Safety evaluation of dermal exposure to phthalates: metabolism-dependent percutaneous absorption

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ABSTRACT

Phthalates, known as reproductive toxicants and endocrine disruptors, are widely used as plasticizers in polyvinyl chloride products. The present study was conducted for **risk identification** of dermal exposure to phthalates. When dibutyl phthalate was applied to the skin of hairless rats and humans, only monobutyl phthalate appeared through the skin, and the permeability of the skin was higher than that after the application of the monoester directly. The inhibition of skin esterases made the skin impermeable to the metabolite following dermal exposure to dibutyl ester, whereas removal of the stratum corneum from the skin did not change the skin permeation behavior. Similar phenomena were observed for benzyl butyl phthalate. The skin permeability of monobenzyl phthalate was higher than that of monobutyl phthalate in humans, although the reverse was observed in rats. Species difference in skin permeation profile corresponded to the esterase activity of the skin homogenate. Di(2-ethylhexyl) phthalate, which was not metabolized by esterases in the skin, was not transported across the skin. These results suggest that highly lipophilic phthalates may be transported easily across the stratum corneum lipids. The water-rich viable layer may become

permeable to these phthalates by their metabolism into monoesters, which are relatively hydrophilic. Skin metabolism is essential to the percutaneous absorption of phthalates. Because esterase activity has large inter-individual differences, further study will be needed for individual **risk identification** of dermal exposure to phthalates.

Abbreviations:

DBP: dibutyl phthalate

BnBP: benzylbutyl phthalate

DEHP: di(2-ethylhexyl) phthalate

BP: monobutyl phthalate

BnP: monobenzyl phthalate

EHP: mono(2-ethylhexyl) phthalate

PA: phthalic acid

DFP: diisopropyl fluorophosphates

HPLC: high-performance liquid chromatography

PBS: phosphate-buffered saline

DMSO: dimethyl sulfoxide

P: permeability coefficient

V: formation rate of metabolites

C: initial concentration

K_m: Michaelis constant

V_{max}: maximum metabolism rate

Introduction

Phthalates are used as plasticizers and solvents in the manufacturing of polyvinyl chloride-containing medical, building, and toy products (Graham, 1973). As these chemicals are non-covalently incorporated into plastics, they can leach out into the environment (Kamarei et al., 2011). We are daily exposed to phthalates owing to their broad use and large production (Blount et al., 2000). Several phthalates are suspected to be endocrine disruptors and reproductive toxicants; thus, their exposure to humans is a public health concern (Heudorf et al., 2007). In Japan, the Ministry of Health, Labor, and Welfare prohibits the use of phthalates in food packages and toys for babies, and limits their concentrations in indoor environment. However, scant attention has been paid to percutaneous absorption compared to ingestion and inhalation as entry routes of phthalates into the body (Hopf et al., 2014).

The skin is the largest organ interfacing directly with the environment, and it acts as a protective barrier against exogenous substances such as pathogens and chemicals (Proksch et al., 2008). Mammalian skin is composed of two primary layers, the epidermis and subjacent dermis. Stratum corneum, the

79 outermost layer of the epidermis, is a stratified squamous epithelium, which
80 comprises enucleated keratinocytes, i.e. corneocytes, and intercellular lipid
81 lamellae linked to the cornified envelope of the cells (Haftek et al., 2011). The
82 structures surrounding the corneocytes contribute greatly to the skin's barrier
83 function and effectively prevent the permeation of numerous molecules across
84 the barrier (Taylor et al., 1984). Conversely, one might say that compounds
85 having adequate lipophilicity and small molecular weight can permeate through
86 the stratum corneum. It is well known that the molecular weight of chemicals must
87 be under 500 to allow skin absorption (Bos and Meinardi, 2000). The optimal
88 lipophilicity of permeants across the stratum corneum was specified around 2.5,
89 which is the logarithm of n-octanol/water partition coefficient (log *P*) (Yano et al.,
90 1986) and 10 $cal^{1/2}/cm^{1/2}$, which is the solubility parameter (Liron and Cohen,
91 1984).

92 After penetrating via the stratum corneum, the molecules meet the viable
93 epidermis comprising stratum granulosum, stratum spinosum, and stratum
94 basale (Haftek et al., 2011). Since highly lipophilic compounds cannot distribute
95 to the water-rich viable epidermis, they remain in the stratum corneum. These
96 compounds hardly reach the blood vessels present in the dermis, and hence the

systemic blood circulation. However, the viable epidermis layer is the most metabolically active region in the skin (Laerum, 1969) and contains many of the same enzymes present in the liver (Kao and Carver, 1990). Therefore, any permeants via the stratum corneum are subjected to the metabolic functions of the living layer. Lack of metabolism would allow only the unchanged compounds to pass into the systemic circulation. Almost all biotransformation processes may produce inactive or less active metabolites, resulting in another barrier function of the skin against exogenous substances. There are reverse cases where the skin enzymes convert inactive chemicals into pharmacological and toxicological active agents (Zhang et al., 2009). If highly lipophilic compounds remaining in the stratum corneum are transformed to metabolites that are hydrophilic and capable to diffuse in the viable epidermis, the compounds will be absorbed into the body as those metabolites. Furthermore, if the metabolites are pharmacologically or toxicologically active, unexpected events can occur depending on the physicochemical properties of the parent compounds.

