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Chemosphere 82 (2011) 424–430

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Viable skin efficiently absorbs and metabolizes bisphenol A ☆,☆☆

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ARTICLE INFO

Article history:
Received 25 May 2010
Received in revised form 17 September 2010
Accepted 20 September 2010
Available online 27 October 2010

Keywords:
BPA
Skin
Dermal absorption
Biotransformation
Human
Endocrine disrupter

ABSTRACT

Skin contact has been hypothesized to contribute to human exposure to bisphenol A (BPA). We examined the diffusion and metabolism of BPA using viable skin models: human skin explants and short-term cultures of pig ear skin, an alternative model for the study of the fate of xenobiotics following contact exposure. ^{14}C-BPA [50–800 nmol] was applied on the surface of skin models. Radioactivity distribution was measured in all skin compartments and in the diffusion cells of static cells diffusion systems. BPA and metabolites were further quantified by radio-HPLC. BPA was efficiently absorbed in short-term cultures, with no major difference between the models used in the study [viable pig ear skin: 65%; viable human explants: 46%; non-viable (previously frozen) pig skin: 58%]. BPA was extensively metabolized in viable systems only. Major BPA metabolites produced by the skin were BPA mono-glucuronide and BPA mono-sulfate, accounting together for 73% and 27% of the dose, in pig and human, respectively. In conclusion, experiments with viable skin models unequivocally demonstrate that BPA is readily absorbed and metabolized by the skin. The trans-dermal route is expected to contribute substantially to BPA exposure in human, when direct contact with BPA (free monomer) occurs.

1. Introduction

Bisphenol A (BPA) is widely used to produce polycarbonate plastics (bottles and food containers) as well as epoxy resins used as internal lacquer in cans. Residual (unreacted) monomers of BPA, but also free BPA released by these polymers under certain pH conditions or during microwave heating (Lim et al., 2009) can migrate into beverages and foods (Brotons et al., 1995; Kubwabo et al., 2009), partly explaining human exposure. Food contamination can also occur along the food chain, due to the very large amounts of BPA-based products manufactured, which result in its presence in the environment. In the last decade, BPA has attracted considerable attention because it is a model xenoestrogen able to trigger reproductive disorders in laboratory animals (Richter et al., 2007). Recently, it was also hypothesized that early exposure to BPA could play a role in the onset of obesity and other metabolic syndromes (Rubin and Soto, 2009), and impact cognitive functions (Palanza et al., 2008). Although a large controversy regarding BPA is still underway (vom Saal and Hughes, 2005; Vandenberg et al., 2009), the amount of evidence supporting the effects of low doses of BPA in animal models is increasing each year. Human exposure to BPA has been clearly demonstrated (Vandenberg et al., 2010) and its possible consequences have become a critical issue in the field of endocrine disruption.

According to recently published reports on risk assessment (EFSA, 2006; Chapin et al., 2008) in the general population, human exposure mostly occurs via residues contained in food or beverages. However, there is ongoing controversy on whether external exposure resulting from food contamination is really a good estimate for internal exposure, because of the discrepancy between the concentrations of BPA in the blood reported by several authors and exposure calculations. Based on PBPK (physiologically-based pharmacokinetic) models, Mielke and Gundert-Remy (2009) noted that the blood concentration calculated for a dose of 0.9 g kg^{-1} d^{-1}, which is the highest daily intake estimated from 3509 biomonitoring samples, is several orders of magnitude lower than the values reported in the literature. Furthermore, Völkel et al. (2002) were unable to measure plasma concentrations above the level of detection of 2.3 ng mL^{-1} after a single oral dose of 5 mg BPA, which is 5000 times higher than the external exposure estimated from biomonitoring data. Several hypotheses can be put forward to explain this inconsistency, among which underestimation of exposure levels.

Additional routes (inhalation and contact) could contribute to the overall human exposure to BPA. Data is still lacking on these issues (Vandenberg et al., 2007), but the contribution of the

Abbreviations: BPA, bisphenol A; HPLC, high-performance-liquid-chromatography.

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☆☆ The authors declare they have no competing financial interests.

