TRANSFER OF PHTHALIC ACID DIESTERS FROM MODEL PVC SHEET TO SKIN SURFACE

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Introduction

Chemicals used in household products may diffuse to indoor environments, and humans may inhale these chemicals. Some of these chemicals are adsorbed on house dust, and humans may ingest the house dust[1]. In addition, some chemicals may be transferred to the skin surface, and human exposure to these chemicals by skin absorption or hand-to-mouth contact has been indicated[2]. Thus, studies investigating the transfer of chemicals, for example, parabens[3], bisphenol-A[4] and fragrances[5], to human skin surfaces have been carried out. Phthalic acid diesters (PAEs) are widely used as plasticizers in various products, particularly those made from polyvinyl chloride (PVC). Since PAEs migrate easily from plastic products, and their content in plastic materials is high, these chemicals are of great concern globally. Although oral exposure of children to PAEs by chewing or licking toys and childcare products has been studied[6], the transfer of PAEs from household products to skin surfaces have not been investigated. Thus, we examined the transfer of PAEs from PVC household products to forearm skin surfaces using model PVC sheets. We have already surveyed the PAE contents of PVC household products in Japan[7], and reported that high detection frequencies and large amounts of di(2-ethylhexyl) phthalate (DEHP). In addition, we reported that diisononyl phthalate (DINP) and di-*n*-butyl phthalate (DBP) were also detected in PVC household products. Thus, we selected these PAEs as the target chemicals for this study. It was thought that sebum was an important factor in PAEs transfer to skin surface, and it has been reported that triglyceride (TG) is the dominant component in human sebum[8]. Therefore, we examined the relationships between the amounts of PAEs transferred to the skin surface and of TG collected from the skin surface.

Materials and methods

Eleven healthy adult subjects, seven men and four women, aged 31-57 years, participated in this study. The ethical review boards of the National Institute of Health Sciences approved this study (No.175). Written informed consent was obtained from all subjects.

Environmental analysis grade DBP, DEHP, and the deuterated PAEs [DBP, DEHP, di-*n*-octyl phthalate] used as internal standards were obtained from Kanto Chemical Co., Inc. DINP (CAS. 28553-12-0) was obtained from Wako Pure Chemical Ind., Ltd. Pesticide residue grade ethanol, acetone, and hexane were obtained from Kanto Chemical Co., Inc., Wako Pure Chemical Ind., Ltd. and Sigma-Aldrich, respectively.

PAEs analysis grade anhydrous sodium sulfate was obtained from Wako Pure Chemical Ind., Ltd. TG analysis was performed using a TG analytical kit purchased from Bio Vision. Triton-X 100 used for TG analysis was obtained from Sigma-Aldrich. Deionized water was produced by Milli-Q Synthesis A10 (Millipore). All utensils made of glass, metal, or Teflon were heated at 250°C for more than 12 h to prevent contamination. DEHP and DBP used for the production of model PVC sheets were obtained from Tokyo Chemical Ind., Co. DINP (Cas.28553-12-0) used for the PVC sheets was purchased from Wako Pure Chemical Ind., Ltd. The PVC polymer (S-1003) and stabilizer (AC-255) used for the PVC sheets were industrial materials. The concentrations of PAEs in the PVC sheet were determined by GC/MS[7] and these are listed in Table 1.

A square model PVC sheet (1.5 cm x 1.5 cm = 2.25 cm^2, weight and thickness approximately 83 mg and 0.3 mm, respectively) was used for this study. The PVC sheet was contacted with inside of the forearm, and fixed by a rectangular silicone mat and surgical tape. After 30 min, the sheet was removed and the part of the skin surface contacted with the sheet was wiped with a clean applicator wetted with ethanol. The applicator was then placed in a test tube and PAE was extracted with acetone. The sample solution was dehydrated with anhydrous sodium sulfate, and the volume was adjusted to 10 ml. Finally, an internal standard solution was added to 1 ml of the sample solution and the sample was analyzed by GC/MS. The transfer experiment for each dose level was carried out in triplicate (n=3). Control samples (using a Teflon sheet of the same size) and blank samples were also analyzed to measure the PAEs background levels and to confirm contamination during the experimental process. The PAE amounts transferred to the skin surface were calculated as the average of three sheets. These experiments were carried out twice per subject [1st: DBP 28%, DEHP 15%, 28%, 37%, DINP 28%; 2nd: DBP 15%, DINP 15%, two-mixed sheets (DEHP and DBP, DEHP and DINP)] on different days. A control sample was used to measure the TG amounts. The control sample solution (9 ml) was concentrated and dried under a gentle N_2 stream. The residue was dissolved in 5% Triton-X aqueous solution and the TG was analyzed using a TG measurement kit according to the Bio Vision protocol.