Phthalates are esters of phthalic acid (PA) and alcohols ranging from methanol to tridecyl alcohol, having either a straight chain or several branching. Their molecular weight ranges from 194.18 to 530.82, and lipophilicity remarkably

115 increases with increase in the length of alcohol chain. We reported that the
116 esterase activity in rodent and human skin was high enough to affect the skin
117 permeation profiles of ethyl nicotinate (Rittirod et al., 1990; Sugibayashi et al.,
118 1996). Several hydrolyzed metabolites of phthlates, i.e. monophthalates, are
119 known as reproductive toxicants (Bonilla and del Mazo, 2010; Muczynski et al.,
120 2012). It is important to know the skin permeation profiles of phthalates, and the
121 influence of metabolism on these profiles from the viewpoint of safety.

122 The present study was conducted for **risk identification** of dermal
123 exposure to phthalates. *In vitro* skin permeation profiles of three popular
124 plasticizers were evaluated in hairless rat skin. Skin permeability and skin
125 concentration of phthalates and their monoester metabolites were measured. The
126 esterase activities in skin homogenate were determined, and the metabolic
127 parameters were compared among phthalates. In addition, the permeation
128 characteristics and enzymatic activity of human skin were assessed and
129 compared with those of rat skin.
130

Materials and methods

Materials

Dibutyl phthalate (DBP), benzyl butyl phthalate (BnBP), di(2-ethylhexyl) phthalate (DEHP), monobutyl phthalate (BP), monobenzyl phthalate (BnP), mono(2-ethylhexyl) phthalate (EHP), PA, and diisopropyl fluorophosphates (DFP) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The physicochemical properties of phthalates used as permeants are listed in Table 1. Other reagents and solvents of special grade or HPLC grade were commercially obtained and used without further purification.

Table 1

Preparation of skin membranes

Male hairless rats (WBN/Ila-Ht, 8–10 weeks of age, with body weight of 220–260 g) were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Fukaya, Saitama). The abdominal skin was freshly excised under anesthesia by an intraperitoneal injection (*i.p.)* injection of pentobarbital (50 mg/kg) immediately before permeation and metabolism experiments. The stripped skin was prepared by stripping the stratum corneum 20 times with a cellophane tape (Cellotape™, No. 405, Nichiban Co. Ltd., Tokyo, Japan) (Flynn et al., 1981). All animal experiments were approved by the Institutional Animal Care and Use Committee of Josai University.

Four pieces of frozen abdominal skin of two female Caucasians (51 and 55 years old; mean skin thickness of 500 and 550 µm, respectively) were purchased from Biopredic International (Saint Grégoire, France) through KAC (Kyoto, Japan). The skins were stored at -50°C and thawed at 32°C just before the experiments. The stripped skins were made by the same method used

for rat skin. All human skin studies were carried out in accordance with the guidelines for the ethical use of human-organs and tissues in KAC.

Skin permeation experiments

Excess subcutaneous fat was trimmed off from the full-thickness or stripped skin of rat or humans. Each skin membrane was mounted on a side-by-side diffusion cell (effective diffusion area of 0.95 cm^2) in which the donor (stratum corneum side) and receiver (dermis side) chambers were prewarmed to 32°C. The skin was hydrated by filling both chambers with 2.5 mL of pH 7.4 phosphate buffered saline (PBS) for 30 min (rat) and 12 h (human) prior to starting BP and DBP permeation experiments. When the permeant was BnP, BnBP, EHP, or DEHP, 10% dimethyl sulfoxide (DMSO-PBS) was used instead of PBS. Under experimental conditions where hydrolysis is inhibited by esterase in the skin, DFP, a serine protease inhibitor, was added to the solutions at a concentration of 2.7 mM (Sugibayashi et al., 1996). After hydration, the solutions in the donor and receiver chambers were replaced with the same volume of phthalate solution and fresh PBS or 10% DMSO-PBS. BP and DBP were applied to the skin in 4.0 and

0.718 mM PBS, respectively. The donor concentration in 10% DMSO-PBS was 4.0, 1.4, 0.4, and 0.256 mM for BnP, EHP, BnBP, and DEHP, respectively. Receiver solutions containing 0.54 mM DFP were used for the complete inhibition of esterase activity in the skin. Donor and receiver solutions were stirred by a magnetic stirrer with a stirring bar and maintained at 32°C throughout the experiments. The receiver solution was periodically sampled, and the same volume of fresh PBS or 10% DMSO-PBS was added to the receiver chamber to keep the volume constant. The samples were stored at 4°C until determination of permeant concentration. The experiments were continued for 6-48 h until permeation reached the steady state.

