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In vitro percutaneous absorption and metabolism of Bisphenol A (BPA) through fresh human skin

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ABSTRACT

Bisphenol A (BPA) is a high production volume compound. It is mainly used as a monomer to make polymers for various applications including food-contact materials. The primary route of exposure to BPA in the general population is through oral intake (EFSA 2015) however, other potential sources of exposure have also been identified, such as dermal contact. In the present study, the percutaneous absorption through human skin has been investigated in an *in vitro* study according to OECD TG 428 (Skin Absorption: *In Vitro* Method). In order to investigate potential dermal BPA metabolism during absorption, radiolabelled BPA was applied to fresh, metabolically competent, human skin samples (ring labelled ^{14}C BPA concentrations tested were 2.4, 12, 60 and 300 mg/L). Measured as total radioactivity the mean absorbed dose (receptor compartment) ranged from 1.7–3.6% of the applied doses and the dermal delivery (epidermis + dermis + receptor compartment), sometimes also named bioavailable dose was 16–20% of the applied doses, with the majority of the radioactivity associated with epidermis compared to dermis and receptor fluid. No metabolism was observed in any of the epidermis samples; however some metabolism was observed in dermis and receptor fluid samples with formation of BPA-glucuronide and BPA-sulfate, and some polar metabolites.

1. Introduction

Bisphenol A (BPA) is a high–production volume chemical. Based on its use as a monomer in the manufacture of polycarbonate plastic and epoxy resins in food packaging containers, the primary route of exposure to BPA in the human population is thought to be oral (European Food Safety Authority (EFSA), 2015). However, other sources of exposure such as dermal contact have also been identified. For example, BPA is used as a developer in thermal paper products, including cash register receipt paper (EFSA, 2015; U.S. Environmental Protection Agency (EPA), 2014; Thayer et al., 2016).

Toxicokinetic studies in mice, rats and monkeys are available for oral and intravenous/subcutaneous exposure. After oral administration to laboratory animals, BPA is rapidly absorbed from the gastrointestinal tract and undergoes first-pass conjugation to BPA-glucuronide (BPA-gluc) and BPA-sulfate (BPA-sulf), which are biologically inactive metabolites (Churchwell et al., 2014; Doerge et al., 2010a, 2010b, 2011a, 2011b, 2012). Studies in humans after oral ingestion of BPA or on high BPA diets are in agreement with the animal data confirming that internal exposure to unconjugated BPA is low after oral exposure with BPA-gluc being the major metabolite (Thayer et al., 2015; Völkel et al., 2002, 2008; Teeguarden et al., 2011, 2015; EFSA, 2015).

So far, no toxicokinetic study in humans involving dermal exposure has been reported that provides information about (i) the extent of dermal absorption of BPA and (ii) the metabolic capacity of the human skin to conjugate BPA.

Concerning dermal absorption of BPA, two *in vitro* studies are reported which were performed according to the Organization for Economic Co-operation and Development test guideline (OECD TG) 428. Both studies used non-viable human skin and report absorptions of 13% (Mørck et al., 2010) and 8.6% (Demierre et al., 2012) of the applied doses. Both studies were considered to exhibit methodological shortcomings and in addition did not investigate potential BPA metabolism by the skin (EFSA, 2015; ECHA, 2015). Additional exploratory,

Abbreviation: BPA, Bisphenol A; BPA-gluc, BPA-glucuronide; BPA-sulf, BPA-sulfate; ECHA, European Chemicals Agency; CoRAP, substance evaluation process by ECHA; EFSA, European Food Safety Authority; EPA, U.S. Environmental Protection Agency; OECD TG, Organisation for Economic Co-operation and Development test guideline; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PBS, buffered saline solution; SULT, sulfatase; UDPGA, uridine 5′-diphosphoglucuronic acid; UGT, UPD-glucuronosyltranferase

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non-guideline, studies are reported in the literature and evaluated by EFSA (2015). Overall, EFSA concluded that the available *in vitro* human skin absorption studies are limited and concluded that absorption of BPA is likely to be in the region of 10% of the applied dose (EFSA, 2015).

Experimental *in vitro* and *in vivo* studies demonstrate that the human skin has metabolic activity. Metabolic activity is reported for different cytochrome P450 isoforms (CPY450), esterases, acetyltranferases, dehydrogenases and conjugating enzymes (Oesch et al., 2014; Gundert-Remy et al., 2014). UPD-glucuronosyltranferase (UGT) and/or sulfatase (SULT) expression and/or activity in *in vitro* skin was measured by different authors (Hewitt et al., 2000; Goetz et al., 2012; Goebel et al., 2009; Hu et al., 2010; Jaeckh et al., 2011; Luu-The et al., 2009; Moss et al., 2000; Sumida et al., 2013).

BPA metabolism in human skin was investigated in two *in vitro* dermal penetration studies with human skin and the authors report inconsistent formation of BPA-gluc in the range of 3 to 27% of the applied dose (Marquet et al., 2011; Zalko et al., 2011). Both studies have limitations and a reliable estimate of the extent of human skin metabolism is not possible (EFSA, 2015).

As part of a substance evaluation process (CoRAP) by the European Chemicals Agency (ECHA) on BPA, a study was requested to assess the rate and extent of absorption and metabolism of BPA following topical application to human skin (ECHA, 2015). In order to fulfil this requirement, an *in vitro* dermal penetration study according to OECD TG 428 (Skin Absorption: *In Vitro* Method) was conducted with fresh, metabolically active human skin, to investigate potential BPA metabolism.