Table 1. Concentrations of PAEs in the model PVC sheet (n=3).

PAEs	Blending ratio of PAE (%)	Average (%)	SD	CV (%)
DBP	15	11.9	0.47	3.9
	28	24.5	0.58	2.4
	14[a]	11.4	0.18	1.6
DEHP	15	12.3	0.22	1.8
	28	23.5	0.14	0.6
	37	33.6	2.0	5.8
	14[a]	12.5	0.13	1.0
	14[b]	12.3	0.54	4.4
DINP	15	13.1	0.16	1.2
	28	26.1	0.72	2.8
	14[b]	13.4	0.24	1.8

[a]mixture of DEHP and DBP. [b]mixture of DEHP and DINP.

Results and discussion:

DEHP was detected in the control samples collected from all subjects (n=22, 0.013-0.11 μg/cm^2). DBP and DINP were not detected in the control samples collected from all subjects, excepting subject D. DBP was detected in the control samples collected from subject D (0.14 and 0.48 μg/cm^2). In a previous study, benzyl butyl phthalate and DBP were detected at levels of 0.79 and 0.90 ng/cm^2 (as median) in the wiped

samples collected from children's skin surfaces[9]. The background amounts of DEHP detected on skin surfaces in this study were higher than those of other PAEs because DEHP is used with high frequency and in large amounts in PVC household products[7]. Stapleton et al. determined brominated flame retardants on skin surfaces (average 251 pg/cm^2)[2]. Methyl paraben has been detected on forefinger skin surfaces (approximately 0.2 μg/cm^2, maximum value)[3]. These differences in detection levels among chemicals used in household products probably reflect their physico-chemical properties and usage.

Noticeable differences in the amounts of PAEs transferred to the forearm skin surface were not observed among all subjects, except in the case of the 28% and 37% sheets for subjects D and I (Fig.1, for example DEHP). Subjects D and I were women, and they did not use cosmetic items before these experiments. The reason for this high transferability is still unknown. The amounts of DEHP transferred from the 28% and 37% sheets were relatively high compared to those transferred from the 15% sheet. However, differences between the 28% and 37% sheets were not observed (Fig. 1). The amounts of each PAE transferred from the mixed sheets were higher than those from single component sheet (15% sheet). The plasticity of the mixed sheet was higher than that of the 15% sheet because of the large total amount of plasticizer. Thus, the PAEs included in the mixed sheet bled easily on contact with the skin. To examine the transferability of PAEs from the PVC sheet to the skin surface, the transferred amounts of DBP or DINP were compared with that of DEHP for the mixed sheets (Fig. 2). The relationships between the transferred amounts of DBP or DINP and that of DEHP were observed; the slopes of their regression lines were 0.503 or 1.16, respectively. The ratios of DBP/DEHP and DINP/DEHP in the mixed sheets were 0.912 and 1.09 (w/w), respectively. Thus, the order of the transferability of these PAEs to the skin surface might be DINP, DEHP, DBP. In addition, the average amount of PAEs transferred to the skin surface from the 15% sheet was correlated with their octanol-water partition coefficient (K_{ow}). Thus, the hydrophobic properties of the PAEs may influence their transferability to the skin surface from PVC sheet.