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doi:10.1016/j.chemosphere.2010.09.058

inhalation route is likely limited in the case of BPA (Wilson et al., 2007; Geens et al., 2009; von Goetz et al., 2010), with the exception of specific occupational exposure, suggested by studies carried out in BPA manufacturing workplaces (He et al., 2009). Large amounts of BPA are used in thermal paper made for printers relying on the thermal transfer technology, in which BPA is often used as a color developer. This printing technique, mainly used in lightweight printing devices, begun to be extensively used in the 1970s. Over the years, the use of thermal printers has increased (cash registers, credit card terminals). Nowadays, most people come in contact with thermal paper on a daily basis. Not all thermal paper (but a large part) contains BPA. When this is the case, a powdery layer of BPA is used to coat one side of the paper. Under heat or pressure, BPA reacts with the thermal paper dye to produce a color-developing complex based on hydrogen bond interactions (Takahashi et al., 2002). Not only can this result in oral contamination (direct contact of unwashed hands with the mouth), but thermal paper is also a major source for the contamination of recycled paper (Gehring et al., 2004).

Because of the extensive use of thermal paper and because large amounts of free BPA can be found in it, skin contact may contribute to the overall exposure of humans to BPA. It is therefore critical to examine if skin can absorb and/or metabolize BPA. The best way to do this is to use radio-labeled BPA, but this approach cannot be used for *in vivo* studies in humans. It is therefore necessary to use the most accurate skin models to address this issue, in accordance with international guidelines. We have developed an *ex vivo* alternative skin model, based on pig ear skins (which can easily be obtained from local slaughterhouses) to examine the fate and penetration of model toxicants. Short-term culture of pig ear skin incubated in proper conditions using static diffusion cells, has been shown to be an efficient skin model that expresses both phase I and phase II functional enzymatic activities and can be used for a period of 72 h with good conservation of the skin barrier function (Jacques et al., 2010a,b,c). In this study, we used this model to examine the percutaneous absorption and biotransformation of BPA, using ^{14}C-BPA. We carried out the experiments using a range of BPA doses (50–800 nmol) applied on the surface of skin explants, and quantified the residues in all compartments (the surface of the skin, the skin itself, and diffusion cells). Similar experiments were conducted using frozen pig skin (control) and viable human dermal explants on which 50 nmol of BPA were applied. Parent BPA and its metabolites were quantified by radio-HPLC. This is the first report of the absorption and metabolism of BPA in viable skin models, with full description of the repartition and metabolic fate of BPA.

2. Materials and methods

2.1. Chemicals

[U-^{14}C]-BPA, with a specific activity of 2.074 GBq mmol^{-1} was purchased from Amersham Biosciences (UK). Its radio-purity was >98.5% based on radio-HPLC analysis. Unlabeled BPA (>99% purity) was purchased from Sigma–Aldrich (St.-Quentin-Fallavier, France). Other chemicals and solvents (analytical grade) were purchased from the following sources: bovine liver β-glucuronidase (type B1 1000 units g^{-1} solid), sulfatase from *Aerobacter aerogenes* (3.9 units mg^{-1} protein), ammonium acetate, sodium acetate, phosphate buffer and sodium hydroxide: Sigma–Aldrich; acetonitrile and ethyl acetate: Scharlau Chemie S.A. (Barcelona, Spain); ethanol and acetic acid: Merck (Briare-Le-Canal, France). Ultrapure water from Milli-Q system (Millipore, Saint-Quentin-en-Yvelines, France) was used for *ex vivo* preparations and for the preparation of HPLC mobile phases.

2.2. Short-term culture of pig ear skin

Skin organ culture used ears of domestic pigs (Pietrain breed, 6-month old females) obtained from a local slaughterhouse. Ears were taken from the animals within 5 min after slaughtering and were kept at 4 °C during the transport to the laboratory, which lasted a maximum of 2 h. After cleaning and shaving, skins were immediately excised with a scalpel, then sectioned at a thickness of 500 μm using a Padgett dermatome (Michael's France, Neuilly-sur-Seine, France) and punched into 28 mm diameter discs. The punch areas were free of structural changes such as scratches, erosions and scars, which could affect the diffusion and metabolism of BPA.

For incubations, skin punches were seeded dermal side down in Transwell® inserts (23 mm diameter, 8 μm pore size; Corning Life Sciences, Avon, France) in 6-well plates prefilled with 1.5 mL culture medium at 37 °C in a 5% CO_2 air incubator. In this *ex vivo* organ culture system, explants are maintained at the air/liquid interface and dermal/epidermal feeding is achieved by the diffusion of nutriments across the insert. The culture medium was Dulbecco's Eagle Modified Medium supplemented with L-Glutamine (0.584 g L^{-1}), streptomycin/penicillin (100 μg mL^{-1}), fungizone (2.5 μg mL^{-1}), gentamycin (50 μg mL^{-1}), all from Sigma–Aldrich.