Determination of skin concentrations

After permeation experiments, the skin was demounted from the diffusion cell, and washed three times with 1 mL of fresh receiver solution to completely remove phthalates remaining on the skin surface. The effective permeation area of the skin was minced with scissors and an appropriate volume of fresh receiver solution was added to make 4 w/v% skin homogenate. Homogenization was

198 performed using a homogenizer (Polytron PT-MR 3000, Kinematica, Switzerland)
199 at 10,000 *g* for 3 min at 4°C. The homogenate was centrifuged at 15,000 *g* for 5
200 min at 4°C, and the resulting supernatant was stored at 4°C until determination
201 of phthalate concentration.
202
203 *Metabolism experiments in skin homogenates*
204
205 Skin homogenates (10% w/v) were made with freshly excised rat skin or
206 thawed human skin and PBS as described above. The homogenates were
207 centrifuged at 9,000 *g* for 5 min at 4°C. The supernatants and various
208 concentrations of phthalate solutions (BP and DBP in PBS, and BnP, BnBP, EHP,
209 and DEHP in 10% DMSO-PBS) were preincubated for 30 min at 37°C and mixed,
210 such that the final concentration of each phthalate was 5–100 μM and that of the
211 skin homogenate was 0.25 (rat) or 0.75% (human), respectively. The solution was
212 kept at 37°C and periodically sampled for 120 min. The sample was stored at 4°C
213 until determination of phthalate concentration.
214
215 *Analytical methods*

The samples were vortex-mixed with the same volume of acetonitrile and centrifuged at 18,800 *g* and 4°C for 5 min. Phthalates in the supernatant (20 µL) were quantified by an HPLC system (Shimadzu Co., Kyoto), which was equipped with CBM-20A system controller, LC-20AD pump, SIL-20AC auto injector, SPD-20AC UV/Visible detector, CTO-20AC column oven, and LC solution analysis software. Cadenza CD-C18 column (4.6 x 75 mm, Imtakt Corporation, Kyoto) packed with C18 silica reversed-phase particles of 3 µm and kept at 40°C was used in this study. The flow rates of mobile phases were set to 1 mL/min. An isocratic mobile phase comprising 65 % mobile phase A (aqueous 0.5 % phosphoric acid) and 35 % mobile phase B (acetonitrile) was used for the determination of BP and DBP, which resulted in retention times of 1.6 and 6.3 min, respectively. Simultaneous analysis of BP, BnP, and BnBP was carried out with a linear gradient elution system as follows: initial mobile phase, comprising 32.5% A and 67.5% B, maintained for 9 min, changed linearly to 20% A and 80% B in 4 min, held for an additional 7 min, and returned to the original composition in 3 min. This resulted in retention times of 9, 11, and 18 min. The elution system for EHP and DEHP included holding 30% A and 70% B for 4 min, a linear gradient

234 to 100% B in 4 min and keeping for another 4 min, and returning to the original
235 composition in 3 min. Their retention times were 3 and 12 min, respectively. The
236 detection wavelength for DBP and BP was 254 nm and that for other phthalates
237 was 280 nm. The lower limit of quantitation was 9.88, 50.0, 25.6, 10.0, 9.62, and
238 89.8 pmol for DBP, BnBP, DEHP, BP, BnP, and EHP, respectively. The extraction
239 ratio of phthalates was almost 1.0.

240 The protein content in skin homogenates used for metabolism
241 experiments of phthalates was determined by Lowry's method (Lowry et al.,
242 1951).

243

244 *Data analysis*

245

246 The skin permeability of phthalates was assessed using the permeability
247 coefficient *P*, which is a parameter calculated as the steady-state permeation rate
248 (flux) divided by the permeant concentration exposed to skin surface. During skin
249 permeation, when the parent phthalates were completely converted into certain
250 metabolites by skin enzymes, the apparent P, which is the steady-state flux of
251 metabolite divided by the concentration of parent phthalate applied to the skin,

was used as an alternative to true *P*. The metabolism of phthalates was quantified by the formation rate (*V*) of metabolites at various initial concentrations (*C*) of parent phthalate in the skin homogenate solution. The *V*-*C* data were fitted to Hanes-Woolf equation [$C/V=C/V_{max}+K_m/V_{max}$] using linear regression to calculate metabolic parameters such as Michaelis constant (K_m) and the maximum metabolism rate (V_{max}).

Results

Skin permeation and concentration of DBP

The skin permeation profiles and skin concentrations, respectively, of phthalates after applying DBP to the excised rat abdominal skin are shown in Figure 1A and B. Although DBP was applied to the skin surface, only BP, an active monoester metabolite of DBP, permeated through the skin. The apparent permeability coefficient, which is the steady-state flux of BP divided by the concentration of DBP applied to skin, is listed in Table 2. BP but not DBP existed inside the skin after the permeation experiments. When the skin was treated with

DFP, a serine-esterase inhibitor, DBP and BP did not transport through the skin and only DBP was detected in the skin.

The corresponding data for **the stratum corneum-stripped skin (stripped skin)** are shown in Fig. 1C and D and listed in Table 2. Despite the absence of stratum corneum, which is the main barrier of skin permeation, the permeation characteristics were similar to those for full-thickness skin. Both permeation and existence of BP were observed in DFP-untreated skin. No permeation was found in DFP-treated skin, but DBP was detected in the skin.

Fig. 1 and Table 2

Skin permeation and concentration of phthalate diesters

Skin permeation and concentration of phthalates other than DBP were also assessed. The skin permeation properties of BnBP, an alkyl aryl ester of PA, after application to full-thickness skin is shown in Figure 2A. BnP and BP, the monoester metabolites of BnBP, were transported across the skin, whereas BnBP was not. The apparent permeability coefficient of BP was 6.5-fold higher than that

of BnP (Table 2). The applied diester and two metabolites were detected in the skin after permeation experiment, although the concentration of BnBP was lower than that of BP (Fig. 2B). Inhibition of skin esterase made two metabolites impermeable across the skin, resulting in the presence of BnBP alone in the skin. Stripped skin had permeation characteristics similar to full-thickness skin (Table 2).

