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INNOVATIVE SOLUTIONS

GRASSE EXPERTISE

Safety testing of cosmetic products: Overview of established methods and new models

SaferWorldbyDesign Webinar 2021

17 June, 2021

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Overview

cosmetics

Published: 11 June 2021

MDPI

Review

Safety Testing of Cosmetic Products: Overview of Established Methods and New Approach Methodologies (NAMs)

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Why should cosmetics be tested?

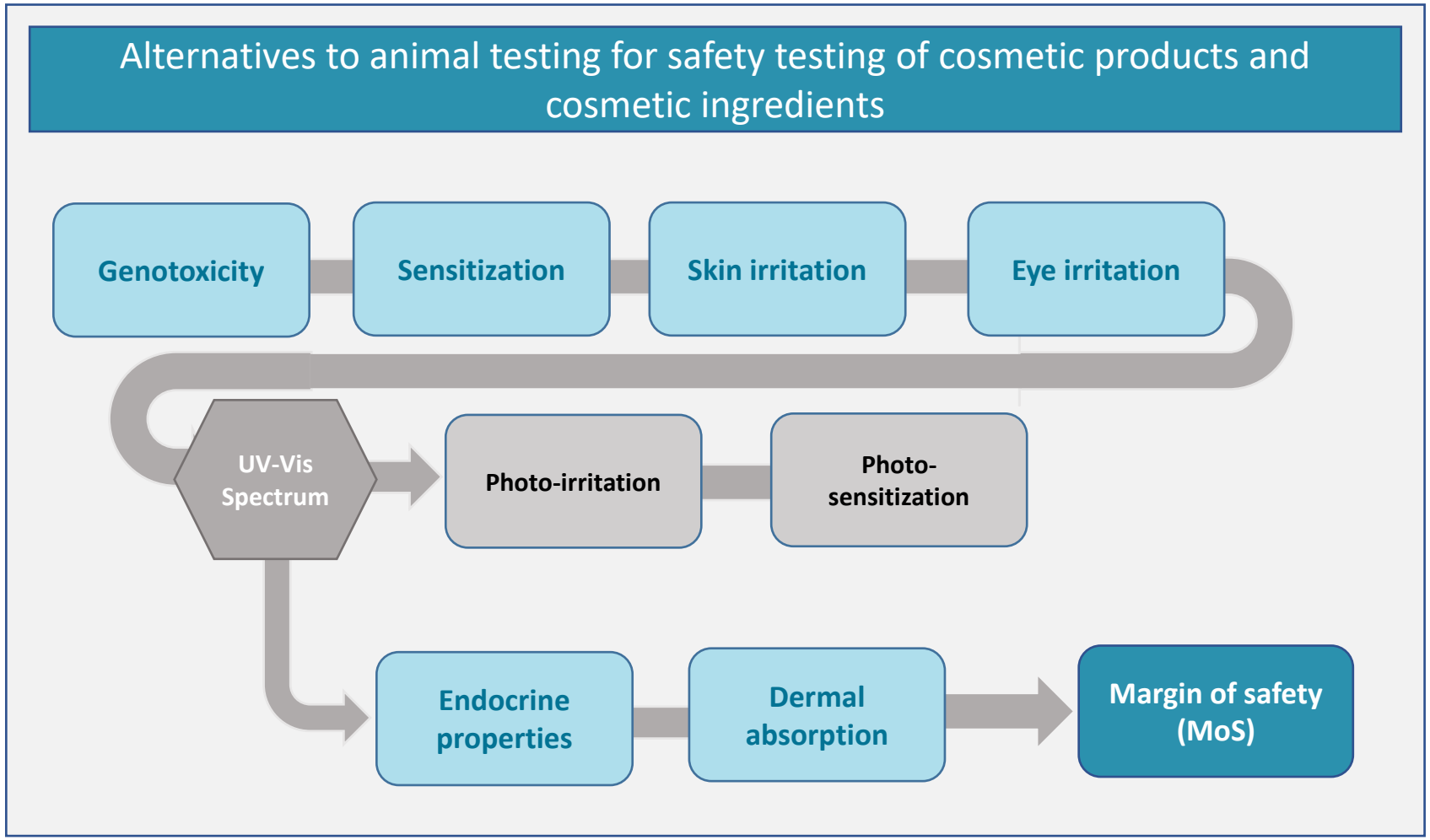
- Cosmetic products need to have a proven efficacy combined with a comprehensive toxicological assessment

  - The 7th Amendment to the European Cosmetics Directive has banned animal testing for cosmetic products and cosmetic ingredients
  - European Cosmetic Regulation EC 1223/2009 & 655/2013 specify the required data to proof the safety and support the claims
  - Business point of view: avoid penalties, build trust company/brand, etc.

