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Skin absorption of Bisphenol A and its alternatives in thermal paper

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Abstract

Introduction: Bisphenol A (BPA) is the most used colour developer in thermal paper for cashiers receipts, labels and tickets. BPA can migrate onto the skin and be absorbed when handling these papers. BPA is a known endocrine disruptor and is therefore being replaced in thermal paper by some alternatives such as Bisphenol S (BPS), D-8 and Pergafast201® (PF201). To our knowledge, no studies have characterised skin permeation of these BPA alternatives.

Methods: We measured/characterised skin absorption for BPA, BPS, D-8, and PF201 through ex-vivo human skin using flow-through diffusion cells according to OECD guideline 428. Skin samples were 7-12 per test substance from three different skin donors. Skin metabolism was studied for BPA. Dermal absorption was expressed as the amount of the BPA alternatives in the receptor fluid over applied dose in percent (%).

Results: The absorbed dose after 24 hours of exposure was 25% for BPA, 17% for D-8, 0.4% for BPS and <LLOQ for PF201. The amount of BPA-glucuronide in the receptor fluid after 24 hours was under the limit of quantification (LLOQ = 0.2 µg/l). Despite the 10-fold lower concentration of the aq solution applied on the skin, D-8's permeation rate J_{MAX} was 5-fold higher than the one for BPS (0.032 vs. 0.006 µg/cm^2/h). Neither D-8 nor BPS permeated readily through the skin (t_{lag} = 3.9 h for D-8, 6.4 h for BPS). None of PF201's skin permeation kinetic parameters could be determined because this BPA analogue was not quantifiable in the receptor fluid in our test conditions.

Conclusions: Skin absorption was in decreasing order: BPA > D-8 >> BPS > PF201. These results are in agreement with their logK_{ow} and molecular weights. We provided here the necessary data to estimate the extent of skin absorption of BPA analogues, which is a necessary step in risk assessment, and ultimately evaluate public health risks posed by D-8, PBS, and PF201.

1. Introduction

Bisphenol A (BPA) is one of the largest high production volume chemicals (OECD). The second largest source of human BPA exposure after food and beverage packaging is thermal paper (cash receipts, parking, airline and cinema tickets, luggage tags, bus and train tickets, grocery weight tickets) (EFSA, 2015). BPA is in the powdery coating on one side of the thermal paper. It has been found in 100/124, 78/100, and 195/308 thermal paper products by Goldinger et al. (2015), Björnsdotter et al. (2017), Vervliet et al. (2019), respectively.

Skin contact with BPA in thermal paper contributed to the overall body burden among occupationally exposed populations, such as cashiers (Braun et al., 2003; Ndaw et al., 2016; Thayer et al., 2016) and workers in industries manufacturing and using BPA (Heinälä et al., 2017; Hines et al., 2017). These studies show that BPA migrates from the thermal paper onto the skin (Biedermann et al., 2010; Hormann et al., 2014) and is absorbed (Marquet et al., 2011; Demierre et al., 2012; Toner et al., 2018; Liu and Martin, 2019). Most of the absorbed BPA is not or is only slightly metabolised by the skin (Marquet et al., 2011; Zalko et al., 2011; Toner et al., 2018; Liu and Martin, 2019), and goes directly into the systemic circulation. BPA metabolites such as glucuronide and sulphate conjugates are not toxic and consequently, metabolism is of importance in assessing BPA toxicity.

In 2016, BPA was classified as a presumed human reproductive toxicant (category 1B), and the European Union decided to restrict BPA in thermal paper to no more than 0.02% (w/w) by 2020 (EU 2016/2235). Several chemicals have been or are in the process of replacing BPA in thermal papers. These alternative developers could potentially migrate from thermal paper and be absorbed by the skin as shown for BPA. Moreover, thermal paper products usually contain one major developer with other developers often used as secondary developers or present in trace levels (Björnsdotter et al. 2017; Vervliet et al. 2019).

The most used BPA alternatives in cash receipts are Bisphenol S (BPS), Pergafast 201® (PF201), and Wincon 8 (D-8) (Table 1). The US EPA has classified BPS and PF201 as high-hazard colour developers for repeated dose toxicity and developmental toxicity, respectively (US EPA 2014). D-8 was considered of moderate hazard for these endpoints and with limited evidence of endocrine activity (US EPA 2014; Goldinger et al. 2015). Other less used developers are: D-90, 4-(4-hydroxy-3-prop-2-enylphenyl)sulfonyl-2-prop-2-enylphenol (TGSA), and 4-(4-phenylmethoxyphenyl)sulfonylphenol (BPS-MAE). Rare developers are urea urethane (UU) and 1,7-bis(4-hydroxyphenylthio)-3,5-dioxaheptane (DD-70). A structural analogue of BPS, 2,4-BPS, was detected in 20.8% of the samples, and it is likely to be an impurity of BPS (Vervliet et al., 2019; Yang et al., 2019).

BPA has the highest concentration in thermal paper (medians 15.0 – 15.9 mg/g (Eckardt and Simat 2017), mean

13.5 mg/g (Goldinger et al., 2015), mean 3.61 mg/g (Yang et al., 2019)), followed by BPS, Pergafast 201® and D-8 . In the study of Eckardt and Simat (2017), BPS, Pergafast 201® and D-8 concentrations ranged from 1.4 to 19.2 mg/g paper (median values between 2.5 and 14.7 mg/g). Similar values were reported by Goldinger et al. (2015) (concentration range 3.3 – 13.2 mg/g, median values between 4.6 – 12.0), while lower values were consistently measured in Chinese samples (mean values range 0.209 – 1.15, Yang et al. (2019)). The Danish Environmental Protection Agency (EPA) found similar concentrations for BPA, BPS and Pergafast 201® in thermal paper where the developers' content had been confirmed by the manufacturer (10.8, 11.6, 10.4 mg/g respectively) (Danish EPA, 2014).