Fig. 2

Neither DEHP nor its monoester metabolite, EHP, was transported through the skin when DEHP was applied to the full-thickness skin. The skin concentrations of two phthalates were under the detection limits 48 h after applying DEHP. Tape stripping and esterase inhibition did not affect the skin permeation properties of DEHP.

Skin permeation of phthalate monoesters

The permeation properties of phthalate monoesters after their application to the skin were evaluated and compared with those after the application of corresponding diesters (Table 2). As mentioned above, little difference was observed in the apparent permeability coefficient of phthalate monoesters (BP and BnP), which were the products of metabolism during the skin transport of phthalate diesters (DBP and BnBP), between full-thickness and stripped skin. In contrast, on removal of the stratum corneum, the permeability coefficient of all tested monoesters (BP, BnP, and EHP) after application to the skin increased remarkably.

Species differences in skin permeation and concentration of phthalate diesters

Skin permeation experiments of phthalate diesters were carried out using excised human skin, and the results were compared with those of rat skin. The results of BnBP permeation experiment using full-thickness human skin is shown in Figure 2C and D. No BnBP permeation was observed through human skin, which is similar to the finding observed in rat skin. However, the permeability of BnP was higher than that of BP contrary to that in rat skin. In agreement with the

permeability data, a higher concentration of BnP than BP was observed in human skin. The permeability of the two monoesters was not changed by tape stripping in human skin, which is the same as that observed in the rat skin (Table 3). The permeation properties of other phthalate diesters through human skin were similar to those through the rat skin. BP permeated through the skin after applying DBP; however, no permeation was found after applying DEHP.

Table 3

Skin metabolism of phthalate diesters

Metabolic experiments of phthalates were carried out in rat and human skin homogenates. DBP and BnBP were hydrolyzed to the corresponding monoesters in skin homogenates and linear Hanes-Woolf plots were obtained. Hanes-Woolf plots for hydrolysis of BnBP to BnP and BP in rat skin homogenate are shown in Figure 3. The enzymatic parameters, K_m and V_{max} were calculated from the slope and intercept of each plot, and the values are listed in Table 4. DEHP was not hydrolyzed to EHP, and PA was not detected in any experiment.

341 The V_{max} value of BnBP to BP was approximately twice that of BnBP to BnP, and
342 approximately half that of DBP to BP. However, almost same K_m values were
343 observed in the rat skin. In contrast, BnBP was metabolized more to BnP than
344 BP, and the K_m value ranged from 13 to 165 in human skin.
345
346 Fig. 3 and Table 4
347
348 **Discussion**
349
350 In the present study, we conducted **a risk identification** of skin
351 permeation of various phthalates following dermal exposure. After application of
352 DBP to the full-thickness skin of hairless rat, BP, an active metabolite, but not
353 DBP permeated through the skin and existed inside the skin after permeation (Fig.
354 1A and B). This shows that DBP was hydrolyzed during skin permeation, and the
355 hydrolysis rate was greater than the skin permeation rate of DBP. Skin treatment
356 with DFP made BP impermeable to the skin and only DBP existed in the skin (Fig.
357 1A and B). Thus, serine esterases are responsible for DBP hydrolysis in the skin,
358 and hydrolysis to BP is essential for skin permeation of this phthalate. Stratum

359 corneum-stripped skin had permeation characteristics almost the same as those
360 in full-thickness skin (Table 2), suggesting that the main permeation barrier of
361 DBP is not the stratum corneum but the viable epidermis and dermis. DBP may
362 be transported easily across the stratum corneum lipids, but hardly across the
363 water-rich viable layer owing to its high lipophilicity (ClogP = 4.73). Unlike DBP,
364 the skin permeability of BP increased on removing the stratum corneum (Table
365 2), indicating the stratum corneum as a main permeation barrier. DBP becomes
366 skin permeable only after metabolizing to BP, which is relatively hydrophilic
367 (ClogP = 2.72). DFP treatment of the skin would make DBP remain in the stratum
368 corneum, because of its high lipophilicity. We previously reported that both ester
369 and its monoester metabolite were transported across the skin after the
370 application of ethyl nicotinate (Rittirod et al., 1990; Sugibayashi et al., 1996). It
371 may be because of the lower lipophilicity of ethyl nicotinate (ClogP = 1.32) than
372 that of DBP and BP.

373 Skin permeability of BP was remarkably higher after applying DBP than
374 BP directly (Table 2). When BP was applied on the skin, permeation through the
375 stratum corneum was the rate-limiting step of the overall permeation process.
376 This step can be overcome by substituting BP with highly lipophilic DBP. Although

377 the amount of DBP reaching the viable epidermis may be small, the quick
378 conversion to BP would maintain a sink condition in the viable epidermis. In
379 addition, the DBP in the stratum corneum would serve as a reservoir of BP. Such
380 a reservoir effect is well known for its association with the delayed
381 pharmacological effect of steroids (Stoughton, 1965; Abidi et al., 2012). The
382 sustained effect of BP may cause serious results because BP is an endocrine
383 disruptor and reproductive toxicant (Heudorf et al., 2007). **We are daily exposed**
384 **to phthalates by touching plastic products such as computers,**
385 **smartphones, carpets, chairs and so on.** Moreover, such phenomenon would
386 depend on the metabolic activity in the skin during cutaneous absorption, as
387 described above.

