Influence of experimental parameters on Bisphenol A skin permeation kinetics

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Introduction

Bisphenol A

Exposure from food alone cannot explain reported BPA blood and urine concentrations.

How much does dermal exposure to BPA contribute to BPA body burden?
In-vitro tests with animal or non-viable human skin

In-vitro tests with animal or non-viable human skin

Mainly Phase II metabolites:
- BPA-monoglucuronide (BPA-Gluc)
- BPA-monosulfate (BPA-Sulf)

Source: Gundert-Remy et al. 2013
Skin absorption/permeation studies in the literature

Six studies on BPA with different results:

• Total absorbed: 37% - 87%
• Skin metabolism: < 2.5% - 27%
• Lag time: 1 h – “long”
• Permeability coefficient: $1 \times 10^{-4}$ cm/h - $3 \times 10^{-2}$ cm/h
Objectives

1. To determine BPA skin permeation kinetics and metabolism through ex-vivo human skin

2. Assess the influence of different variables of the experimental set-up on BPA skin permeation, in order to better understand the different results reported in the literature.
Skin permeation testing

As per the OECD 428 guideline for in vitro testing of chemicals for skin absorption.
Permeation kinetics

At the steady state:

\[ J_{\text{MAX}} = K_p \cdot C_v \]

**Cv**: concentration in vehicle applied on the skin
**Kp**: permeability coefficient = \( K_M \cdot D / L \)
**KM**: partition coefficient
**D**: diffusion coefficient
**L**: skin thickness

**Flux J** [mass/area/time]

**Cumulative amount in the receptor fluid (µg/cm²)**

**Time (h)**

**Lag time (t_{lag})**
BPA skin permeation kinetics

Donor chamber:
• 100 µl/cm² BPA 250 mg/l (25 µg/cm²)

Receptor chamber:
• Physiological saline water
• 50 µl/min

Skin:
• Viable
• Abdomen
• 200 µm thickness
• 3 skin donors (N = 3)
• 12 replicates (n = 12)

Results

\[ J (\pm SD) = 0.712 \pm 0.340 \text{ µg/cm}^2/\text{h} \]
\[ K_p (\pm SD) = 2.84 \pm 1.35 \times 10^{-3} \text{ cm/h} \]
\[ t_{lag} (\pm SD) = 3.6 \pm 0.6 \text{ h} \]
## Summary of BPA skin permeation experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Concentration [mg/l]</th>
<th>Volume [µl]</th>
<th>Dose [µg/cm²]</th>
<th>Skin donors [N]</th>
<th>Total skin samples [n]</th>
<th>Skin thickness [µm]</th>
<th>Skin condition</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>100</td>
<td>25</td>
<td>3</td>
<td>12</td>
<td>200</td>
<td>viable</td>
<td>LC/MS/MS</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>100</td>
<td>25</td>
<td>3</td>
<td>10</td>
<td>800</td>
<td>viable</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>100</td>
<td>25</td>
<td>3</td>
<td>9</td>
<td>800</td>
<td>frozen</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>100</td>
<td>1.8</td>
<td>2</td>
<td>6</td>
<td>800</td>
<td>viable</td>
<td>HPLC-FLD</td>
</tr>
<tr>
<td>5</td>
<td>164</td>
<td>10</td>
<td>1.6</td>
<td>2</td>
<td>6</td>
<td>800</td>
<td>viable</td>
<td>HPLC-FLD</td>
</tr>
</tbody>
</table>
Effects of some variables on BPA skin permeation

Results

• $K_p \uparrow$ 3.5-fold (*)
• $t_{lag} \downarrow$ 2-fold (*)

Skin thickness
800 $\rightarrow$ 200 μm

Vehicle volume
100 $\rightarrow$ 10 μl

• $K_p \downarrow$ 21-fold (*)

Skin condition
Fresh $\rightarrow$ Frozen

Vehicle concentration
18 $\rightarrow$ 250 mg/l

• $K_p \uparrow$ 2-fold (***)

(*) $p \leq 0.05$
(***) $p \leq 0.001$
### BPA-Glucuronide in the receptor fluid was negligible

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Our study</th>
<th>Marquet et al. 2011</th>
<th>Zalko et al. 2010</th>
<th>Toner et al. 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin thickness [µm]</td>
<td>200</td>
<td>400</td>
<td>500</td>
<td>400</td>
</tr>
<tr>
<td>Total skin samples (skin donors)</td>
<td>12 (3)</td>
<td>15 (6)</td>
<td>3 (?)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>System</td>
<td>Flow-through diffusion cells</td>
<td>Static diffusion cells</td>
<td>Transwell inserts in 6-well plates for ex-vivo organ culture.</td>
<td>12-well plate</td>
</tr>
<tr>
<td>Receptor fluid(^1)</td>
<td>Physiological saline water</td>
<td>RPMI, 2% BSA, 1% penicillin/streptomycin</td>
<td>DMEM. L-glutamine, penicillin/streptomycin fungizone, gentamycin</td>
<td>DMEM (ca1% EtOH, v/v) + UDPGA (2 mM) + PAPS(40 μM)</td>
</tr>
<tr>
<td>BPA-gluc analysis method</td>
<td>LC-MS/MS (direct)</td>
<td>HPLC/scintillation counter (direct)</td>
<td>HPLC-fluorescence (direct)</td>
<td>HPLC/scintillation counter (direct)</td>
</tr>
<tr>
<td>BPA-Glucuronide in receptor fluid (% of applied dose)</td>
<td>&lt; LOD (0.2 ng/ml) at 24h</td>
<td>&lt; LOD (1.5 ng/ml)(^2) at 24h</td>
<td>27 % at 72h(^3)</td>
<td>8 % at 24 h(^4)</td>
</tr>
</tbody>
</table>

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\(^1\) Acronyms: RPMI Rosewell Park Memorial Institute medium, BSA bovine serum albumine, DMEM Dulbecco’s modified eagle medium, UDPGA Uridine 5’-diphosphogluconic acid, PAPS 3’-phosphoadenosine-5’-phosphosulfate

\(^2\) BPA accounted for more than 97.5% of the radioactivity detected in the receptor fluid.

\(^3\) Sum of BPA-glucuronide and BPA-sulfate

\(^4\) Sum of BPA-glucuronide, BPA-sulfate and more polar compounds
Using correction factors to compare different studies

Our study: $K_p = 2.8 \times 10^{-3}$

- Skin thickness: $800 \rightarrow 200 \, \mu m$
- Skin condition: Fresh $\rightarrow$ Frozen
- Vehicle volume: $100 \rightarrow 10 \, \mu l$

Demierre et al. 2012: $K_p = 1.0 \times 10^{-4}$

Marquet et al. 2011: $K_p = 3.0 \times 10^{-5}$

Toner et al. 2018: $K_p = 1.1 \times 10^{-5}$

* Calculated from reported $J$ and concentration values.
Using correction factors to calculate internal dose for different scenarios

\[
\text{Internal dose} = \frac{\text{Flux} \times \text{Duration} \times \text{Surface}}{\text{BW}}
\]

\[
\text{Flux} = J = K_p \times C
\]

dry hands \(K_p\) for 10 µl vehicle volume
wet hands \(K_p\) for 100 µl vehicle volume
water, moisturizing lotion, hand sanitizer
Conclusions

• BPA skin permeation kinetics were most influenced by vehicle volume

• The calculated factors correct the permeability coefficient Kp for differences in experimental set up

• Applying the correction factors, different studies get to similar Kp values

• Correction factors could be used to take into account different exposure scenarios in BPA TK modelling and risk assessment
Thank you for your attention

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