- Largely driven by regulatory authorities, a wide range of alternatives to animal testing have been developed and validated for safety testing of cosmetic products and adopted as test guidelines

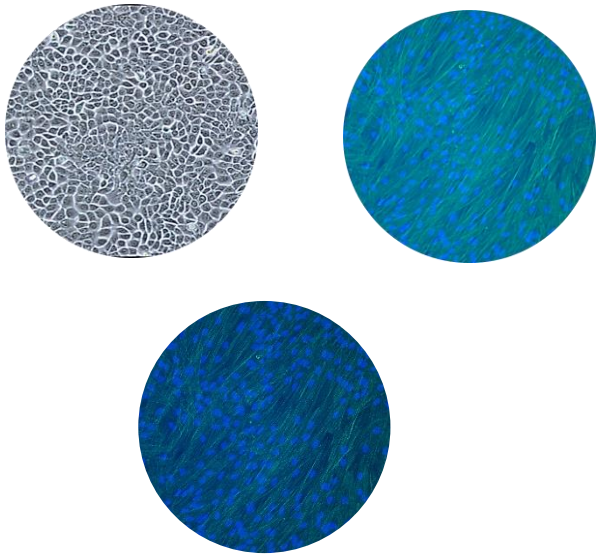
European Commission

Alternatives to animal testing of csmetics

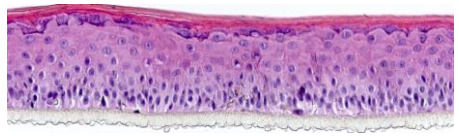


Alternative models for safety testing

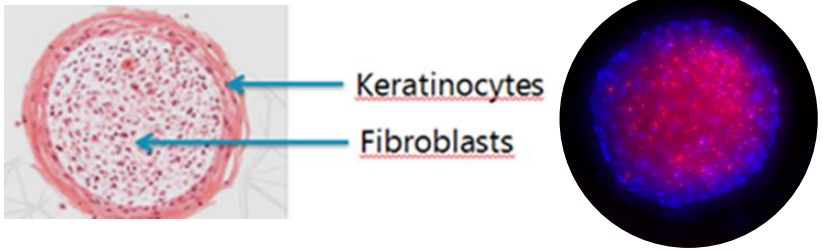
- 2D cell culture
  - Keratinocytes, Fibroblasts
  - Melanocytes, Dendritic cells…



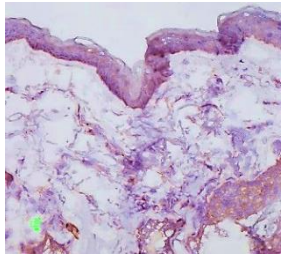
- 3D models of skin equivalent
  - Reconstructed human epidermis
  - More complex models



- Skin microtissues (InSphero)



- Excised human skin
  - “Gold standard”



Alternative models for safety testing

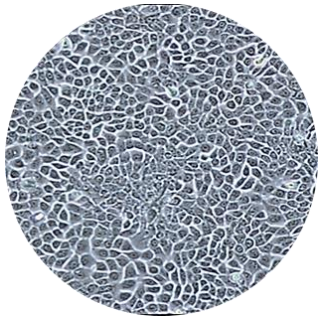
Early stage
Ingredients

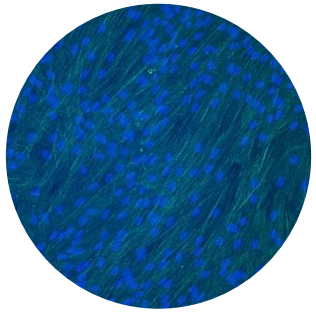
Intermediare stage
Ingredients
Finished product