^{14}C-BPA (8333 Bq sample^{-1}) adjusted with unlabeled BPA to reach the required dose (50, 100, 200, 400 and 800 nmol, corresponding to 2.75, 5.5, 11, 22 and 44 μg cm^{-2}, respectively) was applied in 60 μL ethanol/phosphate buffer 0.1 M pH 7.4 (1:2, vol/vol) on the surface of the skin. Culture media collected at 24, 48 and 72 h (end of the experiment) were kept at −20 °C until analysis. All incubations were carried out in triplicate.

2.3. Control incubations

The influence of skin viability on percutaneous absorption was investigated by comparing BPA diffusion in fresh explants with the corresponding skin preparations which had previously been frozen, following the method of Kao et al. (1985). Skin punches were stored at −20 °C for 1 month. After this enzyme inactivation period, they were incubated in the same conditions as fresh skin punches, with ^{14}C-BPA (8333 Bq sample^{-1}) adjusted with unlabeled BPA to reach the required dose (50, 200 and 800 nmol), in triplicate. Culture media collected at 24, 48 and 72 h were kept at −20 °C until analysis.

2.4. Human skin explants

Human skin from the abdominal region of Caucasian female donors (36–44 years old) with no skin diseases was obtained from surgery and supplied by Biopredic (Rennes, France). The samples were sectioned at a thickness of 500 μm using a dermatome and punched into 28 mm diameter discs. Human skin punches were incubated in the same conditions as pig skin punches with 50 nmol ^{14}C-BPA (8333 Bq), in triplicate. Culture media collected at 24, 48 and 72 h were kept at −20 °C until analysis.

2.5. Radioactivity measurements

At the end of the 72 h experiment, media and skin were removed. Skin surfaces were washed twice with cotton swabs impregnated with ethanol/water (3:1, vol/vol). Cotton swabs were plunged in acetonitrile (2 mL) and placed in a sonicator for 20 min.

Tissue culture inserts were washed with 10 mL acetonitrile. After removal of the media, the wells were washed twice with acetonitrile (1 mL). Radioactivity in the culture media and washing solutions was determined by direct counting of aliquots on a Packard scintillation counter (Model tricarb 2200CA; Packard Instruments, Meriden, CT) using Packard Ultima Gold as the scintillation cocktail. Media were concentrated under nitrogen stream before radio-HPLC analyses. BPA and metabolites were extracted from skin samples after homogenization with a Polytron homogenizer (Kinematica AG, Lucern, Switzerland) in pH 7.4 phosphate buffer and centrifugation at 250*g* (10 min, 4 °C). Then, a second extraction was carried out in the same conditions but using water-saturated ethyl acetate. The organic and aqueous phases were separated and their radioactivity was measured by direct counting of aliquots using the scintillation counter. Residual radioactivity in the skin centrifugation pellets (non-extractable radioactivity) was determined by complete combustion using a Packard Oxidizer 306 (Packard Instruments), as previously described in Jacques et al. (2010a,c). Organic extracts were concentrated under vacuum before radio-HPLC analyses. Aqueous extracts were concentrated using 0.5 g Oasis™ cartridges previously washed with 10 mL methanol and equilibrated with 10 mL sodium phosphate buffer 0.01 M. Extracts were deposited on the cartridges, which were washed with 10 mL sodium phosphate buffer 0.01 M then eluted with 10 mL methanol. Radioactivity in the different fractions was quantified and the methanol fraction was concentrated under vacuum before radio-HPLC analyses.

2.6. Analytical procedure

The HPLC system consisted of a Spectra P1000 pump (Thermo Separation Products, Les Ulis, France) associated with a 250 * 4.6 mm (5 µm) Zorbax Sb-C_{18} column (Agilent, Interchim, Montluçon, France) protected by a Kromasil C18 guard precolumn (Interchim). Mobile phases and analytical conditions were as previously described in Jaeg et al. (2004). ^{14}C-BPA and related metabolites separated by HPLC were monitored by on-line radioactivity detection using a Flo-one (A500, Radiomatic, La-Queue-Lez-Yvelines, France) and Flo-scint II (Packard Instruments) as scintillation cocktail. BPA and metabolites were quantified by integrating the area of the radio-chromatographic peaks.