Reported mean values of amount of BPA transferred from thermal paper to dry (i.e. normal condition) fingers was 1.1 µg/finger (Biedermann et al., 2010), 1.4 µg/finger (Lassen et al., 2011), and 1.1 µg/finger (Danish EPA, 2014). Eckardt and Simat (2017) observed no significant difference in the amount transferred when comparing samples with different colour developers (BPA, BPS, Pergafast 201®, and D-8), and ranged from 0.05 to 6.0 µg/finger (mean 0.8 µg/finger, median 0.3 µg/finger). By contrast, the Danish EPA reported mean values lower than BPA for both BPS (0.8 µg/finger) and Pergafast 201® (0.4 µg/finger). Liu and Martin (2019) reported higher values for BPS (3.9 µg/hand wipe, range 0.71 – 10 µg/hand wipe) than BPA (0.77 µg/hand wipe, range 0.07 – 3.0 µg). Transfer of BPA from thermal paper is greater on humid, sweaty, oily or hand-lotion skin compared to dry skin (Biedermann et al., 2010; Lassen et al., 2011; Danish EPA, 2014). When skin was moistened by water or artificial sweat, Eckardt and Simat (2017) found that the amount of BPA transferred (mean 35 µg/finger, range 21 – 42 µg/finger) was higher than BPS, D-8 and Pergafast 201® (mean range 9 - 14 µg/finger, values range 2.3 – 24.6 µg/finger). Again, the Danish EPA (2014) observed greater amounts of BPA (17.7 µg/finger) and Pergafast 201® (17.1 µg/finger) compared to BPS (3.1 µg/finger).

Colour developers could possibly be absorbed through the skin after migrating from thermal paper. BPA skin absorption has been extensively studied *in vitro* and is shown to be absorbed by the skin (Marquet et al., 2011; Demierre et al., 2012; Toner et al., 2018). Skin absorption of the alternative developers is poorly understood. Only two *in vitro* and *in vivo* study on BPS (Liu and Martin 2019; Champmartin et al. 2020) exist in the literature. To our knowledge, no other alternative developers have been assessed for potential skin absorption. The aim of this study was to characterise skin absorption of the BPA alternatives most frequently used in thermal papers, namely BPS, D-8, and Pergafast 201®, and to compare these to BPA. An *in vitro* study was carried out using human skin and flow-through diffusion cells (Franz cells), and following the "OECD Guideline 428 for Skin absorption". We determined both permeability kinetics and mass balance. The results provide

insights on skin absorption rates and absorbed doses of colour developers replacing BPA that can be used in exposure assessments.

2. Materials and Methods

2.1 Chemicals

BPA, BPA-glucuronide and BPS were purchased from Sigma-Aldrich Chemie GmbH, Buchs, Switzerland. D-8 and PF201 were bought from Santa Cruz Biotechnology, Heidelberg, Germany. The CAS numbers and some chemical and physical properties of the three test substances are shown in Table 2. Bisphenol A-d6 (BPA-[D_6]) and bisphenol A-$^{13}C_{12}$ β-D-glucuronide (BPA-G-[$^{13}C_{12}$]) were obtained from Toronto Research Chemicals, Toronto, ON, Canada. Carlo Erba LC/MS-grade acetonitrile, methanol (MeOH), and water were obtained by Thommen-Furler AG, Büren, Switzerland. Saline was prepared by dissolving 0.9% (w/v) sodium chloride (purissim. p.a. ≥ 99.5%, supplied by Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) in Milli-Q® water (Milli-Q® Advantage ultra-pure water system, Millipore, Milford, MA, USA).

2.2 Test preparations

Water was chosen as a donor vehicle to mimic a solid colour developer deposited on skin. The colour developer will partition into sweat, a necessary step for skin absorption. Aqueous working solutions were prepared separately for BPA, BPS, D-8 and PF201 (250 mg/l, 250 mg/l, 20 mg/l and 3 mg/l, respectively). These concentrations were confirmed by analysis according to the method described in Chapter 2.4 except for calibration solutions that were in water instead of saline, which required no sample preparation. We applied 100 µl/cm^2 of the test preparations on the skin to achieve an infinite dose. This gave the following skin exposures: 25 µg/cm^2 for BPA, 25 µg/cm^2 for BPS, 2 µg/cm^2 for D-8, and 0.3 µg/cm^2 for PF201. All four substances were soluble in donor and receptor fluids at the tested concentrations as required by the OECD guidelines (OECD 2004b, 2004a).

2.3 Skin absorption assays

We did two types of skin absorption assays: skin permeation kinetics assays, where we studied the absorption over time, and mass balance assays, where we studied the absorption in the different system compartments (i.e. skin, skin wash, donor compartment, receptor fluid).

2.3.1 In vitro skin absorption system

We conducted skin absorption assays following OECD Guideline 428 (OECD 2004a) and OECD Guidance notes 28 (OECD 2004b), using jacketed flow-through diffusion cells (11.28 mm internal diameter, PermeGear® obtained from SES Analytical System, Bechenheim, Germany). A heated water-bath circulator (Haake SC 100 Digital Immersion Circulator, 100 °C w/cla, Thermo Scientific, Newington, NH, USA) kept the skin flaps mounted on diffusion cells at 32°C. Receptor fluid was saline continuously stirred (multi-cell V9-CB stirrer, PermeGear® obtained from SES Analytical System, Bechenheim, Germany) and pumped through the receptor chamber (50 µl/min; peristaltic pump from Ismatec IPC-N, IDEX Health and Science GmbH, Wertheim-Mondfeld, Germany). The receptor fluid was collected automatically with a fraction collector (FC 204, Gilson Inc., Middleton, WI, USA) for skin permeation kinetics assays.