Late stage
Finished products and ingredients

2D skin models

- Sensitization
- Inflammation
- Ageing
- Pigmentation
- Wound healing





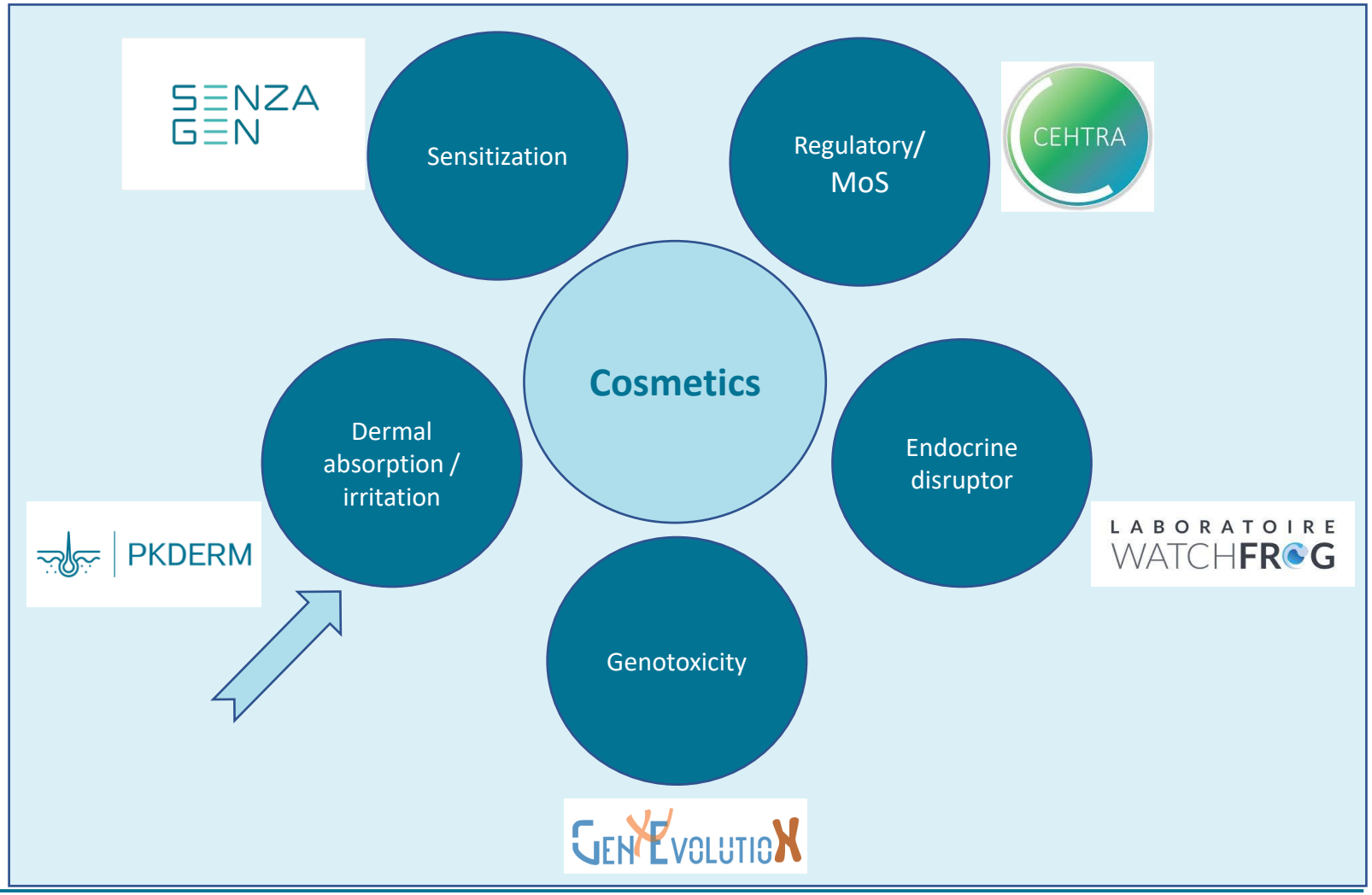
3D skin models

- Irritation
- Sensitization
- Inflammation
- Pigmentation
- Ageing

Excised skin

- Dermal absorption
- Metabolism
- Transporteurs
- Inflammation
- Healing

Consortium of experts in testing of cosmetics

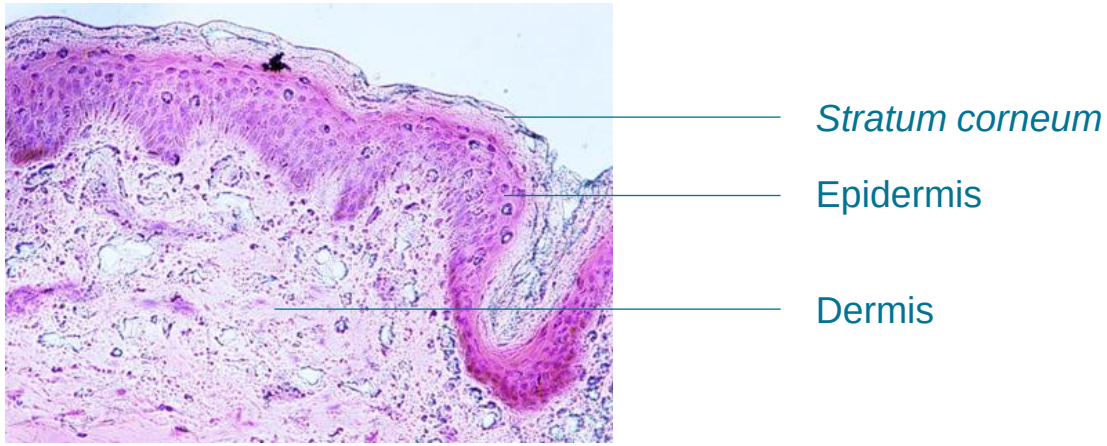


Dermal absorption testing

- Objectives of *in vitro* dermal absorption testing
- Sector of application
- Methodology
- Two examples

In vitro dermal absorption - Objectives

- *Dermal absorption testing is also known as dermal penetration or percutaneous penetration*
- The purpose of the dermal absorption testing is to provide a measurement of the absorption or penetration of a test article through the skin barrier and into the skin.
  - Does the test article penetrate the skin and reach the plasma and to what extent?
  - How the test article is distributed in the skin : *stratum corneum*, epidermis, dermis?

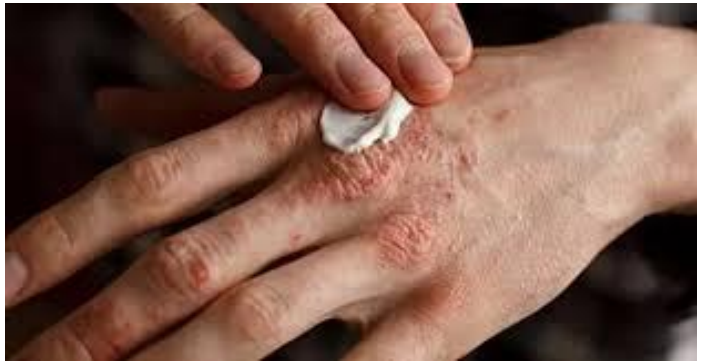


In vitro dermal absorption - Objectives

- Therefore, knowledge of dermal absorption is essential for:
  - Therapeutic aspects:
    - quantities penetrated can be taken into consideration to predict the therapeutic concentration at the target sites in skin tissue.
  - Safety issues:
    - quantities absorbed can be taken into consideration in toxicological risk assessment to extrapolate human exposure and calculate the margin of safety.