2.7. Metabolite characterization

BPA metabolites were characterized based on retention time comparison with authentic standards obtained from *in vitro* incubations with liver S9 fractions as previously described by us (Jaeg et al., 2004), confirmed by specific enzymatic hydrolysis assays on metabolites isolated as described in Zalko et al. (2003). For the confirmation of BPA–glucuronide structure, 500 µL of collected fractions were added to 500 µL 0.2 M sodium acetate buffer at pH 5 and were incubated 3 h with 150 IU β-glucuronidase at 37 °C under gentle shaking. For the confirmation of BPA–sulfate structure, collected fractions were incubated 3 h in 0.01 M Tris buffer at pH 7.4 with 2 IU arylsulfatase at 37 °C under gentle shaking. The hydrolysis reaction was stopped by addition of methanol (1:3, vol/vol) and centrifugation at 8000*g* (10 min, 4 °C). The resulting supernatant was concentrated to 100 µL under nitrogen stream, dissolved in mobile phase, and analyzed by radio-HPLC.

2.8. Statistical analysis

Mean values were compared using a Student Test, using Instat (Graph-Pad software). K_m and V_m values were determined by nonlinear regression analysis as indicated by Loft and Poulsen (1989).

3. Results

3.1. Recovery of radioactivity

Radioactivity levels were measured in culture media every 24 h and at 72 h (end of the experiments) in all other compartments (skin, skin surface, wells and inserts washing solutions). In incubations carried out with fresh pig ear skins (50–800 nmol BPA), total radioactivity recovery ranged from 83.5 ± 4.1% to 87.7 ± 2.4% (Fig. 1a and Table 1). Totals were not significantly different for incubations carried out with human skin explants (92.6 ± 5.8%, 50 nmol BPA) or with previously frozen pig skins explants (93 ± 1.3%, 85.3 ± 1.4% and 91.3 ± 1.3%, for 50, 200 and 800 nmol of BPA applied, respectively) (Fig. 1b).

3.2. ^{14}C-BPA distribution in short-term pig skin cultures

For 72 h incubations carried out with fresh pig ear skin, the majority of radioactivity was in the culture media (Fig. 1a), with

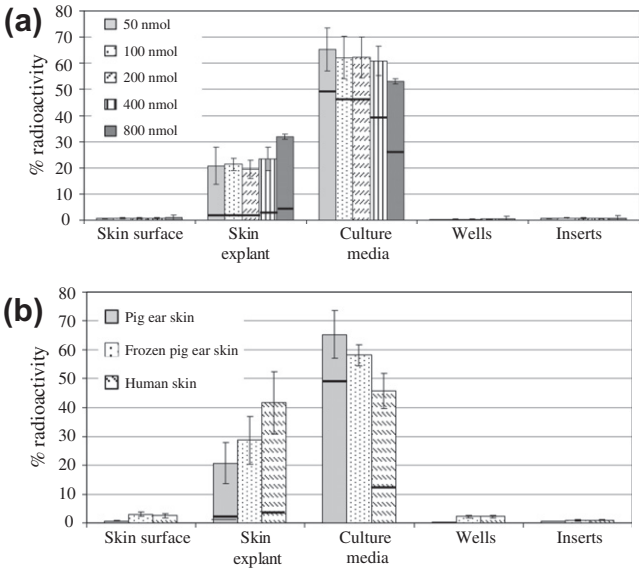

Fig. 1. Distribution of radioactivity in media, skin and washing solutions 72 h after the beginning of the experiment: (a) viable pig skin explants; (b) comparison of the distribution of radioactivity in pig (fresh versus frozen) skin explants, and in human fresh skin, for 50 nmol BPA. Results are expressed as a percentage of the applied dose (mean ± SD, n = 3). Bold horizontal lines within the bars delimit the respective contribution of metabolites (lower part of each bar) and parent BPA (upper part).

Table 1
Radioactivity distribution in media, skin and washing solutions, 72 h after the beginning of the experiment, see M&M section for extracts and pellets preparation. Results expression: % of the recovered dose (mean ± SD, n = 3) and BPA equivalents in nmoles.

	%	nmoles
Skin surface	0.6 ± 0.1	0.3 ± 0.05
Skin explant	20.8 ± 7.1	10.4 ± 3.55
Culture media	65.3 ± 8.2	32.65 ± 4.1
Wells	0.3 ± 0.1	0.15 ± 0.05
Inserts	0.7 ± 0.1	0.35 ± 0.05
Total recovery	87.7 ± 2.4	43.85 ± 1.2

values ranging from 65.3 ± 8.2% (50 nmol BPA) to 53 ± 3.7% (800 nmol BPA). The skin itself contained between 20.8 ± 7.1% (50 nmol BPA) and 31.9 ± 5.7% (800 nmol BPA) of the radioactivity originally applied. There was a slight difference in the distribution of radioactivity with the 800 nmol BPA dose, compared with lower doses, but this tendency was not significant. Whatever the BPA dose, less than 1% of applied radioactivity remained unabsorbed at 72 h, and was consequently recovered on the surface of skin explants. Only residual radioactivity amounts (below 1.5%) were recovered on the inserts and culture wells.