388 Metabolism-dependent characteristics were also observed in skin
389 permeation of BnBP, an alkyl aryl ester of PA. In brief, the metabolites of BnBP,
390 BnP and BP, permeated through the skin, whereas BnBP did not (Fig. 2A). The
391 permeation of these metabolites was inhibited by DFP and only BnBP existed in
392 DFP-treated skin (Fig. 2B). These characteristics were not changed by the
393 removal of the stratum corneum (Table 2). Skin permeability and concentration of
394 BP were a few times higher than that of BnP, following the application of BnBP to

395 the rat skin (Table 2 and Fig. 2B). The V_{max} value of BP in rat skin homogenate
396 was approximately twice as high as that of BnP, although the K_m values were
397 almost the same (Table 4). The permeability through the stripped skin was not
398 very different between BnP and BP (Table 2). The hydrolysis rate of BnBP may
399 reach saturation during skin permeation; thus, the difference in skin permeability
400 and concentration between BnP and BP may be due to the difference in V_{max}
401 values.

402 In humans, BnP and BP were also transported across the skin after
403 dermal application of BnBP, and the transported amounts were not changed by
404 the removal of stratum corneum (Fig. 2C and D, Table 3). However, the apparent
405 permeability coefficient of BP through the human skin was lower than that of BnP,
406 in contrast to that observed in rat skin. The permeability coefficient value of BP
407 was remarkably lower than the corresponding value in rats (Table 2). There is a
408 species difference in skin permeation of BnBP. The human skin homogenate
409 showed a low V_{max} value of BP compared to that of BnP, and the value of BP was
410 about one-fiftieth of that in the rat skin homogenate (Table 4). The K_m values
411 indicated that the esterases in human skin have a lower affinity to BP than BnP,
412 and the affinity of rat esterases to the two phthalates were almost the same. It

413 was reported that species differences exist in the metabolic capacity of DEHP (Ito
414 et al., 2005), and these differences cause pharmacokinetic differences among
415 species (Adachi et al., 2015). However, metabolic data in the skin is not available
416 despite different enzyme expression among the tissues (Imai, 2006). We
417 confirmed that no DEHP is hydrolyzed to EHP in skin homogenates and
418 transported across the skin. Species difference is also observed in the skin
419 permeability of chemicals not being dermally metabolized, but it can easily be
420 predicted from the physicochemical properties of chemicals. Many *in silico*
421 estimation methods have been proposed for dermally unmetabolized chemicals
422 (Potts and Guy, 1992; Vecchia and Bunge 2002; Hatanaka et al., 2014; Chen et
423 al., 2015). Nevertheless, skin permeation characteristics of dermally metabolized
424 chemicals differ quantitatively and qualitatively among species. Therefore, the
425 characteristics of human skin cannot be estimated from those of animal skin.
426 Moreover, inter-individual difference in metabolic activity is often larger than the
427 species difference (Ito et al., 2014). **An accurate estimation of esterase activity**
428 **in the skin is necessary.**

429 **We used the frozen skin to get the human data, because the fresh**
430 **human skin is rather hard to obtain. The influence of freezing was evaluated**

on the skin permeation and metabolism by many researchers, and the extent of influence was remarkably different depending on the permeants, animal species and storage procedures (Hewitt et al., 2000; Lau et al., 2012; Pažoureková et al., 2013; Sintov, 2017). In the present study, the permeability of phthalates through the human skin may be overestimated and the metabolic activity in the skin may be underestimated. Even if those are so, the metabolism-dependent percutaneous absorption of phthalates occurs, as long as phthalates without biotransformation to monoester metabolites cannot be transported across the water-rich viable layers of skin. The obtained permeation and metabolic profiles may not be general in human skin, because only four pieces of skin from two female subjects could be used in the present study. We have already obtained a transgenic mouse to which a certain human esterase gene is transferred. The skin homogenate of transgenic mouse showed a low V_{max} value of BP compared to that of BnP, in contrast to that observed in skin homogenate of wild type mouse (data not shown). These results support the species differences observed in the present study. Further study will be required to clarify the species differences in skin permeation characteristics of dermal

metabolized chemicals such as phthalates.

Conclusion

Phthalates were absorbed percutaneously as well as through oral and transpulmonary routes. Considering the large surface area of the skin and repeated contact with plastics, the percutaneous absorption of phthalates cannot be ignored. Dialkyl phthalates were hydrolyzed to monoester metabolites during skin permeation. Skin metabolism is a key event for the percutaneous absorption of phthalates and their metabolites. Highly lipophilic phthalates may be easily transported across the stratum corneum lipids, and the water-rich viable layers become more permeable to phthalates by metabolizing to monoesters, which are relatively hydrophilic. The permeated amount of phthalates depends on the esterase activity in the skin, which is remarkably different among species and individuals. Further study will be needed for individual **risk identification** of dermal exposure to phthalates.

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Conflict of interest

The authors declare no conflict of interests.