2.3.2 Human skin preparation

We obtained human abdominal skin from the Plastic and Reconstructive Surgery Department at the Vaud University Hospital Centre (CHUV, Lausanne, Switzerland) immediately after surgery (ethical protocol 264/12). Patient data were kept anonymous, and the only known data were the patients' gender and age. The skin samples were excised from three different skin donors (N = 3) for each tested chemical. Occasionally samples from a same skin donor were used for two test substances. A total of eight different skin donors were used for the four test substances. The total number of skin samples per test chemical was n = 12 for BPA, n = 12 for BPS, n = 10 for D-8, and n = 11 for PG201. Skin was rinsed with saline, dried with paper tissues and dermatomed at 200 µm thickness (Acculan®II, B. Braun/Aesculap, Sempach, Switzerland). The use of 200 µm-thick skin allowed the determination of the permeation rate without the depot effect that exist for thicker skin (500 - 1000 µm) (OECD 2011). We cut the skin into circular flaps, mounted them on the diffusion cells, and let them equilibrate for 30 minutes. We followed the same protocol for the mass balance experiments, except that skin had been frozen (-20°C) and dermatomed at 800 µm. Skin was used frozen due to easier availability of skin samples. Frozen skin was easier to dermatome to 800 µm thickness compared to 200 µm. Moreover, the 800 µm-thick skin in mass balance assays allowed the determination of the amount retained in the skin. Skin was thawed at room temperature before cutting and mounting the skin flaps onto the diffusion cells.

2.3.3 Skin integrity

We assessed skin integrity using a trans-epidermal water loss (TEWL) meter (VapoMeter wireless, Delfin Technologies Ltd., Kuopio, Finland) at the beginning and the end of each experiment. Skin flaps with a TEWL above 11 g/m^2/h were discarded (Pinnagoda et al., 1990).

2.3.4 Skin absorption assays

We started the experiment once the skin samples had achieved a steady TEWL reading and skin were exposed (unoccluded) to the test preparations for 24 hours. The experiments started within two hours from retrieving the skin from the surgery unit. The receptor fluid was automatically sampled every hour from 0 to 6 hours, and every two hours from 6 to 24 hours in the permeation kinetics assays. In the mass balance assays, we collected the receptor fluid in glass vial during the 24-hour experiment. We washed the skin by adding water (200 µl) and rubbing gently with a cotton swab that we put into a glass vial. We repeated this washing procedure three times. We added water/acetonitrile 80:20 mixture (3 ml for BPA, 4 ml for BPS, D-8, and PF201) to the vial with the three cotton swabs. We extracted the substances from the cotton swab to the solution by sonicating the vial for ten minutes. We washed the donor chamber with by adding water/acetonitrile solution (0.5 ml), swirling the liquid inside the chamber, and then removing it to a glass vial. We repeated the donor chamber washing three times for each. We cut each skin flap to remove the non-exposed part. We tape stripped the exposed skin five times. We then dropped the skin flap into a glass vial with methanol (1 ml) and sonicated the vials for five minutes. We repeated this extraction process three times, and then pooled the 1 ml x 3 methanol aliquots in one vial.

2.4 Sample analysis

We quantified BPA, BPA-G, BPS, D-8 and PF201 in the receptor fluid and in water/acetonitrile samples by liquid chromatography tandem mass spectrometry (LC-MS/MS). The analytical method for the quantification in the receptor fluid was described in (Reale et al. 2020). Briefly, we prepared receptor fluid samples by solid phase extraction (SPE) (Isolute® C18 6 ml SPE cartridges; Biotage AB, Uppsala, Sweden). A C18 column (100 x 2.1 mm, 1.8 µm, Eclipse Plus, Zorbax, Agilent Technology, Basel, Switzerland) was used to separate the analytes with a HPLC (UltiMate 3000 HPLC system, Thermo Fisher, Fisher Scientific, Reinach, Switzerland) eluting with LC-grade water and acetonitrile containing ammonium hydroxide (5 mM). Triple quadrupole mass spectrometer (MS/MS) (TSQ Quantiva, Thermo Fisher, Fisher Scientific, Reinach, Switzerland) with an electrospray ionization (ESI) source in negative ion mode was used for detection. Internal calibration was done by adding BPA-[D_6] and BPA-[$^{13}C_{12}$] internal standards to samples and calibration standards. The lower limit of quantification (LLOQ) for all analytes was 0.2 µg/l. Method precision (%CV) and accuracy (% of nominal concentration) were tested over five different days. Precision of the quality control solutions at two different concentrations were in the range 93% - 108% and accuracy 3.7% - 15.5%. Extraction recovery corrected by internal standards was calculated as per Matuszewski et al. 2003 and ranged 83% - 110%. All analytes were

stable in receptor fluid matrices as well as in water stored at -20°C at least for 80 days.
Conjugated metabolites of BPS, D-8 and PF201 were not quantified due to the lack of available analytical method. Methanol used in skin extraction was evaporated to dryness under nitrogen stream. The residues were dissolved in water:acetonitrile 80:20 mixture (1 ml). Water/acetonitrile samples were diluted 10 times before injection in the LC system. Skin extraction recoveries were not determined. Cotton swabs extraction recoveries were 102% for BPA, 40% for BPS and D-8, and 49% for PF201. Tape strips were not analysed due to the lack of an analytical method for this matrix

2.5 Data analysis

For permeation kinetics assays, we plotted the permeation curves as cumulative amount of BPA in the receptor fluid per unit skin area over time for each skin sample. For each plot, we determined the maximum permeation rate (J_{MAX}, µg/cm^2/h) as the slope of the steepest linear portion. If the permeation rate achieved a constant value (steady-state) we calculated also the permeability coefficient (K_p, cm/h), and lag time (t_{lag}, h) as follows:

- K_p from Fick's first law for an infinite dose of a chemical applied on the skin $J = K_p \times C$, where C is the concentration (mg/l) of the test preparations ; and
- t_{lag} as the time to reach the steady-state, which is the time (x-axis) intercept of the steepest linear portion of the permeation curve.