3.3. Comparative ^{14}C-BPA diffusion in pig and human skin

For the *ex vivo* pig ear skin model, no significant difference in the distribution of the radioactivity was observed between fresh and previously frozen skin explants, respectively, with the 50 nmol BPA dose (Fig. 1b), or with higher doses of BPA applied on explants (200 and 800 nmol, data not shown). For frozen skin, less than 3% of the applied radioactivity was recovered on the skin surface. The skin itself contained 28.8 ± 8.3% of the applied radioactivity, with a very low proportion of non-extractable radioactivity (^{14}C bound to the extraction pellet): 0.4 ± 0.1%. Most of the radioactivity was located in the culture media (58.1 ± 3.6%). Amounts of residual radioactivity recovered in inserts and culture wells were below 3.2% of the applied dose. For human skin explants exposed to 50 nmol BPA, the percentage of radioactivity recovered at the surface of the skin (2.5 ± 0.8%) was in the same range as that observed for incubations using pig skin explants. Human skin itself was found to contain 41.5 ± 10.8% of the applied radioactivity, which was not significantly higher than the values calculated for the corresponding incubations with pig skin explants. The diffusion of radioactivity (BPA and metabolites) through the skin and into culture media, did not significantly differ in incubations carried out with viable human (45.6 ± 6.2%) or pig skin explants (65.3 ± 8.2%) for 50 nmol of BPA applied.

3.4. BPA biotransformation by pig and human skin explants

Radio-HPLC analyses were carried out for all culture media 24, 48 and 72 h after application of BPA, and, for skin extracts, at 72 h. When using previously frozen pig skin explants, only parent BPA was recovered in the media whatever the dose (Fig. 1b), demonstrating the diffusion of BPA even in the absence of functional metabolism. Fig. 2 shows representative radio-chromatograms of culture media for incubations of BPA with human and pig viable skin explants. In our HPLC system, the retention time of BPA was 42.3 min and two metabolites eluting before parent BPA were detected. Comparison of retention times with authentic standards and confirmation by specific enzymatic hydrolysis assays with β-glucuronidase and sulfatase enabled us to identify these peaks as two BPA conjugates: BPA–glucuronide (peak I) and BPA–sulfate (peak II). Qualitatively, similar profiles were obtained after 24, 48 and 72 h of incubation.

Pig and human skin explants were able to metabolize BPA over a period of 72 h. The same conjugated metabolites were detected in incubations with pig and human skin explants, although the overall proportion of metabolized BPA was lower with human skin. For instance, at 72 h and 50 nmol BPA applied on the explants, 86.5 ± 6.8% and 39.3 ± 7.9% of the radioactivity was recovered as metabolites in media from incubations using pig ear skin and human skin, respectively (Fig. 1b). In the corresponding skin extracts, only 10.2 ± 0.2% and 12.2 ± 3.4% of the radio-chromatographic profiles corresponded to metabolites, respectively (Fig. 1b). The percentage of metabolites in skin itself never exceeded 14.2 ± 2.3% (pig fresh skin explants, 800 nmol BPA, 72 h).

