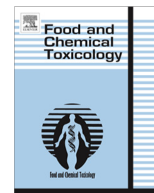
Food and Chemical Toxicology 65 (2014) 105–114

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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



# Dermal toxicity elicited by phthalates: Evaluation of skin absorption, immunohistology, and functional proteomics

Tai-Long Pan [a], Pei-Wen Wang [a,b], Ibrahim A. Aljuffali [c], Yi-Yun Hung [d,e], Chwan-Fwu Lin [f,*], Jia-You Fang [d,e,g,h,*]

[a] *School of Traditional Chinese Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan*
[b] *Research Center for Industry of Human Ecology, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan*
[c] *Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[d] *Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan*
[e] *Chinese Herbal Medicine Research Team, Healthy Aging Research Center, Chang Gung University, Kweishan, Taoyuan, Taiwan*
[f] *Department of Cosmetic Science, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan*
[g] *Graduate Institute of Health Industry Technology, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan*
[h] *Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung, Taiwan*

A R T I C L E   I N F O

*Article history:*
Received 29 August 2013
Accepted 19 December 2013
Available online 31 December 2013

*Keywords:*
Phthalate
Skin absorption
Toxicity
Proteomics
Biomarker

A B S T R A C T

The toxicity of phthalates is an important concern in the fields of environmental health and toxicology. Dermal exposure via skin care products, soil, and dust is a main route for phthalate delivery. We had explored the effect of topically-applied phthalates on skin absorption and toxicity. Immunohistology, functional proteomics, and Western blotting were employed as methodologies for validating phthalate toxicity. Among 5 phthalates tested, di(2-ethylhexyl)phthalate (DEHP) showed the highest skin reservoir. Only diethyl phthalate (DEP) and dibutyl phthalate (DBP) could penetrate across skin. Strat-M® membrane could be used as permeation barrier for predicting phthalate penetration through skin. The accumulation of DEHP in hair follicles was ~15 nmol/cm$^2$, which was significantly greater than DBP and DEP. DBP induced apoptosis of keratinocytes and fibroblasts via caspase-3 activation. This result was confirmed by downregulation of 14-3-3 and immunohistology of TUNEL. On the other hand, the HSP60 overexpression and immunostaining of COX-2 suggested inflammatory response induced by DEP and DEHP. The proteomic profiling verified the role of calcium homeostasis on skin inflammation. Some proteins investigated in this study can be sensitive biomarkers for dermal toxicity of phthalates. These included HSPs, 14-3-3, and cytokeratin. This work provided novel platforms for examining phthalate toxicity on skin.

© 2013 Elsevier Ltd. All rights reserved.

## 1. Introduction

Phthalates are known as plasticizers to give plasticity for rigid materials in many products. Nowadays the industry generates billions of pounds of phthalates every year. Phthalates are widespread contaminants in both indoor and outdoor environments (Guo and Kannan, 2011). These toxicants can be delivered into body via inhalation, dietary intake, and skin absorption (Singh and Li, 2011; Guo et al., 2012; Bekö et al., 2013). Skin is the largest organ of the body, which is easily exposed to a large number of toxicants, such as bisphenol A and semi volatile organic compounds (SVOC) (Mielke et al., 2011; Weschler and Nazaroff, 2012). Dermal absorption occurs at a significant rate for phthalates (Schettler, 2006). Humans can be exposed to phthalates from building materials, household furnishing, soil, and dust. Furthermore, some personal care products provide a direct contact of phthalates to skin. Phthalates are employed in skin care products for the roles as stabilizers, binders, emulsifiers, and lubricants (Koniecki et al., 2011). The products containing phthalates for cosmetic and topical uses include skin moisturizers, mosquito repellents, perfumes, deodorants, shampoos, and nail polishes (Matsuda et al., 2010). Guo et al. (2014) recently demonstrated that hand and body lotions are the main contributors to dermal exposure, especially for diethyl phthalate (DEP). Since phthalates are not chemically bound to most of the products, they are easily released from products to skin (Kamarei et al., 2011).

