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Dermal penetration of bisphenol A in human skin contributes marginally to total exposure

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HIGHLIGHTS

- The aim of the study was to determine the dermal penetration rate of bisphenol A.
- The analysis has been done under GLP conditions and according to OECD guideline 428.
- The test has been performed in conditions close to reality.
- The contribution of dermal exposure to bisphenol A is confirmed to be moderate.

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ABSTRACT

Bisphenol A (BPA) is ubiquitous and many exposure scenarios have been described during the last decades. While oral uptake is considered as the major route of exposure, the contribution of skin penetration has been recently discussed. In the present study, the dermal penetration rate of BPA has been determined in human skin in an *in vitro* test method according to the OECD Test Guideline 428. This analysis resulted in penetration of 8.6% and a total amount of bio-available BPA of 9.3% of the dose applied after 24 h incubation under realistic exposure conditions. This confirms that the systemic exposure to BPA via the skin contributes in a negligible way to total systemic BPA exposure.

1. Introduction

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide, and is known to have weak estrogenic activity (Rubin, 2011). To date, ingestion is considered as the major route of exposure to BPA, but some recent studies raised concern on the contribution of dermal exposure (Geens et al., 2011; Mielke et al., 2011). Indeed BPA is widely used in paper industry as color developer in thermal paper (Geens et al., 2011). BPA has been found in 11 of 13 thermal printing papers collected in Switzerland, with a mean concentration of 13.3 g/kg (Biedermann et al., 2010). A similar study performed in the USA found BPA in 16 of 36 thermal receipts (Environmental Working Group, 2010). Moreover in the USA, the monitoring of pregnant women working as cashiers, and thus handling frequently thermal paper, revealed higher urinary concentration of BPA metabolites. However, this study showed some weaknesses and this correlation needs to be confirmed (Braun et al., 2011). BPA has also been detected in paper currencies (Liao and Kannan, 2011a), as well as in other papers and paper products (Liao and Kannan, 2011b).

Relatively few studies evaluated the penetration of BPA by the skin and these ones resulted in rates ranging from moderate, with up to 15% (Kaddar et al., 2008; Mørck et al., 2010), to substantial, showing more than 35% penetration (Marquet et al., 2011; Zalko et al., 2011). Only the study of Mørck has been performed according to OECD Test Guidelines (TG), but with an exposure time extended up to 48 h. Since the penetration rate is essential to perform a plausible risk assessment of the dermal exposure to BPA, we decided to determine this rate strictly according to OECD TG 428 and good laboratory practices (GLP), under conditions close to reality.

The terminology concerning dermal absorption is often confused in the literature. In the present article the term "penetration" defines the amount of applied BPA that crossed the skin and is recovered in the receptor fluid. On the other hand, the term "absorption" corresponds to the amount of applied BPA which passed through the stratum corneum but is still present in the dermis. Together, the quantities absorbed and penetrated represent the amount of bio-available BPA.

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2. Material and methods

All experiments have been done under GLP conditions at Harlan Laboratories Ltd., Itingen, Switzerland, following the OECD TG 428 (Skin absorption: *in vitro* method).

2.1. Human skin

Full thickness skin was obtained from 2 human cadavers. The samples were taken from the dorsal part of the upper legs. The intact skin samples were then stored at −20 °C for up to one year. After thawing, 7 skin sections of 200 μm thickness were cut off from the top using a dermatome (cordless dermatome GA 643).

2.2. Diffusion cell apparatus

An automated flow-through cell system (Permegear Inc., Riegelsville, USA) was used. Each cell consisted of a donor and a receptor chamber between which the skin was positioned with the stratum corneum uppermost (area exposed: 0.64 cm^2). The apparatus was maintained at 30–32 °C. A physiological saline solution (0.9% NaCl) was used as receptor fluid, with a flow through the receptor cell adjusted to about 3 mL/h.

2.3. Test of skin membrane integrity

The integrity of the skin was checked by applying 50 μL of ^{3}H-water (ARC Inc., St. Louis, USA) to the skin membrane surface. The test has been performed under occluded conditions to limit the evaporation and keep a stable concentration of the solution. The permeability coefficient (Kp) of each skin membrane was calculated using the penetration rate. Human skin membranes with Kp > 2.5×10^{-3} cm/h are excluded. All samples resulted in Kp within the acceptance criteria (between 0.9 and 1.5×10^{-3} cm/h).

2.4. Chemicals and dose

^{14}C-BPA with a specific activity of 3.9 GBq/mmol was purchased from ARC Inc., St. Louis, USA. The purity of the radiochemical has been determined at 99.2% by RHPLC (radio high performance liquid chromatography). A homogenous solution of 193.6 mg/L ^{14}C-BPA has been prepared in H_2O (concentration checked by RHPLC). The limit of solubility of BPA in water has been determined to be approximately 250 mg/L in a previous non-GLP pretest. According to OECD TG 428 recommending the application of up to 10 μL/cm^2, 6 μL aliquot was applied manually to 7 skin membranes, corresponding each to 1.82 μg BPA/cm^2. The donor chambers were then covered by a permeable tape (non-occluded conditions) to mimic real exposure conditions.

