA SET

n=70	Predicted high (%)	Predicted low (%)	TOTAL (%)
High DA (≥ 1.3%)	33	0	33
Low DA (< 1.3%)	54	13	67

SENSITIVITY 100%
SPECIFICITY 19%

PERFORMANCE WHEN COMPOUNDS TRIGGER 2 ALERTS FOR CLASSIFICATION AS HIGH DA

n=70	Predicted high (%)	Predicted low (%)	TOTAL (%)
High DA (≥ 1.3%)	27	6	33
Low DA (< 1.3%)	26	41	67

SENSITIVITY 83%
SPECIFICITY 62%

➡ *↑ SPECIFICITY CAUSES LOSS OF SENSITIVITY*

Ates et al., Reg. Toxicol. Pharmacol. 76(2016)74

SPECIAL CASE

② LOW DERMAL ABSORPTION SUSPECTED

➡ *CUT-OFF VALUE FOR DA: 2%*

PERFORMANCE OF THE RULE SET ON THE DATA SET

N=70	Predicted high (%)	Predicted low (%)	TOTAL (%)
High DA (≥ 2%)	19	0	19
Low DA (< 2%)	68	13	81

SENSITIVITY 100%
SPECIFICITY 16%

⬇

PERFORMANCE WHEN COMPOUNDS TRIGGER 2 ALERTS FOR CLASSIFICATION AS HIGH DA

N=70	Predicted high (%)	Predicted low (%)	TOTAL (%)
High DA (≥ 2%)	19	0	19
Low DA (< 2%)	34	47	81

SENSITIVITY 100%
SPECIFICITY 58%

⬇

SETTING BOUNDARY CRITERIA AT 2% FOR HIGH DA & APPLYING MORE FLEXIBLE RULES
➡ *OPTIMISED SENSITIVITY WITH SPECIFICITY OF 100%*

Ates et al., Reg. Toxicol. Pharmacol. 76(2016)74

SPECIAL CASE

② LOW DERMAL ABSORPTION SUSPECTED

- ❑ *IN CASE OF A COMPOUND TRIGGERING NONE OR ONLY ONE OF THE PHYSICO-CHEMICAL ALERTS, IT IS LIKELY TO HAVE A LOW DA*
- ❑ *STUDY OF LIMITED SET OF COMPOUNDS (n=70) SKEWED TOWARDS LOWER END*
  - ➡ *NOT APPLICABLE TO OTHER DOMAINS*
- ❑ *TO WAIVE SYSTEMIC TOXICITY TESTING FOR A COSMETIC INGREDIENT CLASSIFIED AS LOW DA (≤ 2%), A CONFIRMATORY AND EXTENDED IN VITRO DA STUDY IS NECESSARY*

Ates et al., Reg. Toxicol. Pharmacol. 76(2016)74

CONTENTS

- WHAT IS DERMAL ABSORPTION ?
- WHY TO MEASURE DERMAL ABSORPTION ?
- FACTORS AFFECTING DERMAL ABSORPTION
- HOW TO MEASURE DERMAL ABSORPTION ?
- SPECIAL CASES
  - ① NO DERMAL ABSORPTION DATA AVAILABLE
  - ② LOW DERMAL ABSORPTION SUSPECTED
- CONCLUSIONS

CONCLUSIONS

- *DA OF COSMETIC INGREDIENTS AFFECTS:*
  - *LOCAL TOXICITY e.g. first step in potential for skin sensitising process*
  - *SYSTEMIC TOXICITY e.g. critical in MoS calculation (systemic exposure)*
- *DA IS INFLUENCED BY MANY FACTORS TO BE TAKEN INTO CONSIDERATION*
- *DA CAN BE MEASURED USING A VALIDATED 3R-REPLACEMENT METHOD: IN VITRO METHOD OECD 428*
- *WHEN NO DA DATA ARE AVAILABLE, THE DEFAULT VALUE OF 50% CAN BE USED FOR HEALTHY SKIN*
- *WHEN A LOW DERMAL ABSORPTION OF A COSMETIC INGREDIENT IS EXPECTED BECAUSE NONE OR ONLY ONE OF THE PHYSICO-CHEMICAL ALERTS IS TRIGGERED, THIS NEEDS TO BE CONFIRMED BY AN EXTENDED DA STUDY*
  - *→ WAIVING OF SYSTEMIC TOXICITY TESTING IS THEN POSSIBLE*