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575 Figure Legends

576

577 **Fig. 1.** Skin permeation profiles and skin concentrations of phthalates after
578 applying DBP to excised hairless rat skin. (A), cumulative amount of DBP and its
579 monoester metabolite, BP, that permeated through full-thickness skin; (B),
580 phthalate concentrations after permeation experiment in full-thickness skin
581 untreated or treated with DFP; (C), cumulative amount of DBP and BP that
582 permeated through stripped skin; (D), phthalate concentration after permeation
583 experiment in stripped skin untreated or treated with DFP. N.D. means “not
584 detected.” Each point represents the mean ± S.D. (n = 4).

585 **Fig. 2.** Skin permeation profiles and skin concentrations of phthalates after
586 applying BnBP to excised hairless rat and human skins. (A), cumulative amount
587 of BnBP and its monoester metabolites, BP and BnP, that permeated through full-
588 thickness rat skin; (B), phthalate concentration after permeation experiment in
589 full-thickness rat skin untreated or treated with DFP; (C), cumulative amount of
590 BnBP, BP, and BnP that permeated through full-thickness human skin; (D),
591 phthalate concentration after permeation experiment in full-thickness human skin.
592 N.D. means “not detected.” Each point represents the mean ± S.D. (n = 4).

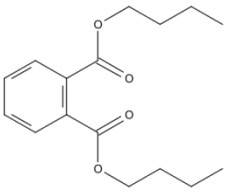
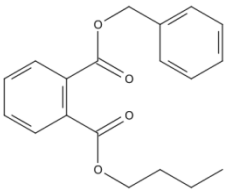
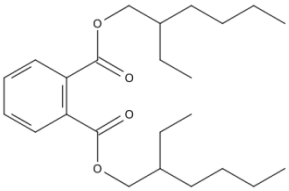
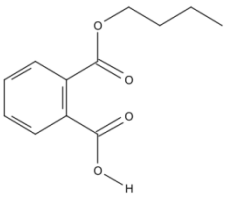
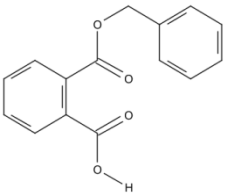
593 **Fig. 3.** Hanes-Woolf plot for hydrolysis of BnBP to BnP and BP in rat skin

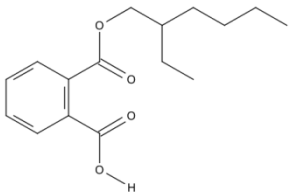
594 homogenate.

595

596 Table 1

597 Physicochemical properties of phthalates used as permeants in this study.

CAS No.	Compounds name (abbreviation)	Structural fomula	Chemical formula (Molecular weight)	Clog*P*[a]
84-74-2	Dibutyl phthalate (DBP)		$C_{16}H_{22}O_4$ (278.3)	4.73
85-68-7	Benzyl butyl phthalate (BnBP)		$C_{19}H_{20}O_4$ (312.4)	4.97
117-81-7	Di (2-ethylhexyl) phthalate (DEHP)		$C_{24}H_{38}O_4$ (390.6)	8.71
131-70-4	Monobutyl phthalate (BP)		$C_{12}H_{14}O_4$ (222.2)	2.72
2528-16-7	Monobenzyl phthalate (BnP)		$C_{14}H_{10}O_4$ (256.25)	3.23

4376-20-9	Mono (2-ethylhexyl) phthalate (EHP)		$C_{16}H_{22}O_4$ (278.3)	4.71

[a] Logarithm of n-octanol/water partition coefficient calculated by Chem Draw Ultra 12.2® (PerkinElmer informatics, Cambridge, MA).

601 Table 2

602 Permeability coefficients of phthalates through hairless rat skin[a].

Applied chemical	Permeated chemical	$P_{full\text{-}thickness}$[b] (cm/s)	$P_{stripped}$[c] (cm/s)
DBP	BP	$7.3\times10^{-5} \pm 1.4\times10^{-5}$	$6.8\times10^{-5} \pm 2.2\times10^{-5}$
BnBP	BnP	$9.2\times10^{-7} \pm 6.1\times10^{-8}$	$9.0\times10^{-7} \pm 1.5\times10^{-7}$
	BP	$6.0\times10^{-6} \pm 2.2\times10^{-6}$	$3.5\times10^{-6} \pm 1.1\times10^{-6}$
BP	BP	$2.4\times10^{-7} \pm 1.4\times10^{-7}$	$4.2\times10^{-6} \pm 2.1\times10^{-6}$
BnP	BnP	$3.6\times10^{-8} \pm 8.7\times10^{-9}$	$4.4\times10^{-6} \pm 8.2\times10^{-7}$
EHP	EHP	N.D.[d]	$4.5\times10^{-8} \pm 6.9\times10^{-9}$

603 [a] Mean ± S.D. of 3–5 experiments. When the permeated chemical differed from

604 the applied chemical, the values were the apparent permeability coefficients.

605 Refer to *Data analysis* for details.

606 [b] Permeability coefficient through the full-thickness skin.

607 [c] Permeability coefficient through the stripped skin.

608 [d] Not detected.