2.5. Sampling

The receptor fluids were collected at 1 h intervals up to 6 h, and then at 2 h intervals. After 24 h the skin membranes were rinsed 3 times with 0.5 mL of a mild washing solution (1% shower gel, Nivea, Beiersdorf, DE) and once with 0.5 mL pure H_2O. The skin membranes were removed from diffusion cells and consecutively stripped with adhesive tape until the stratum corneum was removed (15 tape strips). The first five strips were analyzed individually, whereas the strips 6–10 and 11–15 were pooled. The donor cells were finally washed each with 15 mL ethanol/water (1:1).

2.6. Measurement

Skin samples (tape strips corresponding to stratum corneum and residual skin membranes) were first solubilized with tissue solubilizer (Solvable, Perkin Elmer, Boston, USA). In all samples (i.e. skin surface rinse, stratum corneum, residual skin membrane, receptor fluids and donor cell rinse), radioactivity was quantified by liquid scintillation counting on Packard Tri-Carb scintillation counters (Packard Instruments Comp. Inc., Meriden, USA).

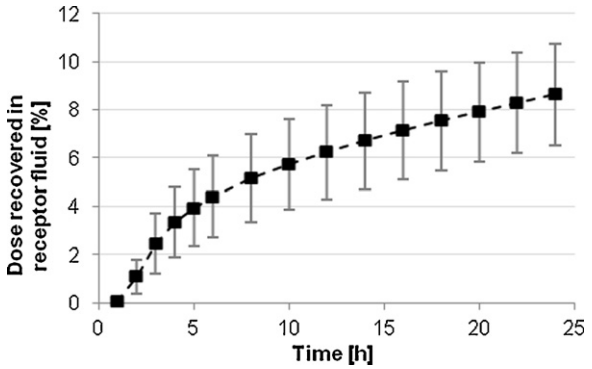

Fig. 1. Kinetics of ^{14}C-BPA penetration. The mean percentages of dose recovered in receptor fluid have been cumulated ($n = 7$).

3. Results

Within 8 h after application of ^{14}C-BPA, 0.093 μg/cm^2 (5.1% of the applied dose) penetrated into the receptor fluid. After 24 h, at the end of the experiment, this rate reached 0.157 μg/cm^2, corresponding to 8.6% of the applied dose (Fig. 1).

The maximum penetration flux, reflecting the penetration rate under steady state conditions (linear phase, from 1 to 4 h) was measured to be 0.022 μg/cm^2/h. In the stratum corneum, 34.9% of the applied dose was recovered, mainly in the most external layers (Fig. 2). The amount of BPA absorbed, i.e. measured in the remaining skin membrane after tape stripping, was 0.012 μg/cm^2 (0.6% of the applied dose). Accordingly the amount of bio-available BPA after 24 h exposition reached 0.169 μg/cm^2, accounting for 9.3% of the total dose applied. The distribution is relatively similar in both donors skin samples, with amounts of bio-available BPA of 8.3% and 10.0%, respectively (Table 1). The mean total recovery was 101.5%. The detailed distribution of the dose recovered in each samples independently can be found in supplementary data 1.

4. Discussion

It is known that ingestion is the main exposure route to BPA, but recently the awareness of dermal exposure increased. Up to now none of the previous evaluations of BPA skin penetration have been

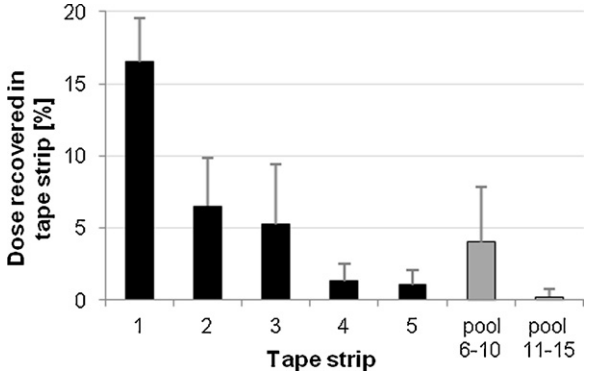

Fig. 2. Distribution of ^{14}C-BPA in stratum corneum after 24 h incubation (mean percentages of dose applied, $n = 7$). The tape strips correspond to layers from most external to internal. The samples 6–10 and 11–15 have been pooled.

Table 1
Distribution of dose recovered after 24 h incubation [% of dose applied]. The mean results ± SD of the two donors are shown, as well as the limit of quantification (LOQ) in each type of sample.