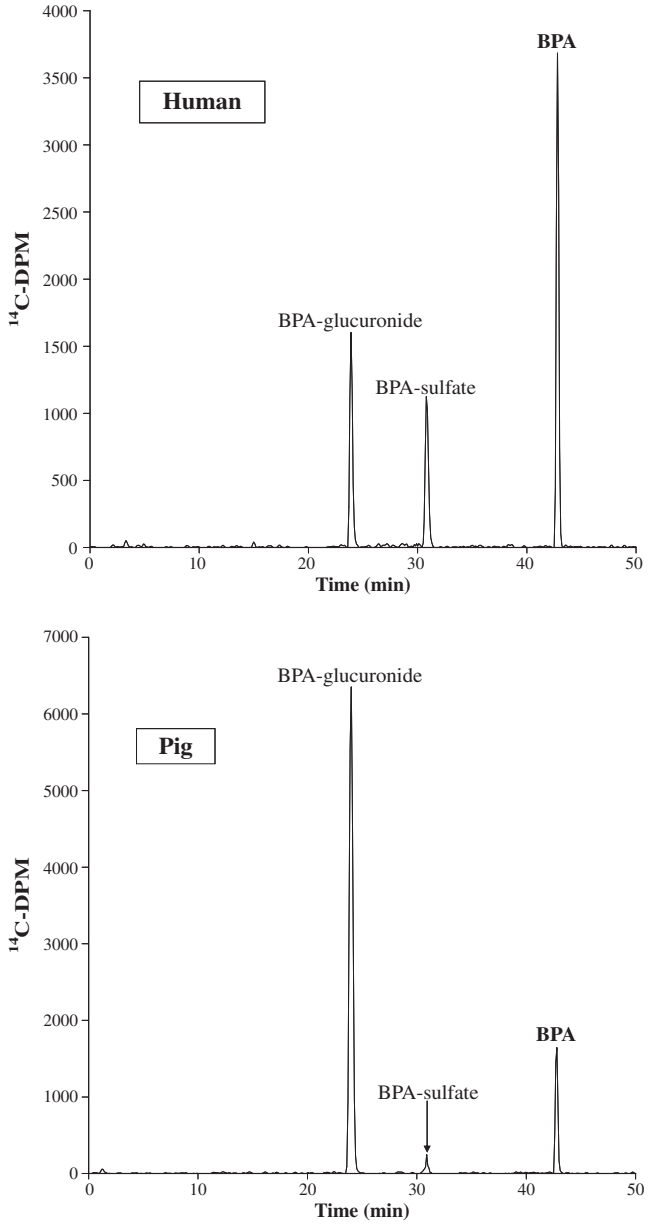

Fig. 2. Typical radio-chromatographic profile obtained when analysing a culture medium from human and pig fresh skin explants on which BPA was applied (50 nmol, 72 h).

3.5. Kinetic data

Following radio-HPLC separation, metabolites were quantified by integrating the area of the radio-chromatographic peaks. Fig. 3 shows the respective amounts (nmol) of BPA–glucuronide and BPA–sulfate produced by pig ear skin and human skin explants 24, 48 and 72 h after the beginning of the incubations with the 50 nmol dose of BPA. For pig skin (Fig. 3a), the major metabolite detected in culture media was BPA–glucuronide. The amount of BPA–glucuronide formed, corresponding to 7.15 ± 1.73, 15.83 ± 2.77 and 30.4 ± 3.3 nmol at 24, 48 and 72 h, respectively, gradually increased with an increase in incubation time up to the end of the experiment. Only small amounts of BPA–sulfate were formed by pig explants (always less than 1.5 nmol). The overall BPA conjugation rate was lower in human skin explants (Fig. 3b), but in this case, equal amounts of BPA–glucuronide and BPA–sulfate were formed. Again, the quantity of both metabolites increased with

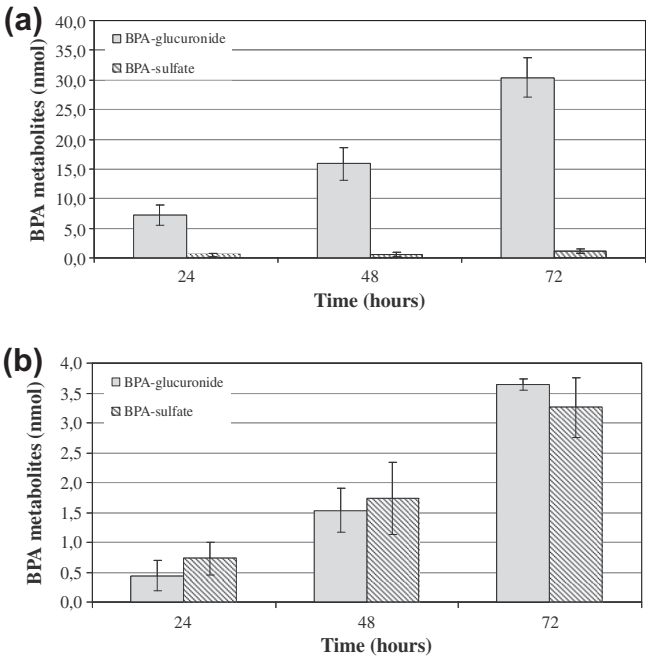

Fig. 3. Effect of the incubation time on the quantity of metabolites in culture media after the application of 50 nmol BPA on pig ear (a) and human; (b) skin explants. Data are expressed in nmol h^{-1} (mean ± SD, $n = 3$).

an increase in incubation time, indicating that conjugation enzymes were still functional after 72 h of incubation. At the end of the experiments, 3.64 ± 0.09 nmol of BPA–glucuronide, and 3.26 ± 0.49 nmol of BPA–sulfate had been formed by human skin explants, after the application of 50 nmol BPA.