In vitro dermal absorption – sector of application

- *In vitro* dermal absorption studies are applied in different sectors and for different purposes:



  - Pharmaceutical products:
    - Part of safety and efficacy assessment
    - Formulation Screening
    - Bioequivalence: to determine if the new product has the same degree of dermal absorption as reference product

  - Cosmetics and consumer products:
    - *in vitro* dermal absorption studies are part of safety assessment of a test item
  - Chemical/agrochemical:
    - *in vitro* dermal absorption studies are part of
    - safety assessment purposes

In vitro dermal absorption – Types of formulations

- Different types of formulations can be assessed through *in vitro* dermal absorption studies:
  - Creams, gels, ointments, suspensions, foam, patches, aqueous, solvent, hair dyes, shampoo, foundation, moisturizer, cleansers, soaps, sunscreen etc.

In vitro dermal absorption – Methodology

- Dermal absorption studies are conducted according to OECD Test Guideline 428 using Franz-type diffusion cells

  - The test substance it is applied to the surface of the skin. The skin sample is placed between two chambers (a donor chamber and a receptor chamber) of a diffusion cell which may be either a static or flow-through set up.

Diffusion cell

In vitro dermal absorption – Methodology

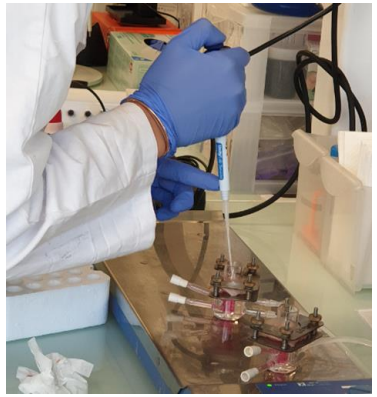
- Origin of skin samples
  - Excised human skin are the gold standard
    - From plastic surgeries, abdomen area
  - Skin samples from animal models (rat or pig) can be used
- Fresh or frozen skin samples can be used
  - Metabolism or drug transporters: fresh tissues
- Number of donors : 3 to 6 donors in 3 to 4 times
  - N = 9 or N = 24

Human skin samples

In vitro dermal absorption – Methodology

- Full thickness or split thickness (dermatomed) skin
  - Need to measure skin thickness of each sample
- Ensure skin barrier integrity
  - Need to measure TEWL of each sample
- Treatment duration : according to use conditions
  - 5 min to 24 hours
- Application amount:
  - Finite dose (5 to 10 mg/cm²)

Measurement of skin thickness



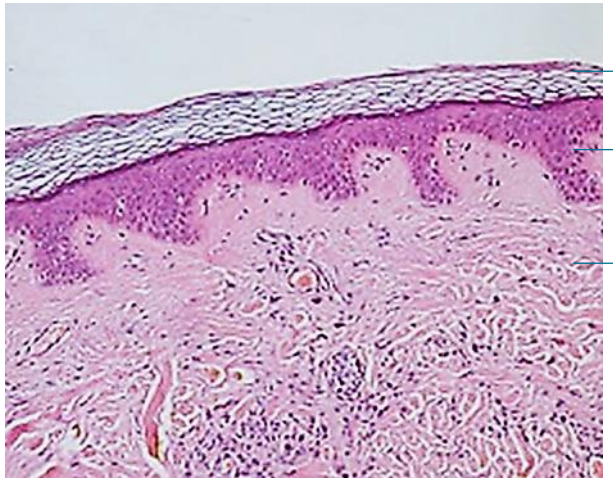

Measurement of TEWL

In vitro dermal absorption – Methodology

- Choice of receptor liquid
  - Need to be chosen carefully to ensure solubility and stability of TA
  - PBS with surfactant
  - Check the solubility
  - Check the stability

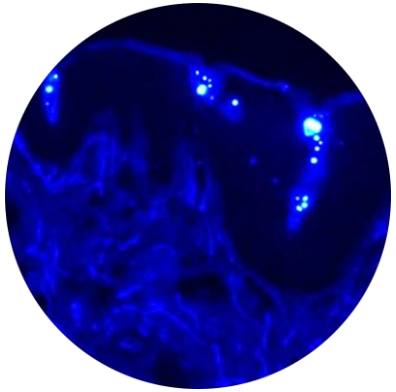
In vitro dermal absorption – Methodology

- Sample analysis
  - Analyses can be done in different compartments according to the objective of the study:
    - Receptor liquid (Absorbed dose)
    - Dermis
    - Epidermis
    - *Stratum corneum*

