In vitro rat skin to predict human dermal absorption: influences of aging and anatomical site

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INRS : reference body for occupational prevention in France
Introduction

• Workers are exposed to chemicals at their workplace

• Large surface area of the human body: dermal absorption is a route of exposure to consider when assessing occupational risks

• Freshly-excised human skin samples are regarded as the gold standard (difficult to obtain, experiments are restrictive)

• Rat skin is frequently used as a model for percutaneous absorption studies (easy to obtain, large amount of data available in the literature)

• Transposition of the results obtained in rats to humans is often subject to discussion

• Guidelines are not always very precise, what can lead to different experimental conditions depending on the studies
Objectives

• Under which conditions rat skin can be used as a model for percutaneous absorption?

• Reduce variations in results by reducing variations in samples used in vitro
  ■ Determine an « ideal » age to take rat skin samples
  ■ Determine the more relevant location to excise rat skin samples

• Based on previous findings, comparison of percutaneous absorption between rats and humans for 3 molecules
Methodology: skin layers thicknesses

- **Samples used**
  - Sprague Dawley rats (4 weeks, 7 weeks, 4, 8 and 12 months), 3 locations
  - Human abdominal skin (abdominal plastic surgery, 3 different donors)
Methodology: skin layers thicknesses

- Frozen sections were prepared and cut with a cryostat
- Stained with eosin-hematoxylin-saffron
- Thicknesses
  - stratum corneum
  - viable epidermis
  - total epidermis

0.6 cm² skin sections
Results

Stratum corneum thickness in rat

Thickness (µm)

- Shoulders
- Back
- Hips

4 weeks 7 weeks 4 months 8 months 12 months
Results

Total epidermis thickness in rat

- Shoulders
- Back
- Hips

Thickness (µm)

- 4 weeks
- 7 weeks
- 4 months
- 8 months
- 12 months

Thickness range:
- 20 µm to 70 µm
Results

Viable epidermis thickness in rat

Thickness (µm)

Shoulders
Back
Hips

8 weeks
7 weeks
4 months
8 months
12 months

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Choice of skin samples for *in vitro* experiments

- Skin thickness depends on the age of the rats and the location

- Samples taken from the back are relatively uniform from 7 weeks

→ Back samples from at least 7 weeks old rats were chosen for *in vitro* percutaneous absorption experiments
Methodology: *in vitro* flux

• Comparison of *in vitro* percutaneous absorption fluxes in rats and humans for three $[^{14}C]$-radiolabelled molecules:
  - N-methyl-2-pyrrolidone (NMP, neat, 40 µL/cm²)
  - N,N-dimethylformamide (DMF, neat, 400 µL/cm²)
  - Bisphenol A (BPA, 200 µg/cm², 50 µL/cm² in acetone)

• Dermatomed skin samples (about 500 µm)

• Static Franz diffusion cells

• Skin integrity (Trans Epidermal Water Loss)
Methods: *in vitro* flux

Franz diffusion cells

Receptor cell + rat skin

Automatic fraction collector

**Franz cell characteristics**

- Static cells
- Deposit area: 0.45 cm²
- Receptor cell volume: 2 mL
- Aliquots volume: 200 µL

Receptor fluid: RPMI cells culture medium, 0.2% gentamycin, 2.5% penicillin streptomycin (+ 2% Bovine Serum Albumin for BPA)
NMP *in vitro* absorption

![Graph showing NMP cumulative absorption and absorption flux for Rat and Human over time.](image)
DMF *in vitro* absorption

**DMF cumulative absorption (mg/cm²)**

- Time (h):
  - 0, 4, 8, 12, 16, 20, 24

**DMF absorption flux (mg/cm²/h)**

- Time (h):
  - 0, 4, 8, 12, 16, 20, 24

**Graphs**

- **DMF cumulative absorption**
  - Rat: Red dots
  - Human: Green squares

- **DMF absorption flux**
  - Rat: Red dots
  - Human: Green squares
BPA \textit{in vitro} absorption

BPA cumulative absorption ($\mu$g/cm²) vs Time (h)

- Rat
- Human

BPA absorption flux ($\mu$g/cm²/h) vs Time (h)

- Rat
- Human
Conclusions

• Rat skin samples from the back of at least 7 weeks old animals

• Limits of the rat skin to predict percutaneous absorption in human
  ▪ Good prediction for NMP and DMF
  ▪ 3-fold overestimation for BPA

• Rat is probably not the best model, but not the worst

• By combining *in vivo* and *in vitro* results in rat with *in vitro* results in humans, it allows a “triple pack approach” for extrapolation to humans
Capacity of an *in vitro* rat skin model to predict human dermal absorption: Influences of aging and anatomical site

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Thanks for your attention