609 Table 3

610 Permeability coefficients of phthalates through human skin[a].

Applied chemical	Permeated chemical	$P_{full\text{-}thickness}$[b] (cm/s)	$P_{stripped}$[c] (cm/s)
DBP	BP	$8.6\times10^{-6} \pm 4.7\times10^{-7}$	$7.2\times10^{-6} \pm 1.1\times10^{-6}$
BnBP	BnP	$1.1\times10^{-6} \pm 2.4\times10^{-7}$	$1.5\times10^{-6} \pm 6.0\times10^{-7}$
	BP	$2.7\times10^{-7} \pm 4.3\times10^{-7}$	$2.5\times10^{-7} \pm 1.5\times10^{-7}$

611 [a] Mean of 3–5 experiments. When the permeated chemical differed from the

612 applied chemical, the values were the apparent permeability coefficients. Refer

613 to *Data analysis* for details.

614 [b] Permeability coefficient through the full-thickness skin.

615 [c] Permeability coefficient through the stripped skin.

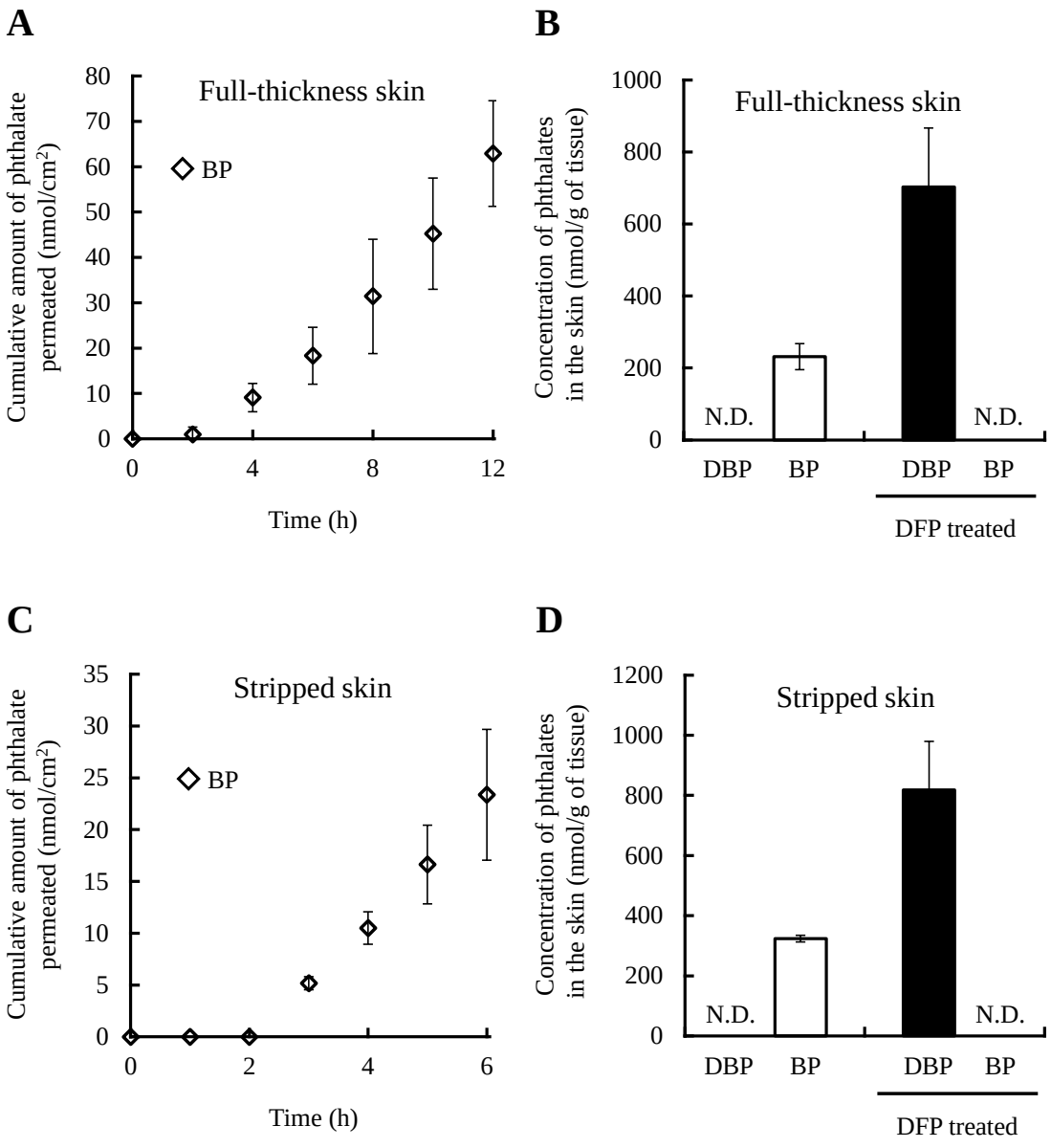
616

Table 4

Metabolic parameters for hydrolysis of DBP and BnBP in rat and human skin homogenates.