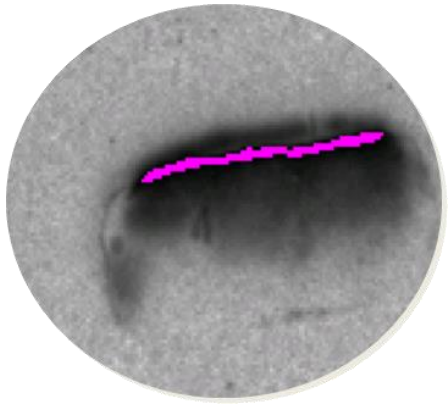


In vitro dermal absorption – Methodology

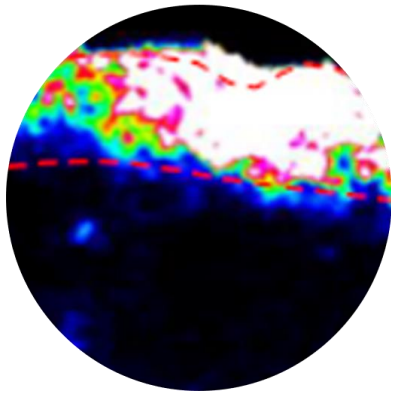
- Sample analysis
  - Different analytical methods can be used to quantify concentration of test substance in different skin compartments according to physicochemical properties of the test substance such as lipophilicity, molecular weight, charge, and concentration of the test substance:
    - **LC-MS/MS**; ICP-MS/MS, LC-UV; LC-Fluo; LSC (radiolabelled)
    - Imaging methods can also be used to visualize the distribution of test substance in skin compartments: Fluorescence microscopy; Autoradiography; MALDI-MSI



Fluorescence microscopy



Autoradiography



MALDI-MSI

In vitro dermal absorption – Methodology

- Sample analysis
  - LS-MS/MS
    - Need to highly Sensitive and Accurate analytical method (LOQ < 1 ng/mL when possible)
    - Validation in every matrix (receptor liquid, SC, epidermis, dermis, washing)

Examples of application

Bioequivalence study

In vitro dermal absorption – Skin distribution

- Objective of the study: To compare skin distribution of two different TA
  - One with low dermal absorption rate
  - One with high dermal absorption rate

In vitro dermal absorption – Skin distribution

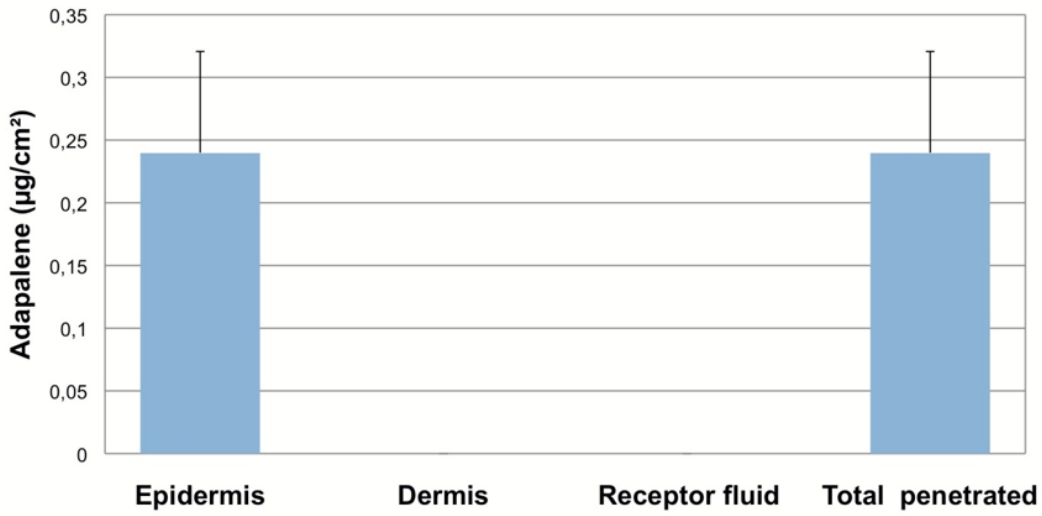
- *Ex vivo* skin samples
  - Full thickness human skin
  - 3 human donors
  - Each condition performed in 3 replicates (N = 9)
- Diffusion cells
  - Surface area: 1 cm^2
  - Volume of receptor compartment: 2 mL
  - Receptor fluid: PBS pH 7.2
- Skin thickness and TEWL measurement

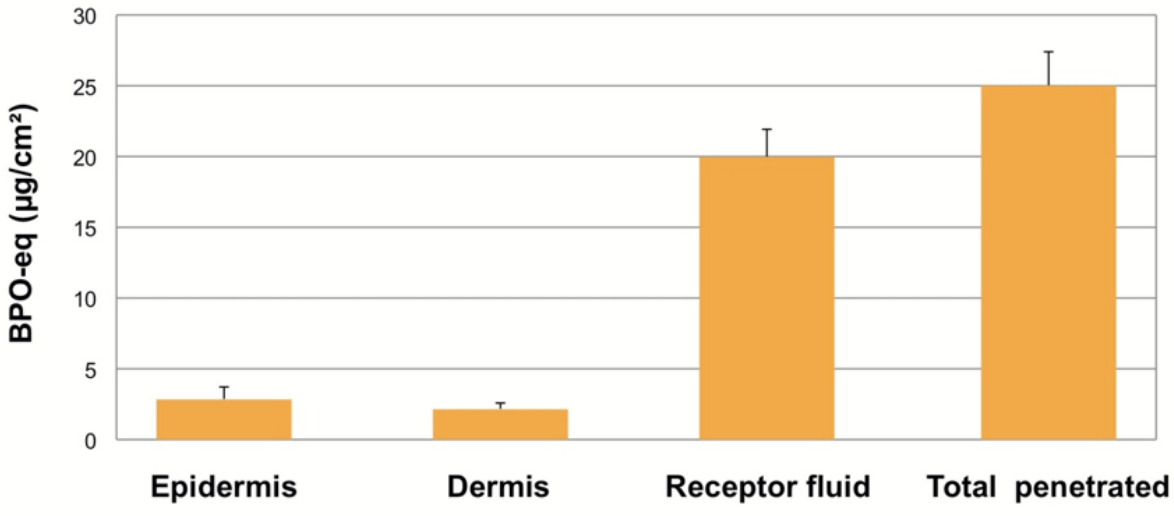
- Treatment conditions
  - Static conditions
  - Application: 10 mg/cm^2
  - Treatment duration: 24 hours
  - 32°C
- Sample analysis
  - Epidermis
  - Dermis
  - Receptor liquid
- Analysis performed using validated HPLC method