2. Materials and methods

2.1. Regulatory citations

This study was performed in accordance with Good Laboratory Practice regulations (GLP); OECD Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: *In Vitro* Method (OECD, 2004a); OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28; Guidance Document for the Conduct of Skin Absorption Studies (OECD, 2004b) and the Scientific Committee on Consumer Safety basic Criteria for the *In Vitro* Assessment of Dermal Absorption of Cosmetic Ingredients (SCCS, 2010).

2.2. Chemicals

Carbon-14 labelled Bisphenol A ([ring^{14}C(U)]-Bisphenol, specific activity: 474 μCi/mg, radiochemical purity: 99.8%) was supplied by Moravek Biochemicals Inc., 577 Mercury Lane, Brea, California 92821, USA. Non-radiolabelled Bisphenol A (CAS No: 80-05-7, Chemical Purity: 99.9%) was supplied by Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA. Metabolite reference standard bisphenol A mono-beta-(D)-glucuronide sodium salt was obtained from Ultrafine Chemicals, Manchester, UK and bisphenol A sulfate sodium salt was obtained from ISOTEC (Miamisburg, OH, USA). Adenosine 3,5-diphosphate disodium salt, uridine 5′-diphosphoglucuronic acid trisodium salt, acetonitrile, 2 propanol, thiazolyl blue tetrazolium bromide, MTT formazan, phosphate buffered saline, hydrochloric acid, trifluoroacetic acid, methanol and tin (II) chloride dihydrate, were obtained from Sigma Aldrich Ltd., 3050 Spruce Street, Saint Louis, MO 63103, USA. Dimethyl sulfoxide (DMSO) was supplied by VWR, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire LE17 4XN, UK. Tissue culture medium DMEM was obtained from Lonza, Wheldon Rd., Castleford, West Yorkshire, WF10 2JT, UK. GlutaMAXtm-1 (100 ×) was obtained from Gibco®, 3 Fountain Drive, Inchinnan Business Park, Paisley, PA4 9RF, UK. Solvable® was obtained from Perkin Elmer Inc., 549 Albany St., Boston, Massachusetts 02118, USA. Physiological saline was obtained from Baxter, 20 Kingston Road, Staines-upon-Thames, TW18 4LG, UK. Ethanol was obtained from Hayman Ltd., 70 Eastways Park, Witham, Essex, CM8 3YE, UK. Aquasafe 500 plus liquid scintillation fluid was obtained from Zinsser Analytic, Howarth Road, Maidenhead, Berkshire SL6 1AP, UK. Simple Kind to Skin Antibacterial Handwash was obtained from Simple Health and Beauty Limited, Unilever, Unilever House, 100 Victoria Embankment, London, EC4Y 0DY, UK.

2.3. Receipt and preparation of skin

Full-thickness abdominal human skin samples were obtained from 4 patients (3 female and 1 male, aged 33 to 46 years) that attended the Plastic Surgery Unit of St John's Hospital (Livingston, Scotland UK). Immediately following surgery, the excised skin samples were transferred on ice to the Charles River Laboratories (Edinburgh, UK) within 4 h of surgery and cleaned of subcutaneous fat and connective tissue using a scalpel blade. The skin samples were washed under cold running water and dried using tissue paper. Split-thickness membranes were prepared (350–400 μm) using a Zimmer® electric dermatome supplied by Zimmer Ltd., Swindon, SN2 4FP, UK. The split-thickness skin was used immediately. The processed skin was cut into sections of approximate dimensions 1.5 cm × 1.5 cm.

2.4. MTT formazan assay

For each donor, following receipt and preparation, the split-thickness skin was tape stripped with 20 successive tape strips using Scotch® 3 M tape. Twelve skin disks were then cut. Six of the prepared skin disks were heat deactivated in boiling water for *ca* 30 min. A MTT formazan standard curve in DMSO (concentration ranging from 0.002 to 0.1 mg/mL) and quality control (QC; 0.005 and 0.075 mg/mL) solutions were prepared and protected from light. Three fresh and three heat deactivated skin disks were weighed and added to individual amber capped tubes containing MTT in supplemented DMEM solution (0.3 mg/mL; 0.5 mL). The reaction was allowed to proceed for 180 min at *ca* 37 °C. The skin disks were then removed, blotted dry on tissue paper and transferred to a fresh amber capped tube containing acidified isopropanol (800 μL) to extract. Duplicate aliquots (200 μL) of each sample were transferred to a 96 well plate and read alongside MTT formazan standard curve and quality control solutions at 550 nm with a reference reading at 630 nm, using Multiskan® Spectrum plate reader (Thermo Fisher Scientific Oy, Vantaa, Finland).

2.5. Flow-through cell apparatus

The Scott-Dick diffusion cells and automated flow-through system were manufactured at the University of Newcastle (Newcastle, UK). Each diffusion cell had an exposure area of 0.64 cm^2 and a receptor compartment volume of 0.25 mL. The diffusion cells were incorporated into a steel manifold that was heated *via* a circulating water bath so that skin surface temperatures were maintained at 32 ± 1 °C. The cells were connected to multi-channel peristaltic pumps from their afferent ports with the receptor fluid effluent dropping *via* fine bore tubing into vials on a fraction collector. The peristaltic pumps were adjusted to maintain a flow-rate of 0.75 ± 0.1 mL/h.

2.6. Barrier integrity assessment

The barrier function of skin sample was confirmed by measuring the electrical resistance before the start of the experiment. Skin samples were allowed to equilibrate at 32 °C ± 1 °C for *ca* 30 min. One milliliter phosphate buffered saline solution (PBS) was then added to the donor chamber and the skin samples were allowed to equilibrate for a further *ca* 30 min. The electrical resistance was then measured using a Tinsley Databridge (Model 6401) supplied by Tinsley Instrumentation Ltd., Braintree, Essex CM7 2YW, UK.

A barrier integrity assessment was also conducted for selected skin