Species	Substrate	Product	K_m (μM)	V_{max} (nmol/min/ mg protein)
Rat	DBP	BP	18	2.9×10^{-1}
	BnBP	BnP	19	5.2×10^{-2}
		BP	15	1.3×10^{-1}
Human	DBP	BP	13	4.1×10^{-2}
	BnBP	BnP	59	4.7×10^{-2}
		BP	165	2.6×10^{-3}

621 Fig. 1



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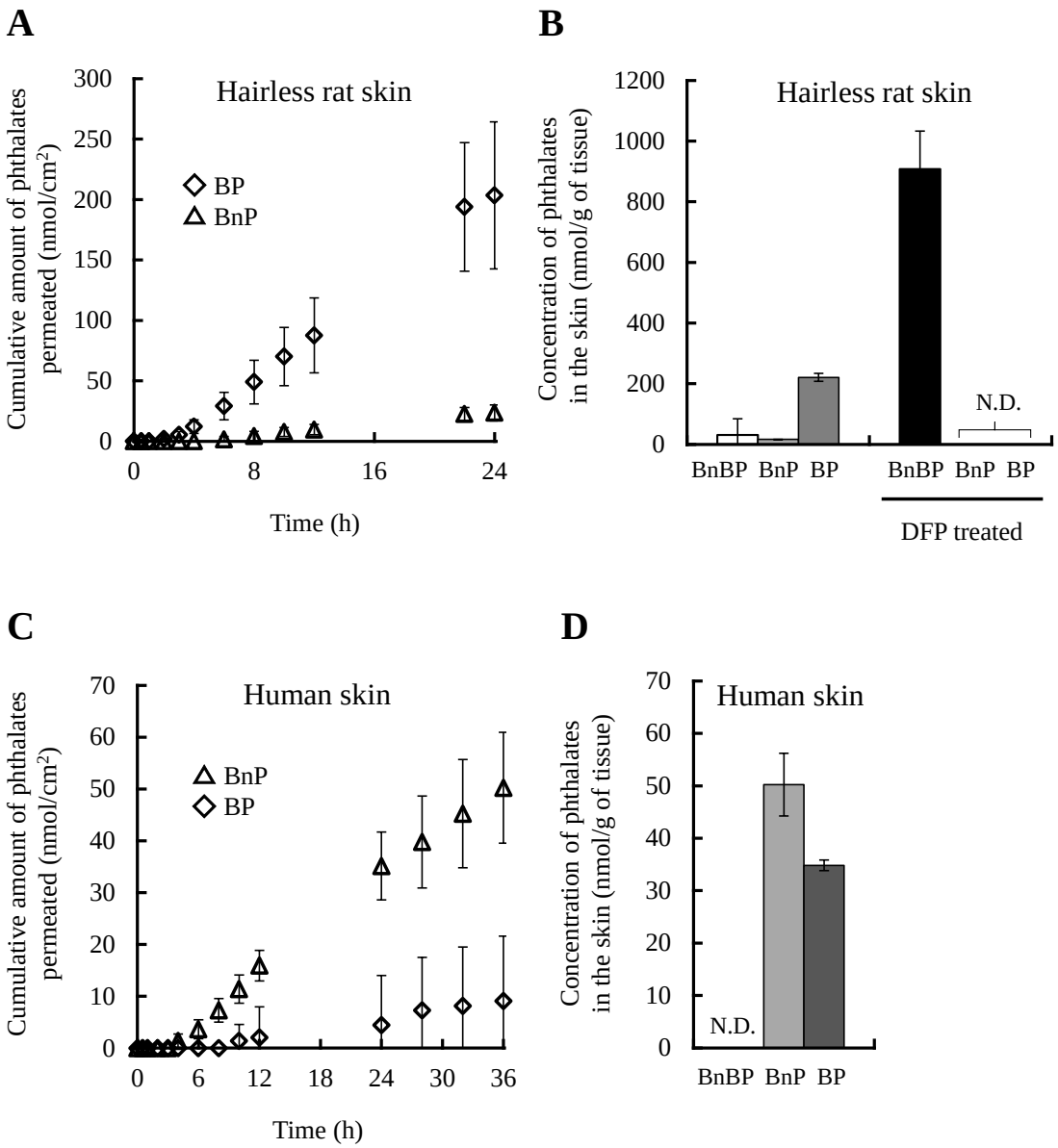
624 Fig. 2



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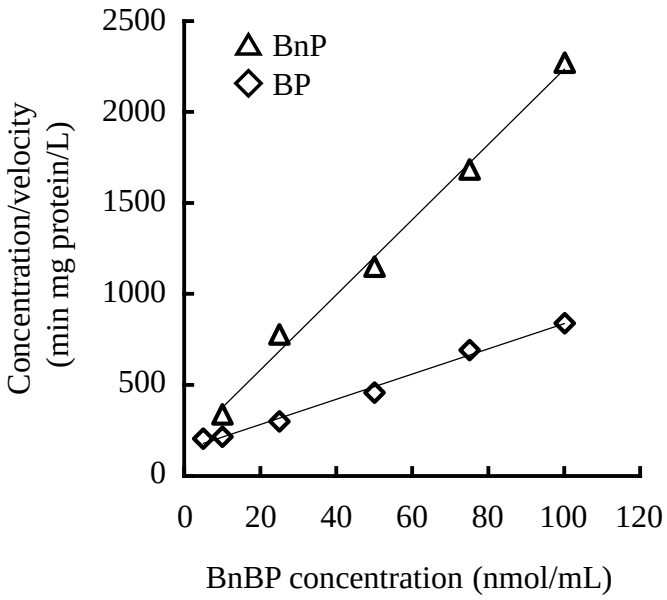
628 Fig. 3


2500
2000
1500
1000
500
0
Concentration/velocity
(min mg protein/L)
BnP
BP
0
20
40
60
80
100
120
BnBP concentration (nmol/mL)

629