Fraction	Donor 1 (n = 3)	Donor 2 (n = 4)	Total (n = 7)	LOQ
Skin surface rinse	52.4 ± 2.8	60.3 ± 2.7	56.9 ± 4.9	0.30
Donor cell rinse	0.2 ± 0.0	0.6 ± 0.4	0.5 ± 0.4	0.12
Stratum corneum	41.0 ± 3.9	30.3 ± 3.2	34.9 ± 6.6	0.05
Residual skin membrane	0.5 ± 0.1	0.7 ± 0.3	0.6 ± 0.3	0.03
Receptor fluids	7.8 ± 0.1	9.3 ± 2.7	8.6 ± 2.1	0.09
Total recovery	101.8 ± 2.2	101.3 ± 1.3	101.5 ± 1.6	

done strictly according to OECD TG and under GLP conditions. The present study fills this gap and can be used for BPA risk assessment.

The study has been performed in conditions supposed to be as close as possible to reality. The amount of BPA transferred from thermal paper to a single finger has been evaluated at 1.13 μg. Even in case of prolonged or repeated contacts, the dose of BPA transferred to the finger tip does not increase (Biedermann et al., 2010). Accordingly, the dose tested in the present study is slightly higher with 1.82 μg/cm^2. The time of exposure recommended in the OECD TG and used in the present study (24 h) is also longer than expected in reality, as normally cashiers are working up to 10 h per day and wash their hands sometimes during this period. Concerning the medium used for the application of BPA, it is very difficult to mimic a real situation since the composition of sweat is different for each individual and since other factors such as the use of hydrating cream can influence the penetration. In order to be closer to sweat composition, ^{14}C-BPA has been dissolved in pure water and not in alcohol as in Mørck et al. (2010), Zalko et al. (2011) and Marquet et al. (2011).

After 24 h of application, only 8.6% of the applied dose has been measured in the receptor fluid. These results are comparable to those in pig skin published by Kaddar et al. (2008) and in full thickness human skin published by Mørck et al. (2010), i.e. respectively, 0.7% after 10 h and 13.0% after 48 h. The study from Zalko et al. (2011) resulted in a much higher penetration rate, up to 65.3% in pig skin and 45.6% in human skin. This discrepancy can be explained by a longer incubation time (72 h) and the use of an alternative model based on skin organ culture in static diffusion cells using Transwell inserts rather than Franz type diffusion cells. Substantial penetration has also been observed by Marquet et al. (2011), with up to 38.6% penetration after 30 h. However this result is not comparable, since the analysis has been performed *in vivo* in rat and with an extreme high dose applied (about 2000× higher than the dose used in the present study). Moreover, they demonstrated *ex vivo* that the penetration flux in rat skin samples is about 10× higher (1.48 μg/cm^2/h) than in human skin (0.12 μg/cm^2/h). The flux calculated in the present study on human skin is slightly lower (0.022 μg/cm^2/h) but the high dose used in the study of Marquet et al. (2011), which was about 100× higher than the dose applied here, can explain this discrepancy. Since skin penetration results from passive transfer, it depends on the gradient between the applied concentration and the concentration in the receptor fluid. Thus use of a high concentration could induce a higher and faster penetration than when using a realistic field concentration (Moser et al., 2001).

Considering the amount of BPA transferred to each finger by Biedermann et al. (2010) and assuming a worst case, with a large palm surface entering in contact with thermal paper, the external exposure could reach up to about 100 μg. Considering the amount of bio-available BPA of 9.3% measured in the present study, the total daily uptake via the skin in this case would correspond to 9.3 μg/day, which is far below the TDI (tolerable daily intake) set by the WHO at 50 μg/day per kilo body weight, i.e. 3000 μg/day for a person weighing 60 kg. Moreover, for its risk assessment of BPA updated in 2008, the European Union based its evaluation on a penetration rate of 10%, since no data were available for the dermal penetration of BPA at that time. Using this rate, which is slightly higher than the one determined in the present study, they concluded there is no concern arising from dermal exposure in comparison to other sources of exposure (European Union, 2008).

BPA has been monitored in human in several studies, covering different countries and different population groups (Braun et al., 2011; Vandenberg et al., 2010). Only few of them used these data to extrapolate the BPA daily intake, estimating mean exposures to BPA in the range of 0.01–0.07 and in worst cases of up to 0.50 μg/day/kg body weight (Geens et al., 2011; Koch and Calafat, 2009; Lakind et al., 2012). These values are all well below the TDI set at 50 μg/day/kg body weight, but are only slightly higher than the data presented here. However, a comparison with the penetration rate presented in this study is not really feasible. Indeed the method used here is an *in vitro* method, meaning that the substance is not metabolized as in the human body. It is well known that ingested BPA is rapidly metabolized in human, forming non-estrogenic compounds that are excreted within few hours (Dekant and Volkel, 2008), but the fate of BPA absorbed by the skin is still unclear. Zalko et al. (2011) presented data showing that BPA is highly metabolized after penetration through viable skin. Moreover in the present study, the exposure time has been extended up to 24 h, which is much longer than in reality.

In conclusion, the present study confirms that dermal exposure to BPA is moderate and contributes in a negligible way to total body burden.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxlet.2012.07.001.

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