Finally, we calculated the mean values of J_{MAX}, K_p and t_{lag} over all skin samples. For mass balance assays, we expressed the absorption as percent of the applied dose as well as µg/cm^2 in the different compartments i.e. skin, skin rinsing, donor chamber rinsing, and receptor fluid.

3. Results

3.1 Permeation kinetics assays

Results of the skin permeation kinetics assays are presented in Figure 1, and in Table 3. BPS reached steady-state after 7 hours. The permeation curves of BPA and D-8 started plateauing approximately after 12 hours. These plateaus suggest that steady-state conditions were not achieved. BPA had the greatest permeation rate J_{MAX} (0.67 µg/cm^2/h). BPS's J_{MAX} was approximately 110-fold lower than BPA's. D-8's skin absorption was slightly lower than BPA's: D-8's J_{MAX} was 16-fold lower than BPA due to the 12-fold difference in the concentration of the test preparation. D-8's J_{MAX} was 7-fold higher than BPS', despite the 10-fold lower applied concentration. After 24 hours of exposure, 32% (8.0 µg/cm^2) of BPA's applied dose had been absorbed in the receptor fluid versus 20% (0.4 µg/cm^2) of D-8 and only 0.2% (0.05 µg/cm^2) of BPS. BPA-glucuronide in the

receptor fluid, after 24 hours of skin exposure to BPA, was detectable, but under the LLOQ of our analytical method in almost all the tested samples. PF201's skin permeation kinetic parameters could not be determined because it was not quantifiable in the receptor fluid in our test conditions.

3.2 Mass balance assays

Mass balance assays results are presented in Table 4. The amount of applied dose quantified in the receptor fluid after 24-hour exposure (i.e. absorbed dose) was 0.4% (0.1 µg/cm^2) for BPS and 17% (0.5 µg/cm^2) for D-8 compared to 25% (6.4 µg/cm^2) for BPA. These values were similar to the absorbed dose observed in the skin permeation kinetics assays (0.2% BPS, 20% D-8, 32% BPA). The dislodgeable dose (amount removed from skin by cotton swabs and donor chamber rinsing) was greater for BPS (73% of applied dose, 18 µg/cm^2) compared to BPA (58%, 15 µg/cm^2) and D-8 (43%, 1.2 µg/cm^2). The percent of applied dose quantified in the skin was similar for all three substances (11 – 16%, 0.4-3.7 µg/cm^2, Table 4). These do not include the tape strips samples, as we did not analyse these. Mass balance was 84% for BPS and 76% for D-8, which was lower compared to BPA (98%). For PF201 mass balance was 24%, which corresponded to the dislodgeable dose (22% of the applied dose in the skin swabs (0.1 µg/cm^2), and 1.5% in the skin (0.01 µg/cm^2)). PF201 was under the LLOQ in the donor chamber rinsing and in the receptor fluid.

4. Discussion

We studied the skin absorption of BPA and its main alternatives used in thermal paper. Our results show that the absorbed dose in the receptor fluid through frozen 800 µm skin after 24 hours of exposure was 25% ± 13% (6 µg/cm^2) for BPA, 17% ± 12% (0.5 µg/cm^2) for D-8, 0.4% ± 0.2% (0.1 µg/cm^2) for BPS and <LLOQ for PF201. Similar values were determined for viable 200 µm-thick skin. BPA skin absorption kinetics were similar to D-8, and 100 times greater than BPS. PF201 kinetics across the skin could not be determined because it was not quantifiable in the receptor fluid.

The reported mean transfer of BPA from thermal paper on hands is 1.4 µg/finger for dry skin (range 0.58 – 3.8 µg/finger), and 12.8 µg/finger for humid skin (range 2.63 – 30 µg/finger) (Lassen et al., 2011)). Here, we adopted the infinite dose strategy to be able to study the kinetics of the test substances through the skin, and to increase the probability of detecting these substances with our analytical method. Using the infinite dose approach, our BPA applied dose was 18 times greater than the reported finger exposure for dry skin, and in line with wet skin. No data exist in the literature on D-8 migration from thermal paper to the skin. The Danish

Environmental Protection Agency (Danish EPA, 2014) estimated D-8's potential skin migration based on its available physico-chemical properties. According to the Danish EPA, D-8 is expected to have a migration potential similar to that of BPA as it has chemical structure, molecular mass, and octanol-water partition coefficient ($logK_{ow}$) similar to BPA. In our study, D-8 dose was 2 µg/cm^2, which was in the range of dermal exposures reported for BPA (Lassen et al., 2011). PF201 dose (0.3 µg/cm^2) was in the reported range of dermal exposures to PF201 (0.28 – 0.50 µg/fingertip (Danish EPA, 2014)). For BPS, the reported range of migration on the skin is 0.73 – 0.85 µg/dry finger, and 1.6 – 4.8 µg/sweaty finger (Danish EPA, 2014)). BPS has a high water solubility (1,100 mg/l (US EPA, 2014)). Applying 100 µl of BPS in saturated solution would result in a dose 22 times greater than BPA, 140 times greater than reported finger exposure of dry skin to BPS, and 30-70 times greater than reported fingertip exposure of dry skin to BPS (0.73 – 0.85 µg/dry fingertip and 1.6 – 4.8 µg/sweaty fingertip (Danish EPA, 2014)). Therefore, we chose to use a BPS solution not at saturation but at the same concentration and dose used for BPA (250 mg/l, 25 µg/cm^2) to directly compare the permeation rate, which is dependent on the concentration. The applied BPS dose was 5 to 34 times higher than the reported range of BPS migration on the skin.