The average amount of TG collected from the skin surfaces of all subjects was 9.3 nmol/cm^2 (n=22, 3.4-18.3 nmol/cm^2). This value was relatively high compared to those in a previous study that measured forearm sebum using a sebumeter[10], and slightly lower than in another previous study which used standardized adhesive patches designed to collect sebum[11]. Generally, men have higher amounts of sebum than woman[12]. However, differences in sebum amounts, based on age and sex, were not observed among

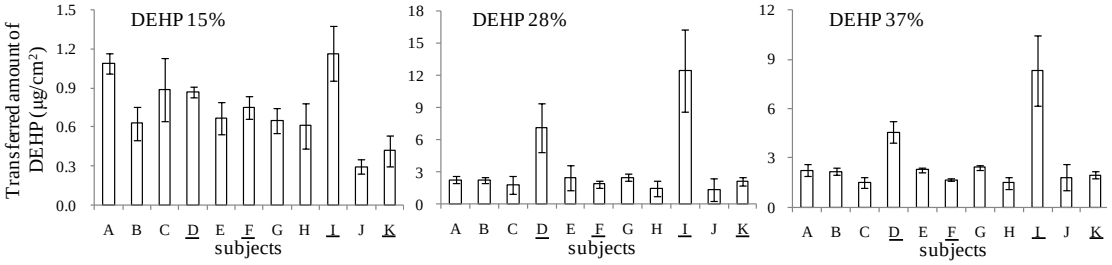

Fig. 1. Amounts of DEHP transferred from model PVC sheet to the skin surface (average ±SD).
(Alphabetical order means in order of age and underline means female subject)

the subjects in this study. No obvious relationship between the amounts of PAEs transferred to the skin surface and the amounts of TG collected from subject's skin was observed. It was thought that the PAEs were transferred to a very thin layer of skin surface, although the TG collected in this study might be collected not only from this very thin layer but also from under the thin layer of surface skin. Therefore, the method of sampling sebum on the skin surface might affect examination of the relationship between PAEs transferred and TG. In addition, TG is composed of several kinds of fatty acids and other compounds such as wax-esters and squalene, which are also components of sebum on the skin surface. Thus, it is necessary to examine the relationships between PAEs transferred to the skin surface and sebum components to evaluate the transferability of PAEs to the skin surface.

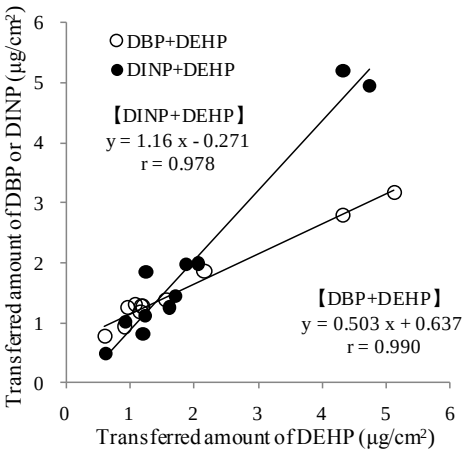

Fig. 2. Relationships between amount of DEHP and DBP or DINP transferred from mixture sheet.

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References:

1. Kanazawa A, Kishi R. (2009); *Jpn J Hyg*. 64: 672-682
2. Stapleton MH, Kelly MS, Allen GJ, Mcclean DM, Webster FT. (2008); *Environ Sci Technol*. 42: 3329-3334
3. Shibata H, Tsuda T. (2002); *BUNSEKI KAGAKU*. 51: 397-401
4. Biedermann S, Tschudin P, Grob K. (2010); *Anal Bioanal Chem*. 398: 571-576
5. Api MA, Bredbenner A, McGowen M, Niemiera D, Parker L, Renskers K, Selim S, Sgaramella R, Signorelli R, Tedrow S, Troy W. (2007); *Regul Toxicol Pharmacol*. 48: 279-283
6. Sugita T, Kawamura Y, Tanimura M, Matsuda R, Niino T, Ishibashi T, Hirabayashi N, Matsuki Y, Yamada T, Maitani T. (2003); *J Food Hyg Soc Japan*. 44: 96-102
7. Kawakami T, Isama K, Matsuoka A.(2011); *J Environ Sci Health Part A.* in press
8. Greene SR, Downing TD, Pochi EP, Strauss SJ. (1970); *J Invest Dermatol*. 54: 240-247
9. U.S. EPA. *http://www.epa.gov/heasd/ctepp/* (accessed April 14, 2011)
10. Tanaka A, Seki T, Ochiai H, Tazawa K. (1999); *J Nurs Soc Toyama Med Pharma Univ*, 2: 49-58
11. Thiele JJ, Weber US, Packer L. (1999); *J Invest Dermatol*. 113: 1006-1010
12. Inomata N. (1975); *Rinsho Derma*. 29: 349-357