There are more than 10 articles reporting the cause of allergic contact dermatitis by phthalates (Yanagisawa et al., 2008; Lampel and Jacob, 2011). Takano et al. (2006) suggested that phthalates

* Corresponding authors. Address: Department of Cosmetic Science, Chang Gung University of Science and Technology, 261 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan (C.F. Lu). Address: Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan. Tel.: +886 3 2118800; fax: +886 3 2118236 (J.Y. Fang).
*E-mail address:* fajy@mail.cgu.edu.tw (J.-Y. Fang).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.12.033

may be responsible for recent increment of dermatitis in the developed countries. Another concern is that babies and children are more exposed as compared to adults (Wormuth et al., 2006; Kolarik et al., 2008). Sathyanarayana et al. (2008) have demonstrated that ⩾7 phthalates are detected in urines of 81% infants after using baby care products. Although it is approved that skin exposure is a major source of phthalate toxicity, the mechanism and severity of the toxicity have not been conclusively identified. The absorption level of phthalates via topical route also has not been systemically examined. We aimed to establish permeation profiles of a series of phthalates in this study. These included DEP, dibutyl phthalate (DBP), di(2-ethylhexyl)phthalate (DEHP), diisononyl phthalate (DINP), and trioctyl trimellitate (TOTM). This work was also designed to explore possible mechanisms governing phthalate toxicity on skin.

To facilitate risk assessment of phthalates, the skin permeation and adverse effects of phthalates should be determined by using validated and relatively new methodologies. The dermal absorption of phthalates was evaluated by Franz cell assembly. The skin acts as a signaling interface between xenobiotics and the body. Any stress may contribute to alteration of genome conformation and protein expression (Huang et al., 2005). Functional proteomics can serve as a potent tool to elucidate cellular process at protein level and identify target proteins involved in the process. We examined proteomics of skin with phthalate treatment. Different responses such as inflammation, differentiation, and apoptosis are accompanied with skin disorders. Another purpose of this study was to seek some biomarkers which show significant change of expression after topical phthalate application. We had utilized immunohistology and Western blotting as platforms for searching biomarkers.

## 2. Materials and methods

### 2.1. Materials

DEP, DBP, DEHP, DINP, and TOTM were all commercially available from Sigma–Aldrich (St. Louis, MO, USA) with a purity of >99%. Strat-M® membrane for transdermal diffusion test was purchased from Merck Millipore (Darmstadt, Germany).

### 2.2. Animals

Female nude mouse (ICR-Foxn1nu) aged eight weeks were provided by National Laboratory Animal Center (Taipei, Taiwan). Specific pathogen-free (SPF) pigs of one week old were supplied by Animal Technology Institute Taiwan (Miaoli, Taiwan). The protocol for animals was reviewed and approved by Institutional Animal Care and Use Committee of Chang Gung University (approval number: CGU-10-030). The committee confirmed that the animal experiment followed the guidelines as set forth by the Guide for Laboratory Factlines and Care. Ethical issues with animal experiments complied with Directive 86/609/EEC from European Commission. Animals were housed in cages in a room with controlled temperature (25 ± 1 °C) and relative humidity (60 ± 5%). A laboratory diet and water were given ad libitum.

### 2.3. In vitro skin absorption

Full-thickness skin on dorsal region was excised from mice or pigs after sacrifice. The skin was mounted between donor and receptor of Franz cell with stratum corneum (SC) facing upwards into donor side. The receptor medium (5.5 ml) contained 40% ethanol in pH 7.4 buffer for maintaining sink condition. The donor medium was 5.4 mM phthalates in 40% ethanol/pH 7.4 buffer. The diffusion area between compartments was 0.785 $cm^2$. The stirring rate of the stirrers in receptor was 600 rpm. The temperature of receptor was maintained at 37 °C. A 300-μl aliquot was taken from receptor at determined intervals and immediately replaced with an equal volume of medium. The sample number in this experiment was four.

The skin was removed from the cell after a 12-h permeation. The skin was washed and weighed, cut with scissors, and positioned into a glass homogenizer containing methanol. The duration for homogenization was 5 min. Then the resulting mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was collected for quantifying phthalate content by high-performance liquid chromatography (HPLC).

### 2.4. HPLC setup

The HPLC system was Hitachi 7-series (Tokyo, Japan) with ultraviolet detector. A C18 column was employed as stationary phase (LiChrospher®, Merck, Darmstadt, Germany). The mobile phase consisted of methanol and water. The ratio of methanol and water was 70:30, 90:10, 95:5, 100:0, and 100:0 for DEP, DBP, DEHP, DINP, and TOTM, respectively. Flow rate and wavelength was set to 1 ml/min and 220 nm, respectively. The limit of detection (LOD) for DEP, DBP, DEHP, DINP, and TOTM was 10, 5, 15, 20, and 40 ng/ml, respectively.