Even under dose conditions ten times higher than the ones indicated in the OECD TG428 (100 µL/cm^2 of a saturated solution), steady-state was not reached for BPA and D-8. This has also been shown for other lipophilic permeants (Selzer et al. 2013) and was explained by donor depletion (high dermal delivery resulting in a reduction of the amount in the donor chamber). Actually, the amount of test substance to reach steady-state conditions depends on its permeability: the lower the permeability, the lower the dose necessary to reach steady-state. BPS was less permeable than BPA and D-8 and applying 100 µl/cm^2 of a BPS solution not at saturation led to steady-state.

Our results are in agreement with skin absorption estimates based on the test substances' molecular weight (MW) and $logK_{ow}$. These physico-chemical properties play an important role in passive diffusion of chemicals through the stratum corneum. The stratum corneum is the lipidic layer of the skin and is composed of corneocytes and inter-cellular lipid lamellae. The most efficient route of diffusion through the stratum corneum is the intercellular route among the lipid lamellae. Chemicals that follow this route have a MW lower than 500 Da and are lipophilic. BPS, D-8 and BPA have similar MWs in the range of 250-300 Da. BPA and D-8 have in addition similar $logK_{ow}$, while BPS has a lower $logK_{ow}$. Although BPA had an approximately 1.5-fold higher permeation rate and absorbed dose than D-8, this can be explained by the higher BPA concentration and dose applied onto the skin. Thus, we conclude that D-8's absorption was similar to BPA. As expected, BPS's skin

absorption was consistently lower than BPA and D-8 (Figure 1 and Table 3). Percent dose of BPS in the skin and receptor fluid (11%) was a third compared to D-8 (33%).

BPA

Mass balance assays achieved an excellent recovery of BPA (98% ±13%). A comparison of our BPA mass balance results with literature data (Table 5) shows that all studies agree on the absorption in the skin (dermis + epidermis + stratum corneum excluding the first two tape strips) in the 12 % - 27% range. The largest difference among these studies is in the absorbed dose. The greatest absorbed dose (46%, 3.5 µg/cm^2) is reported by (Liu and Martin 2019), probably because of the use of a thinner (120 µm) 3D model skin and a high vehicle volume (1538 µl/cm^2). Also (Zalko et al. 2011) reported an absorbed dose of 46% (1.3 µg/cm^2), using 500 µm-thick skin and a low vehicle volume (10 µl/cm^2). The second greatest absorbed dose is reported by (Champmartin et al. 2020) (41%, 8.2 µg/cm^2) with a 476 µm-think skin and a 50 µl/cm^2 volume, followed by this study (25%, 6.4 µg/cm^2), using a thicker skin (800 µm) and a 100 µl/cm^2 volume. The lowest absorbed doses have been reported by (Kaddar et al., 2008; Demierre et al., 2012; Toner et al., 2018) (2%, 0.03-0.06 µg/cm^2) and (Mørck et al., 2010) (13%), and were probably due to the smaller vehicle volume used.

Our results show that BPA metabolism by skin into its glucuronide conjugate was negligible. A limitation of our study is the use of saline as the receptor fluid in the experiments where skin metabolism was studied. For skin metabolism studies cell culture media are the recommended receptor fluids (OECD, 2004b). Cell culture media support longer skin viability and it does not hinder the quantification of radiolabelled analytes, which are often used in this type of studies. In our study, we preferred the use of a simpler matrix (saline) because our test substances were not radio-labelled and were quantified by LC-MSMS. However, our experiments started no more than two hours from retrieving the skin. Such a short delay led to results in agreement with Marquet et al. (2011), who used cell culture media receptor fluid and reported that BPA was not at all or only very slightly metabolised by viable human skin (< 2.5% of the dose). Other two studies show higher values of BPA-conjugated metabolites and more polar compounds: 27% (Zalko et al., 2011) and 8% (Toner et al., 2018). The main difference is that these two studies did not use diffusion cells but a multi-well system. In this system, the skin sample soaks in receptor fluid containing the chemical of interest. This allows the study of the overall skin metabolic capacity neglecting chemical's skin permeation kinetics. The CEF-EFSA Panel considered this method an overestimation of *in vivo* skin metabolism (EFSA, 2015).