In vitro dermal absorption – Skin distribution

- Skin distribution of 2 different compounds: Adapalene and Benzoyl peroxide (BPO)

Mean and SEM, N = 9





- Dermal absorption of adapalene is very low and mainly distributed in epidermis.
- Dermal absorption of BPO is high, and is distributed in both epidermis and dermis compartments

In vitro dermal absorption – Bioequivalence

- Objective of the study: To compare 2 products
  - One marketed product
  - One new formulation

- Bioequivalence purpose

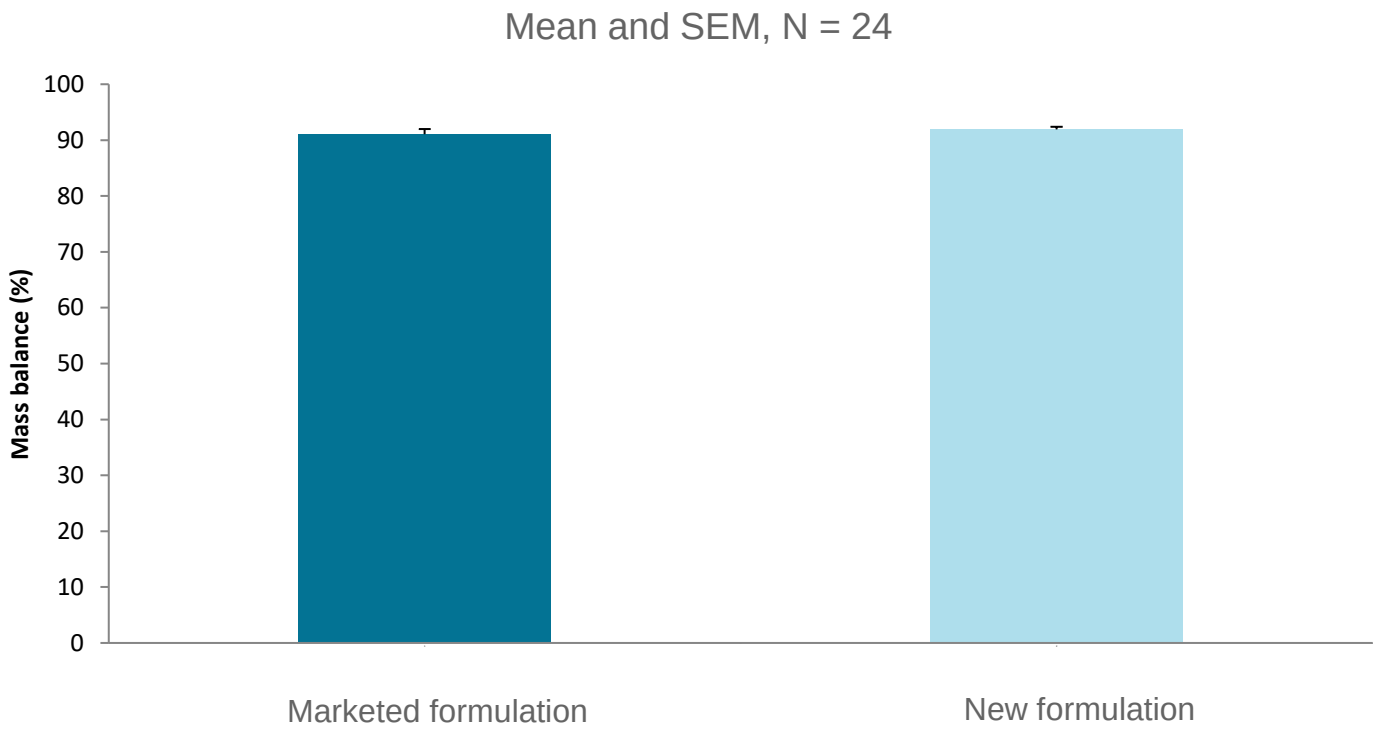
In vitro dermal absorption – Skin distribution

- *Ex vivo* skin samples
  - Full thickness human skin
  - 6 human donors
  - Each condition performed in 4 replicates (N = 24)
- Diffusion cells
  - Surface area: 2 cm^2
  - Volume of receptor compartment: 3 mL
  - Receptor fluid: PBS pH 7.2 + 0.25% Tween® 80
- Skin thickness and TEWL measurement

- Treatment conditions
  - Static conditions
  - Application: 5 mg/cm^2
  - Treatment duration: 5 minutes
  - Washing
  - Incubation for 24 hours at 32°C
- Sample analysis
  - Formulation excess:
  - Washing
  - Cotton swab
  - 1 tape strip
  - Total skin (including stratum corneum, epidermis and dermis)
  - Receptor liquid samples
- Analysis performed using validated HPLC method with UV detection

