Table 1. List of the Desiccant Samples Studied

Sample name	The product enclosed with the desiccant sachet	Country	The time obtained the sachet	How to obtain[a)]	Remarks
S1	Sports shoes	China	Mar-09	V	The sachet stayed for long time under room temperature.
S2	Ink toner	Unknown	Mar-09	V	Laser color printer
S3	Leather bag	Vietnam	Mar-09	V	The sachet stayed for long time under room temperature.
S4	Children sofa	China	Sep-09	V	Synthetic leather
S5	Ornament for the new year	Japan	Dec-09	V	
S6	Sandals	China	May-10	P	"Do not eat" and "mold-proof desiccant" were printed on the sachet in Japanese, English, and Chinese.
S7	Leather bag	China	May-10	V	Tote bag for woman, synthetic leather
S8	Belt	China	May-10	V	Synthetic leather
S9	Sofa	China	May-10	V	Clay type desiccant
S10	Rack	China	May-10	V	Synthetic leather with mount paper
S11-1 S11-2	Sports shoes	China	Jun-10	P	"Do not eat" and "mold-proof desiccant" were printed on the sachet in Japanese, English, and Chinese. The two sachet with same notes were enclosed together.
S12	Shoes	Unknown	Jun-10	V	The detail of products was unknown
S13	Shoes	Unknown	Jun-10	V	The detail of products was unknown
S14	Shoes	Unknown	Jun-10	V	The detail of products was unknown
S15	Shoes	Unknown	Jun-10	V	The detail of products was unknown
S16	Shoes	China	Jul-10	P	Synthetic leather and fiber
S17	Sandals	China	Jul-10	P	Synthetic leather, sandal for woman
S18	Shoes	Unknown	Jul-10	P	Synthetic leather and fiber
S19	Sandals	China	Jul-10	P	Synthetic leather
S20-1 S20-2	Sports shoes	China	Jun-10	P	"Do not eat" was printed on the sachet in English, French, Dutch, and German. However, the notes of these sachets were written by different font type.

a) V: provided from volunteer, P: purchased from retail store.

temperature of the water bath below 40°C. The sample solution was passed through a graphite carbon cartridge (InertSep GC 300 mg/6 ml, GL Science, Tokyo, Japan) washed with 4 ml of ethyl acetate and 4 ml of methanol before sample loading. Next, 2 ml of methanol containing 50% ethyl acetate was passed through the cartridge twice, and 6 ml of eluate was concentrated to approximately 1 ml with a rotary evaporator while maintaining the temperature of the water bath below 40°C. Then, to change the dominant solvent, 5 ml of ethyl acetate added to sample solution and concentrated to 2 ml with a rotary evaporator while maintaining the temperature of the water bath below 40°C. The sample solution was passed through a neutral alumina cartridge (InertSep AL-N 1 g/6 ml, GL Science) washed with 4 ml of ethyl acetate before sample loading. Next, 2 ml of ethyl acetate was passed through the cartridge and 4 ml of eluate was concentrated to below 0.5 ml by a gentle N_2 stream. Twenty-five micro liters of ethyl acetate solution containing 1 μg/ml of naphtalane-d_8 as an internal standard was added, and the sample volume was adjusted to 0.5 ml. This solution was then analyzed by GC/MS.

GC/MS Analysis —— All the samples in this study were analyzed using a Focus GC with a DSQII MS (Thermo Fisher Scientific, Waltham, MA, U.S.A.). A VF-5 ms fused silica capillary column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm, Varian-Agilent, Santa Clara, CA, U.S.A.) was used. The carrier gas used was He with a flow rate of 1.0 ml/min. The temperatures of the injec-

Table 2. List of the Product Samples Studied[a)]

Type of product[b)]	Sample name	Remarks
Sandals (S6)	P6-1	Sole surface
	P6-2	Mesh cloth strap inside
	P6-3	Synthetic leather strap inside
Rack (S10)	P10-1	Synthetic leather (outside)
	P10-2	Synthetic leather (inside)
	P10-3	Mount paper bonded to synthetic leather (inside)
Sports shoes (S11)	P11-1	Mesh cloth that covers the inside of shoe
	P11-2	Insole surface
Shoes (S16)	P16-1	Mesh cloth that covers the inside of shoe
	P16-2	Insole surface
Sandals (S17)	P17-1	Sole surface
	P17-2	Inside of strap
	P17-3	Strap lining of heel
Shoes (S18)	P18	Insole surface
Sandals (S19)	P19-1	Sole surface
	P19-2	Inside of strap
Sports shoes (S20)	P20-1	Mesh cloth that covers the inside of shoe
	P20-2	Insole surface

a) The sample was obtained the product part that can come into contact with skin, except for P10-3. *b*) The sample name in parenthesis corresponded to Table 1.