BPA alternatives

The low D-8 and BPS recoveries in mass balance assays could be due to various factors: tape-stripping samples not being included in the mass balance, low cotton swabs recoveries, metabolism, skin protein binding, or photo-degradation. Studies on BPS have reported metabolism into its glururonide and hydroxylated forms (Skledar et al., 2016), and photodegradation (Kovačič et al., 2019). For D-8, no data are available on its metabolism or stability. Considering that Liu and Martin (2019) and Champmartin et al. (2020) found no or limited BPS conjugated metabolites in their assays, and that we took special care to avoid photo-degradation (laboratory lights turned off during skin exposure to BPS, use of amber glass vials), plausible causes of low BPS mass balance are the exclusion of tape stripping samples, and the extraction efficiency of the skin and cotton swabs. D-8 and BPS mass balance results were close to the recommended range of 100 ± 20% for non-radiolabelled test substances (OECD, 2011). Our results for BPS mass balance showed that most BPS was in the dislodgeable dose (73%) and less in the skin (11%). This is in contrast with Champmartin et al. (2020) who found a large amount of BPS to remain in the skin (47%). The low recovery of PF201 in the mass balance assays could be due to metabolism, binding with skin proteins, and/or to its instability at skin pH (5.5 in humans). While there are no data on PF201 metabolism in the literature, it is known that PF201 is unstable in protic solvents at pH < 7 (Eckardt and Simat, 2017). Although we did not conduct stability tests, we observed that PF201 was stable in the aqueous test preparation and in the receptor fluid. Therefore, a hypothesis is that PF201 hydrolysed in direct contact with the skin (pH = 5.5). If dermal absorption of PF201 is expected to be very limited or negligible, as already mentioned, the dislodgeable dose should be close to the applied dose, which is not the case. Therefore, even if PF201's metabolism by the skin and protein binding cannot be excluded, the low PF201 amount quantified in the skin rinsing (22% of applied dose) supports the hypothesis of PF201's hydrolysis in contact with skin. Overall, PF201 skin permeability is likely to be very low. Future studies are needed to better understand the possible PF 201 hydrolysis, metabolism and binding in the skin.

Our results for BPS are in line with Liu and Martin (2019) and Champmartin et al. (2020) in that BPS dermal absorption is consistently lower than BPA. However, in our study, BPS' K_p was two orders of magnitude lower than that of Liu and Martin (2019) (0.24×10^{-4} cm/h vs. 30×10^{-4} cm/h). This could be due to several differences in the experimental setup, as discussed for BPA. First, different skins and diffusion systems: our study used ex-vivo human skin and flow-through diffusion cells, while Liu and Martin (2019) used a 3D skin model consisting of organized human keratinocytes on tissue culture inserts and static diffusion cells designed specifically for the 3D skin model. Second, different skin thicknesses (200 µm in our study vs. 120 µm in Liu and Martin (2019)) and different vehicle volumes applied on the skin (100 µl/cm^2 in our study vs. 1538 µl/cm^2 in Liu and Martin (2019)). The effect of skin thickness on K_p is well-known as K_p is by definition inversely proportional to the skin

thickness. The effect of hydration is known to increase skin permeability (Bunge et al., 2012; Frasch et al., 2014; Zhu et al., 2016). A higher skin thickness and a lower skin hydration (hence the lower volume of aqueous solution applied on the skin) could explain the lower BPS skin absorption in our study. Accordingly, Champmartin et al. (2020) used thicker skin (476 µm thickness) than both Liu and Martin and our studies as well as a vehicle volume (50 µl/cm^2) that was half compared to our experiments and 30 times lower than Liu and Martin (2019). Champmartin et al obtained a K_p value only one order of magnitude lower than in our study (0.026 x 10^{-4} cm/h).

Limitations

Some limitations of this study have already been acknowledged, such as not assessing metabolism when viable skin was used, and not validating the skin extraction recovery. However, the BPA amount extracted from skin was in line with data reported in the literature. This suggests that the method used for skin extraction was efficient. Another limitation was not quantifying the amount of chemicals in the tape-strip samples, thus omitting this value in the recovery calculation. Colour developers are present as neat powder in thermal paper. We did not assess skin permeation of the neat powder or as in receipts per se. Applying colour developers as a powder on the skin might be closer to a scenario of a person touching thermal paper, although, this experimental set-up would not take into account the pressure applied and rubbing of the receipts when they are handled. Applying an infinite dose in aqueous solution of the colour developers, as we did in our study, would simulate an exposure scenario of a solid on sweaty or moistened hands.

5. Conclusion

Overall, we can conclude qualitatively that skin absorption was in increasing order: PF201 < BPS << D-8 < BPA. These results are in agreement with these chemicals' log K_{ow} and MW. BPA metabolism by the skin was negligible in our test conditions. The absorbed dose in the receptor fluid was 25%, 17%, 0.4% and <LOQ for BPA, D-8, BPS and PF201, respectively. Low mass balance results for Pergafast 201® suggest that this molecule could undergo hydrolysis and/or binding when in contact with the stratum corneum.

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Conflict of interest

The authors declare no competing interests.

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Table 1 – Detection frequency of colour developers in thermal paper products.

	Björnsdotter et al. (2017)	**Eckardt and Simat (2017)***	**Goldinger et al. (2015)**	**Vervliet et al. (2019)**	**Yang et al. (2019)**
n	100	114/98/99	124	308	120
Countries	Norway, Netherlands, Spain and Sweden	Germany	Switzerland	14 countries in Europe, Asia, North America and Oceania	China
	% of n	**% of n**	**% of n**	**% of n**	**% of n**
BPA	78	48/47/53	81	63	77
BPS	49	11/9/6	3	22	72
Pergafast 201	49	34/34/40	9	12	NA
D-8	24	6/7/1	7	8	33
D-90	7	NA	NA	4	19
TGSA	7	NA	NA	4	25
BPS-MAE	2	NA	NA	2	52
UU	NA	0/0/3	NA	0.6	NA
DD-70	NA	NA	NA	0.3	NA

(*) Numbers listed by collection years 2015/2016/2017

Table 2 - Some physicochemical properties of BPA and its alternatives used in thermal paper: BPS, D-8, and Pergafast 201® (Source: EPA, 2014).