In vitro dermal absorption – Bioequivalence

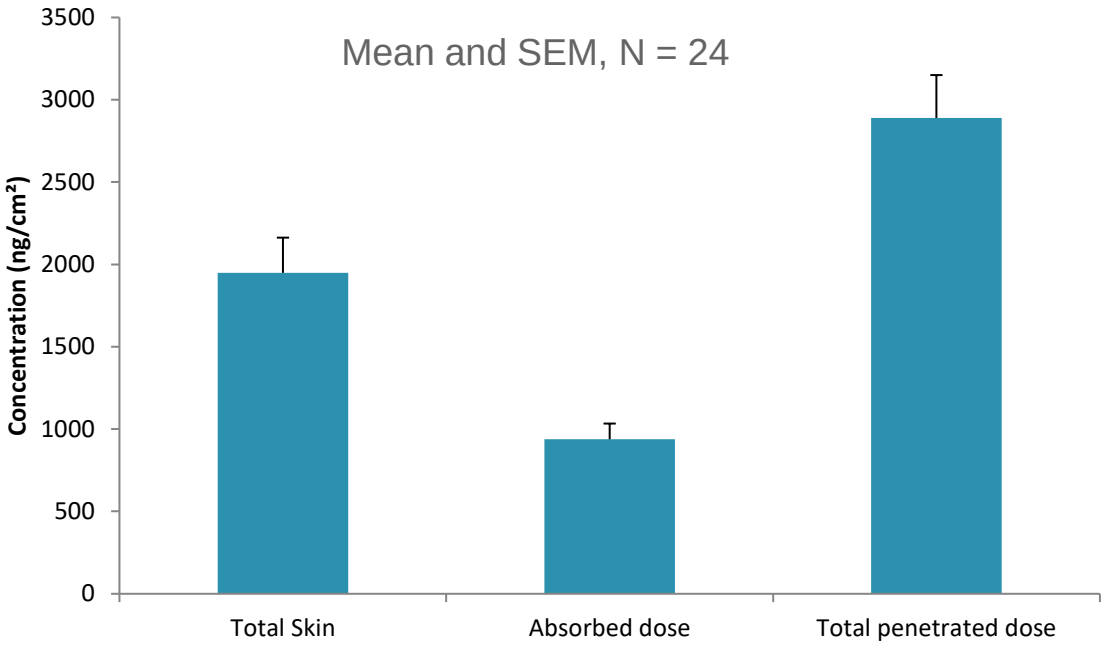
- Mass balance



- Mass balance ranged between 91 and 92% (acceptance criteria : 100 ± 20%)

In vitro dermal absorption – Bioequivalence

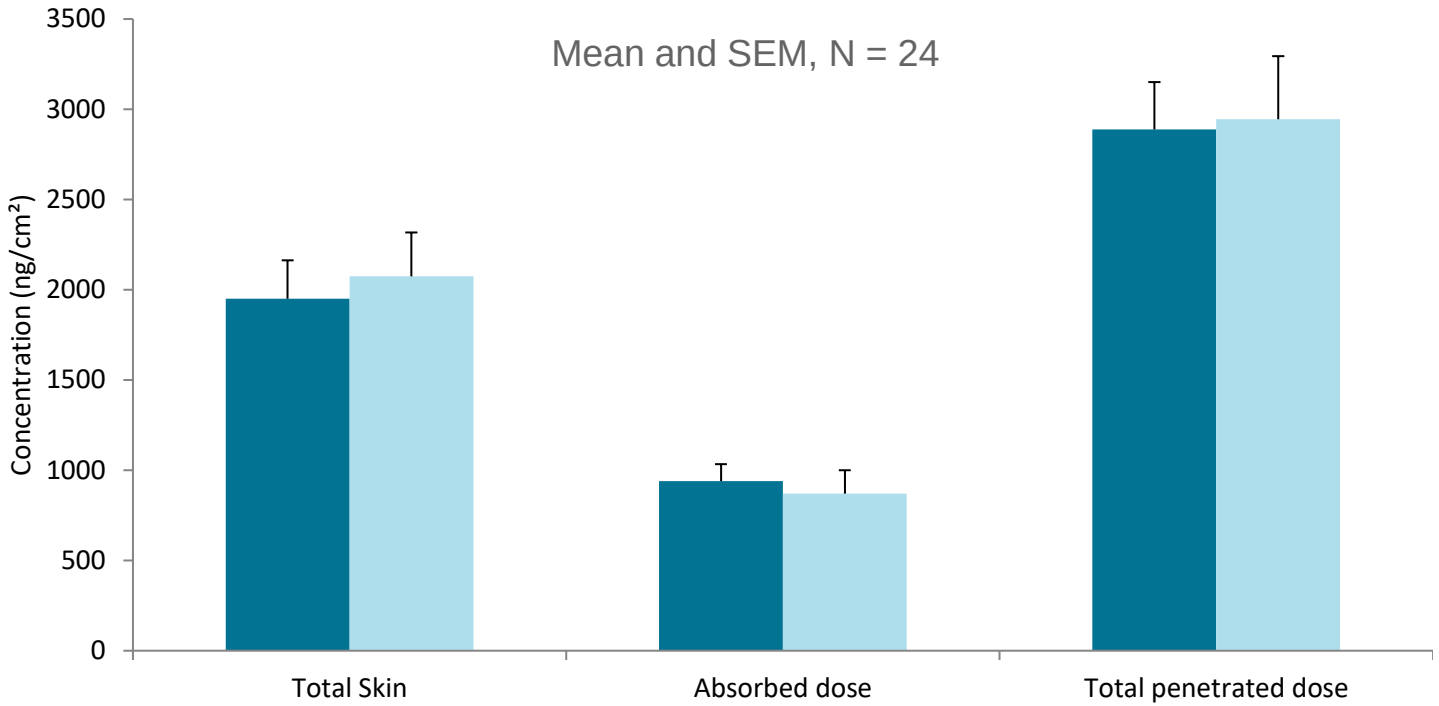
- Skin distribution profile of marketed formulation