### 2.5. Phthalate content in hair follicles

The skin removed from Franz cell was stripped by adhesive tape 20 times to ablate SC. Subsequent to stripping, follicular cast was prepared by pipetting a drop of superglue (ethyl cyanoacrylate 7004T, 3M, Taipei, Taiwan) on a glass slide. This slide was pressed onto surface of the skin. The cyanoacrylate polymerized, and the slide was removed with quick movement after 5 min. The superglue remaining on slide was scrapped off, subsequently placed in a test tube with 2 ml methanol. The tube was shaken for 3 h, and then evaporated methanol by vacuuming. The mobile phase was used to dilute the samples for HPLC determination.

### 2.6. Cell viability test

Human keratinocytes (HaCaT) and foreskin fibroblasts (Hs68) were seeded in 96-well plates by appropriate culture medium for 24 h. Phthalates in dimethyl sulfoxide (DMSO) were added to wells to make final concentrations of 0.5, 1, and 5 mM. DMSO was used as the control (100% viability). After a 24-h incubation, tetrazolium salt in isopropanol was added to the wells. The optical density of the dissolved material was measured by spectrophotometer for calculating viability. The number of replications was three.

### 2.7. Determination of caspase-3 and poly(ADP ribose) polymerase (PARP) in cells

After pretreatment of phthalates with cells at 6 and 12 h, the proteins were isolated using cell lysis buffer and measured by Bradford protein assay kit (Amresco, Solon, OH, USA). Total proteins were separated with 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A Western blot analysis was performed using caspase-3 and PARP. The levels of GADPH expression was used as a gel loading control.

### 2.8. In vivo topical administration

Phthalates (5.4 mM) in 40% ethanol/pH 7.4 buffer at a volume of 0.6 ml was spread on a nonwoven polyethylene cloth (1.5 × 1.5 $cm^2$), and then applied to dorsal region of nude mouse. The cloth was fixed by Tegaderm® adhesive dressing (3M, St. Paul, MN, USA) and Fixomull® stretch adhesive tape (Beierdorf AG, Hamburg, Germany). The cloth was changed by a new one every 24 h. After 7 days, the cloth was withdrawn, and the treated skin area was cleaned for the following physiological examination, immunohistology, and proteomic profiling. There were six animals tested for each group.

### 2.9. Physiological examination of nude mouse skin

The skin treated by phthalates was examined by physiological parameters such as transepidermal water loss (TEWL) and skin surface pH. A Tewameter® (TM300, Courage and Khazaka, Köln, Germany) was employed for recording TEWL ($g/m^2/h$). The pH of skin was detected by Skin-pH-Meter® pH 905 (Courage and Khazaka). An adjacent untreated skin was used as a baseline standard for each examination. The sample number was six for each parameter.

### 2.10. Immunohistology

The skin excised from nude mouse treated by phthalates was fixed in a 10% buffered formaldehyde at pH 7.4. The samples were dehydrated with ethanol and embedded by paraffin wax. The skin was vertically cut to slices with a thickness of 3 μm. For cyclooxygenase (COX)-2, proliferating cell nuclear antigen (PCNA), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, the antibodies (Santa Cruz, CA, USA) were used. The procedures for immunostaining were performed according to a previous report (Lin et al., 2009).

### 2.11. Two-dimensional gel electrophoresis (2DE) and image analysis

The tissue samples (250 μg) extracted from phthalate-treated skin were thawed and diluted in immobilized pH gradient sample buffer containing 7 M urea, 2 M thiourea, 2% 3-[3-(cholamidopropyl) dimethylammoniol]-1-propanesulfonate (CHAPS), 65 mM dithiothreitol, and 1% IPG buffer to a volume of 350 μl. After rehydration for 12 h at 30 V, isoelectrofocusing (IEF) was automatically conducted with a total of 75 kV h. Following IEF separation and equilibration, electrophoresis was

Download English Version:

https://daneshyari.com/en/article/5850629

Download Persian Version:

https://daneshyari.com/article/5850629

Daneshyari.com