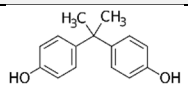
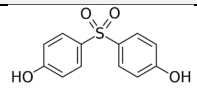
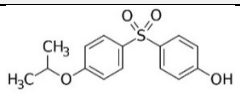
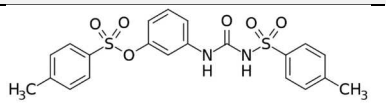
	Bisphenol A	**Bisphenol S**	**D-8**	**Pergafast 201®**
Structure				
Acronym	BPA	BPS	D-8	PF201
CAS number	80-05-7	80-09-1	95235-30-6	232938-43-1
Molar mass (g/mol)	228.29	250.27	292.35	460.52
Physical description	Dry powder	Dry powder	Dry powder	Dry powder
Solubility in water (mg/l)	120 – 301 (measured)	500-1100 (20°C)	19.7 (measured) at pH 6.85 21 (measured)	35 (measured) 35 at 20°C (measured)
Log k_{ow}	3.3 (measured)	1.2 (measured)	3.36 (measured)	2.6 (measured)

Table 3 - Summary of skin exposure conditions and results of skin permeation kinetics assays for the tested chemicals.

Chemical	n[a]	Concentration (aq) [mg/l]	Volume [µl/cm²]	Mean amount in receptor fluid after 24 h of exposure		t_{lag} [h]	J x 10^{-2} [µg/cm²/h]	K_p x 10^{-4} [cm/h]
				µg/cm² (±SD)	% of applied dose (±SD)			
BPA (aq)	12	250	100	8.00 (±3.05)	32 (±12)	ND	67 (±30)	ND
BPS (aq)	12	250	100	0.05 (±0.03)	0.2 (±0.1)	7.6	0.6 (±0.9)	0.24 (±0.35)
D-8 (aq)	10	20	100	0.41 (±0.18)	20 (±9)	ND	4.1 (±2.0)	ND
Pergafast 201® (aq)	11	3[b]	100	<LLOQ	<LLOQ	ND	ND	ND

[a] Number of skin samples tested from three skin donors.

[b] Water solubility has been reported to be 35 mg/l but we could only dissolve 3 mg/l.

ND, not determined.

Table 4 - Mean and standard deviation (SD) of the amount of test chemicals quantified in the different compartments at the end of the experiments (24 h) following an application of 100 µl/cm² aq solution on 800 µm-thick frozen human skin. Results are expressed as percent of applied dose and as µg/cm²

Chemical	BPA (n = 8)		BPS (n = 7)		D-8 (n = 7)		Pergafast 201® (n = 8)	
	Mean [%]	SD	Mean [%]	SD	Mean [%]	SD	Mean [%]	SD
Skin swabs	57	4.2	71	14	39	18	22	20
Donor chamber	1.0	1.8	2.1	2.4	3.9	5.0	< LLOQ	
Skin	15	14	11	6.8	16	5.4	1.5	0.9
Receptor fluid [a]	25	13	0.4	0.2	17	12	< LLOQ	
Recovery (total)	98	13	84	14	76	13	24	22
	Mean [µg/cm²]	SD	Mean [µg/cm²]	SD	Mean [µg/cm²]	SD	Mean [µg/cm²]	SD
Skin swabs	14.22	1.0	17.7	3.7	1.1	0.7	0.09	0.07
BPA in donor chamber	0.5	0.6	0.4	0.6	0.09	0.10	<LLOQ	
Skin	3.7	3.4	2.5	1.6	0.4	0.1	0.01	0.00
Receptor fluid [a]	6.4	3.1	0.1	0.0	0.5	0.3	<LLOQ	
Recovery (total)	24.8	3.1	20.7	3.5	1.7	0.6	0.09	0.07

[a] Sum of the amount quantified in the receptor fluid collected for 24 hours and in the receptor fluid remained in the receptor chamber.

Table 5 - Experimental set up and results of BPA skin absorption studies in the literature.

Authors	**Marquet et al. (2011)**	**Zalko et al. (2011)**	**Kaddar et al. (2008)**	**Mørck et al. (2010)**	**Demierre et al. (2012)**	**Toner et al. (2018)**	**Liu and Martin (2019)**	**Champmartin et al. (2020)**	**Our study**
Skin parameters									
Skin type	Human	Human	Pig	Human	Human	Human	Human skin model	Human	Human
Skin thickness (µm)	400	500	Full thickness	800-1000	200	350-400	120	476	200, 800[a]
Skin condition	Frozen and viable	Viable	Frozen	Frozen	Frozen cadaver	Viable	3D model with metabolic activity	Viable	Viable, frozen[a]
Skin temperature (°C)	32	37	32	32	32	32	NA	32	32
total skin samples (n)	15	3	6	11	7	12	3	10	12
Skin donors (N)	6	Not reported	Not reported	Not reported	2	4	NA	Not reported	3
Methods									
System	Static diffusion cells	6-well plates for *ex vivo* organ cultures.	Static diffusion cells	Static diffusion cells	Flow through-diffusion cells	Flow through-diffusion cells, 12-well plate for metabolism	Static diffusion device in 6-well plates	Static diffusion cells	Flow through-diffusion cells
BPA concentration [mg/l]	4000	284[b]	10	3995[b]	194	300 (60, 12, 2.4)	1, 5	400	250
Vehicle	Acetone	DMEM[c]	Saline	Saline with 2% ethanol	Water	Phosphate buffered saline (PBS)	Water	Water	Water
Vehicle volume (µl/cm^2)	50	9.7	Not reported	Not reported	9.4	10	1538	50	100
BPA dose (µg/cm^2)	200	2.75	Not reported	Not reported	1.82	3 (0.6, 0.12, 0.04)	1.5, 7.7	20	25