- Quantity penetrated in the skin was 0,77% of the applied dose
- Quantity of the absorbed dose (receptor liquid) was 0,37% of the applied dose
- Total penetrated dose represented 1,14% of the applied dose

In vitro dermal absorption – Bioequivalence

- Comparison of two formulations



- No statistical difference was observed between the two formulations

In vitro dermal absorption – Bioequivalence

- Conclusion
  - Both formulations, the current marketed formulation and the new one are considered similar
  - Both formulations are equivalent in:
    - Total penetrated dose, absorbed dose and total skin
    - Similar efficacy and tolerance at action site with similar safety profile
- Overall, the new formulation should be as safe as the marketed one in terms of systemic absorption with the same efficacy
- No need to perform clinical trial

Examples of application

Cosmetic active ingredient (Safety assessment)

In vitro dermal absorption – Safety assessment

- Objective of the study
  - To measure in vitro dermal absorption of a cosmetic ingredient formulated in three different products
    - Formulation 1: 30%
    - Formulation 2: 5%
    - Formulation 3: 3,38%

- Safety assessment purpose

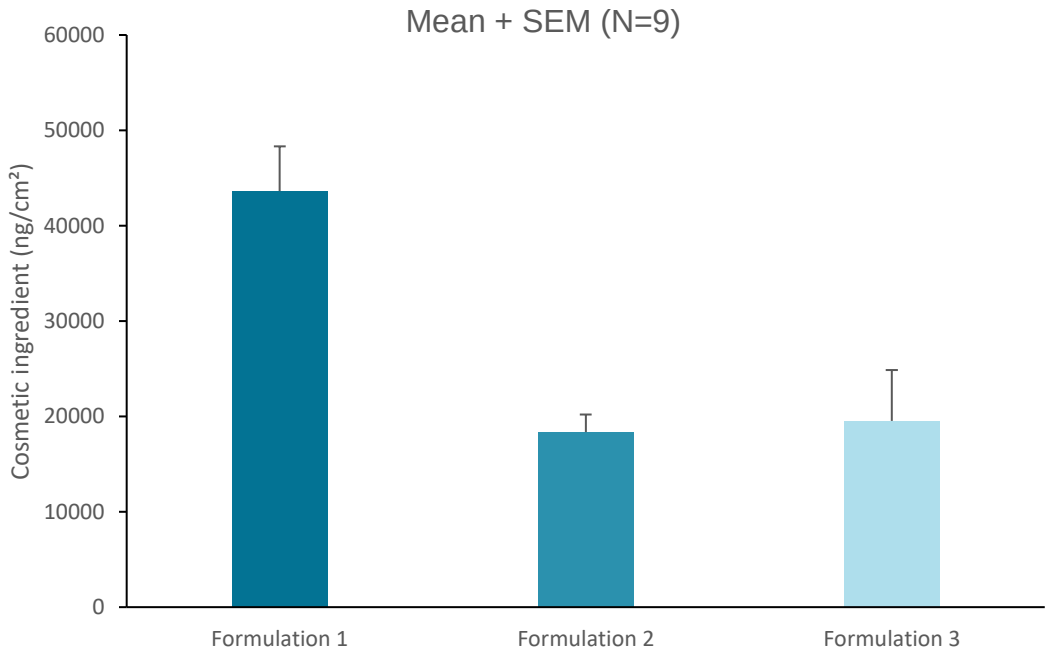
In vitro dermal absorption – Safety assessment

- *Ex vivo* skin samples
  - Full thickness human skin
  - 3 human donors
  - Each condition performed in 3 replicates (N = 9)
- Diffusion cells
  - Surface area: 2 cm^2
  - Volume of receptor compartment: 3 mL
  - Receptor fluid: PBS pH 7.2 + 1% Tween® 80
- Skin thickness and TEWL measurement

- Treatment conditions
  - Static conditions
  - Application: 10 mg/cm^2
  - Treatment duration: 24 hours
  - Washing
  - Incubation for 24 hours at 32°C
- Sample analysis
  - Total skin (including *stratum corneum*, epidermis and dermis)
  - Receptor liquid samples
- Analysis performed using LC-MS/MS method

In vitro dermal absorption – Safety assessment

- Comparison of three formulations



- Concentration of cosmetic ingredient penetrated the skin is proportional to the concentration in the formulation and does not exceed 10% of the applied dose
- Dermal absorption data are used in toxicological risk assessment to extrapolate human exposure

Conclusion

- Many examples are available and can be shared upon request

Thank you

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