In the pig ear skin model, the formation rates of BPA metabolites in media were expressed in nmol h^{-1} and were plotted as a function of the BPA dose (Fig. 4). BPA–glucuronide formation increased with an increase in the dose of BPA applied, and the enzymatic kinetic curve showed the beginning of saturation around 200 nmol. Complete saturation was not reached, but the formation of both metabolites followed Michaelis–Menten kinetics. The apparent K_m and V_m values were calculated based on the concentration of metabolites in the receptor fluid. K_m and V_m values were 197.6 ± 44.7 μM and 16.77 ± 2.02 nmol h^{-1} for BPA–glucuronide (Fig. 4), and 73.6 ± 35.9 μM and 0.3 ± 0.05 nmol h^{-1} for BPA–sulfate.

4. Discussion

The hypothesis that skin contact could contribute to human exposure to BPA has been repeatedly raised and debated at recent international conferences on endocrine disruptors. It relies, among others, on the fact that BPA residues are known to be present in many manufactured goods and certain types of paper in daily use. Today, *in vitro* skin models enable a better understanding of the many functions of the skin and it is now well established that this large tissue does not only act as a physical barrier. Because it is difficult to evaluate skin metabolism based solely on *in vivo* experiments, *ex vivo* and/or *in vitro* models are now being developed with the aim of improving characterization of the biotransformation capabilities of the skin, as well as percutaneous diffusion dynamics. Regarding endocrine disrupters, the case of thermal paper containing BPA deserves special attention, since it can be heavily loaded with BPA. Unlike in plastics and epoxy resins, BPA is present in this type of paper primarily as a free monomer, and is therefore directly available for skin uptake.

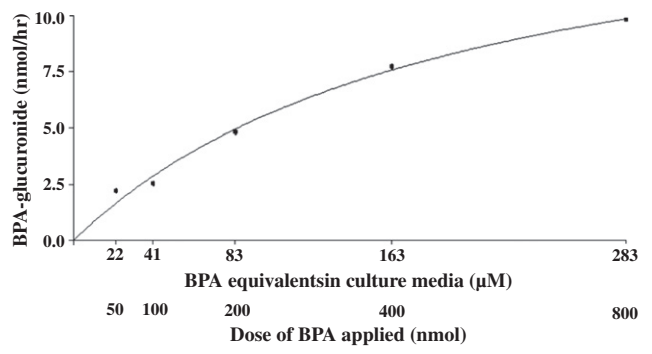

Fig. 4. Effect of the BPA dose deposed on skin, on the production of BPA–glucuronide by fresh pig skin explants, at 72 h. Data are expressed in nmol h^{-1} (mean ± SD, $n = 3$).

To date, the fate of BPA at the level of the skin has only been examined in three studies. One was carried out using deuterium-labeled BPA on isolated perfused bovine udders (Kietzmann et al., 1999), a model that is poorly representative of human skin and that is not recommended by current guidelines for dermal absorption studies. The two others were carried out with ^{14}C-BPA, using non-viable (e.g. previously frozen) pig skin (Kaddar et al., 2008), or human skin explants (Mørck et al., 2010). Excised human skin is the most relevant choice for the study of the penetration and biotransformation of xenobiotics. However, samples are often not available when required, and only in limited amounts. In addition, marked variability is observed due to gender, age, or ethnic differences, and depending on the site of the body where the skin was removed (Bronaugh et al., 1982; Vallet et al., 2007). Several reconstructed human skin models have been developed, but one of their main limits is their weak barrier function (Netzlaff et al., 2005), which can lead to an overestimation of diffusion and biotransformation rates (van de Sandt et al., 2000).

We examined the fate of BPA in an alternative *ex vivo* model (short-term culture of pig ear skin) and compared the results with incubations carried out with commercially available human skin explants, and with non-viable (previously frozen) pig ear skin. The histological and biochemical properties of porcine skin have been repeatedly shown to be close to that of human skin (Wester and Maibach, 1989; Jacobi et al., 2007). In addition, the thickness of both the *stratum corneum* and the epidermis of pig ear skin and human skin are in the same range. We recently documented the absorption, diffusion and biotransformation capabilities of short-term cultures of pig ear skin, using 7-ethoxycoumarin and benzo(a)pyrene as model compounds. This *ex vivo* skin model could be efficiently used for the simultaneous study of the penetration and metabolism (both oxidation and conjugation pathways) of water soluble as well as more lipophilic compounds (Jacques et al., 2010a,c).