Receptor fluid	RPMI, 2% BSA, 1% penicillin/ streptomycin[d]	DMEM[d]	Saline	Saline with 5% BSA[d]	Saline	DMEM[d] + 1% ethanol + UDPGA[d] 2mM + PAPS[d] 40 µM	Phosphate-buffered saline	RPMI 1640 solution + 0.2% gentamycin + 2.5% penicillin-streptomycin + 2% BSA[d]	Saline
Exposure time (h)	24	72	10	48	24	24	25	24	24
Results									
Metabolism (%)	< 2.5 (viable skin)[e]	27[f]	NA	NA	NA	8[g]	Not significant	88 was not metabolised	Not significant
Unabsorbed dose (%)	NA	3	65	45	57	72[h]	38[i]	Not reported	60
Skin (%)	NA	42	14	25	0.6 (epidermis + dermis), 24 (tape strips 1-2), 11 (tape strips 3-20)	13 (epidermis + dermis), 7.5 (tape strips 1-2), 2.5 (tape strips 3-20)	13	27	12 (epidermis + dermis)
Receptor fluid (%)	NA	46	1	13	8.6	3	46	41	29
Recovery (%)	NA	92	84	82	100	99	97	98	102

[a] Viable 200 µm-thick skin was used in the metabolism assay, frozen 800 µm-think skin in the mass balance assay.

[b] Calculated from the reported value 17.5 mM BPA.

[c] Calculated from the reported value 50 nmol BPA.

[d] Acronyms: RPMI Rosewell Park Memorial Institute medium, BSA bovine serum albumin, DMEM Dulbecco's Modified Eagle Medium, UDPGA Uridine 5'-diphosphoglucuronic acid, PAPS 3'-phosphoadenosine-5'-phosphosulfate

[e] BPA accounted for more than 97.5% of the radioactivity detected in the receptor fluid.

[f] Sum of BPA-glucuronide and BPA-sulphate

[g] Sum of BPA-glucuronide, BPA-sulphate and more polar compounds

[h] Results are reported only for the 3 µg/cm^2 dose

[i] Results are reported only for the 7.7 µg/cm^2 dose

NA not available

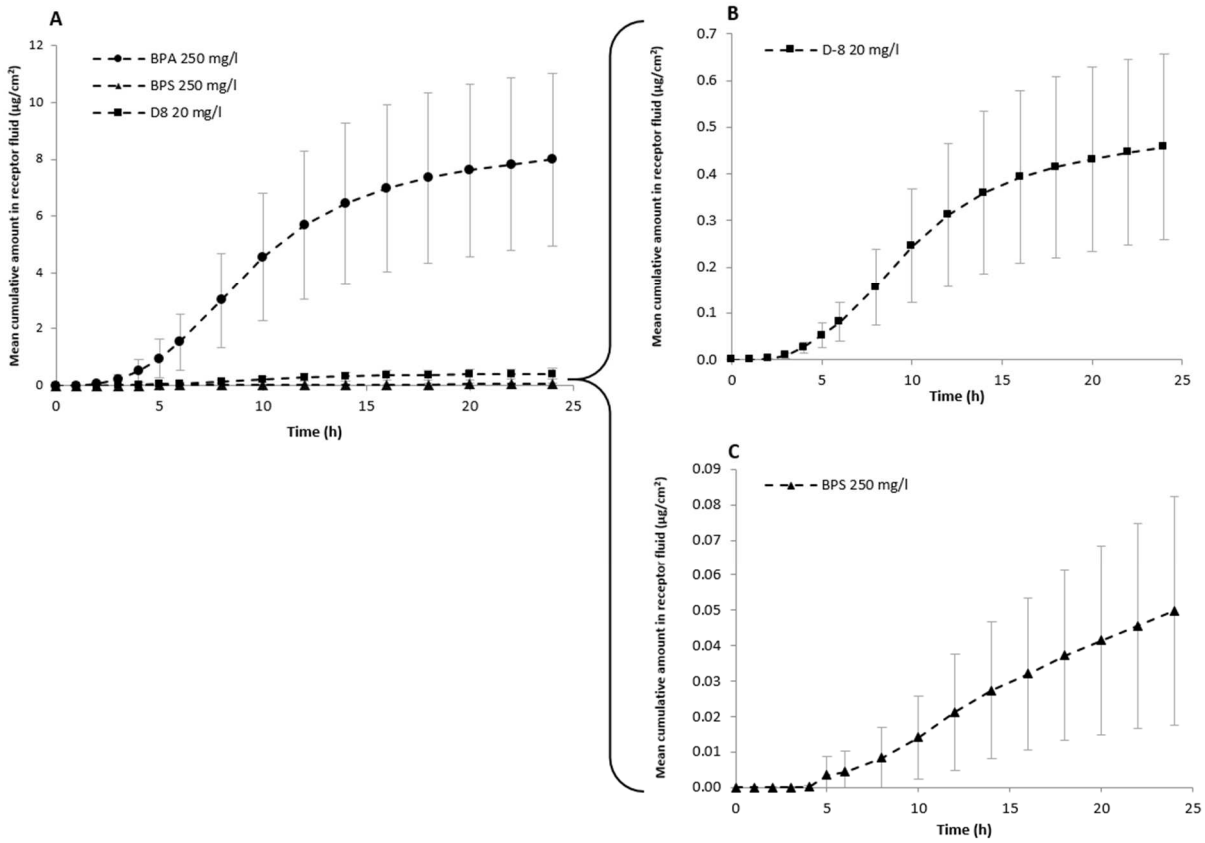


Figure 1 - Mean permeation curve of BPA, BPS and D-8 (A), and expanded view of D-8 (B) and BPS (C). n = 12 for BPA, 12 for BPS, and 10 for D-8. Error bars represent the SD.