Fresh pig ear skin was exposed to a wide range of BPA concentrations in short-term cultures. We characterized the diffusion of ^{14}C-BPA (skin barrier function) as well as the production of metabolites. The proportion of radioactivity diffused into culture media (trans-dermal passage) was within a range of 50–60%, most of which was associated with BPA–glucuronide (ca. 90%). Skin contained 20–30% of the radioactivity applied on explants, whatever the BPA dose, but a much lower proportion of metabolites (ca. 10%). These results, and the fact that metabolic capabilities were conserved over a period of 72 h, clearly demonstrated that the barrier effect of the *ex vivo* pig ear skin model was preserved throughout the experiment.

The percentage of radioactivity reaching culture media was not found to significantly differ in control (previously frozen) pig ear skin (58.1 ± 3.6%), or when BPA was applied on human skin explants (45.4 ± 6.18%). All these values are well above those

determined by Kaddar et al. (2008), using pig skin incubation over a maximum period of 10 h. In these experiments, radioactivity recovery in the receptor fluid and in the dermis did not exceed 0.7% and 8.8% of the radioactivity applied on the skin, respectively. Our values are also above those reported by Mørck and co-workers (2010) using human skin over a period of 48 h with 17.5 mmol of BPA applied at the surface of explants. In the latter study, the percentage of radioactivity recovered in the epidermis, the dermis and the diffusion cells was 7.45, 17% and 13%, respectively. However, no information was provided about the surface area of the skin explants and therefore, about the concentration of BPA used. Previously frozen skin was employed in Kaddar et al. study, which very likely explains the low trans-dermal passage rates observed. In 2003, the European Union reviewed unpublished data from a human dermal absorption study, estimating that the bioavailability of BPA applied on skin was around 10%. Again, our results suggest that this value may be considerably underestimated.

In short-term cultures of fresh pig skin, the majority of the radioactivity recovered in culture media corresponded to metabolites. In contrast, when previously frozen pig skins were used, only the parent BPA was present in media, due, as expected, to the inactivation of biotransformation enzymes during storage. Human skin explants were shown to metabolize BPA, but to a lesser extent than viable pig skin. Human skin also metabolized BPA via phase II reactions, with the production of the same conjugates. But in the human skin, BPA–sulfate and BPA–glucuronide were formed in equal amounts. No comparison could be made with previous studies, in which metabolism was not addressed. However, since the systems used in these studies relied on skin samples that had been stored frozen previous to the incubations (like our negative control), there is little chance that they would express any functional biotransformation enzymes.

At the level of the skin, the optimal penetration of xenobiotics is influenced by several factors. Molecules that are highly water-soluble hardly diffuse through the epidermis and the dermis. Conversely, lipophilic molecules do not easily pass the epidermal barrier. In the latter case, skin metabolism plays a critical role, through the production of more water-soluble metabolites. For instance, this was demonstrated for the pig ear skin model with benzo(a)pyrene. Using fresh skin, benzo(a)pyrene is readily absorbed due to extensive biotransformation, while in control studies with non-viable skin, very little trans-dermal passage and no biotransformation are observed (Jacques et al., 2010a). The characteristics of BPA are a moderate water solubility (K_{ow} of 3.2), and a relatively low molecular weight. Such physico-chemical properties greatly favor skin penetration, and to a large extent explain why BPA trans-epidermal penetration rates were found to be high in our study, even when non-viable skin was used. In other words, biotransformation processes are not expected to be a major determinant of the percutaneous penetration of BPA compared to more lipophilic compounds.

BPA was found to be metabolized by viable *ex vivo* models only. The lower biotransformation rate observed for human skin is likely due to the delay between the collection of human skin and the experiments (usually 36–48 h for skin explants from commercial sources). We hypothesize that the biotransformation rate of BPA would be even higher than 27% if freshly collected human skin could be used, although, for the above mentioned reasons, its absorption would be expected to remain in the same range (ca. 60%). The conjugation of BPA, at least with glucuronic acid, results in the cancellation of the estrogenic activity of the parent compound (Matthews et al., 2001). Therefore, it could be concluded that the high biotransformation rate of BPA by the skin does correspond to metabolic detoxification. This remains highly speculative, as BPA conjugates may be converted back into the parent compound at the level of target tissues.

5. Conclusions

Experiments carried out with viable skin models demonstrated extensive uptake and biotransformation of BPA following topical application. Regardless of the ongoing debate on the adverse effects of BPA, these results demonstrate that skin contact could contribute to human exposure to BPA, at least when contact occurs with the free monomer. Thermal paper can be a direct source for free BPA. Whether the extensive use of thermal paper raises concerns regarding human health is a question that was not under the scope of the present study, and which remains to be investigated.

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