Table 3. GC Retention Times, Quantifying and Qualifying Ions, Recovries and its Coeficients of Variation (C.V.%, $n = 3$) and LOD[a)] and LOQ[b)] of the Compounds Studied

Compound	Retention time (min)	Quantifying ion (m/z)	Qualifying ion (m/z)	Desiccant sample				Product sample			
				Recovery (%)	C.V.(%)	LOD	LOQ	Recovery (%)	C.V.(%)	LOD	LOQ
DMF	5.92	113	59, 85	77	1.1	0.0032	0.012	54	1.3	0.0058	0.012
DEF	7.20	127	99, 126	77	2.0	0.0031	0.0097	87	3.2	0.0060	0.017
DBF	9.94	117	99, 156, 177	73	1.0	0.0096	0.0098	56	3.8	0.017	0.45
DMM	5.88	113	59, 85	62	2.6	0.00058	0.011	40	2.3	0.0020	0.021
DEM	7.06	99	126, 127	91	2.2	0.00087	0.010	61	2.5	0.0013	0.012
DBM	9.64	117	99, 156, 177	77	0.21	0.011	0.012	60	3.6	0.027	0.36
Naphthalene-d_8	7.43		136								

a) LOD (mg/kg): (3.3 × standard deviation)/(slope of calibration curve × relative sensitivity) ($n = 3$). *b*) LOQ (mg/kg): 10 × standard deviation ($n = 3$).

tor, transfer line, and ion source were 250, 280, and 250°C, respectively. The sample was injected in the splitless mode, and the injected volume was 1 μl. The GC oven temperature was initially maintained at 40°C for 0.5 min and the temperature increased to 310°C at a rate of 20°C/min. The oven temperature was then maintained at 310°C for 10 min. The MS was operated in the electron ionization (EI) mode at 70 eV, and the analysis was performed using the selected ion monitoring (SIM) mode. The retention times and the quantifying and qualifying ions are listed in Table 3.

Relatively small amounts of DEF and DBF were observed on the GC/MS chromatogram of DEM and DBM, respectively. We assumed that the DEF and DBF observed on the GC/MS chromatogram were impurities of DEM and DBM or that they were generated by photo-translation under room light or heat-translation in the GC injector; however, the ratios of DEF/DEM and DBF/DBM were almost constant after prolonged exposure to room light or injection at the various temperatures of the GC injector. Therefore, we thought that the concentrations of these compounds could be determined by the sample processing described above and GC/MS conditions adopted in this study. The standard curves of fumaric acid diesters and maleic acid diesters were prepared separately.

RESULTS AND DISCUSSION

Examination of Sample Preparation

First, the effect of N_2 dryness on behavior of DMF was examined, and the recovery of DMF after N_2 dryness was 11% (coefficient of variation: C.V. = 36%, n = 3). Thus, we performed the sample preparation process to prevent evaporation to dryness. Since acetone and ethyl acetate were used as extraction solvents for desiccant samples in previous studies,[18–20] the recoveries of DMF, DEF, and DBF extracted by acetone and ethyl acetate were examined (added 0.1 mg/kg, n = 3). The recoveries using acetone were 78–82% (C.V. = 2.4–9.1%), and those of ethyl acetate were 73–77% (C.V. = 1.0–2.0%). In this study, because reproducibility was considered to be of greater importance, ethyl acetate was selected for as the extraction solvent for the desiccant samples.

Although DMF was extracted from components of shoe samples by ultrasonic extraction using ethyl acetate in a previous study,[8] ethyl acetate could not be used as the extraction solvent in this study because the extracted solution had high viscosity, preventing next sample processing and GC/MS analysis. A high-viscosity solution may have been obtained because of the elution of adhesive and resin components from the product samples. It was observed that the solution extracted from product samples using methanol was not highly viscous; hence, methanol was used as the extraction solvent for product samples in this study. The sample solution was purified by a graphite carbon cartridge because several extracted solutions were colored and muddy. Pure methanol, methanol containing 25% ethyl acetate, and methanol containing 50% ethyl acetate were examined as eluted solutions from the cartridge for DMF, DEF, and DBF (Fig. 1). The results indicated that methanol containing 50% ethyl acetate was the most suitable solution for elution. Although several sample solutions remained colored, further purification was performed using a neutral alumina cartridge that has been reported to purify the extract from leather products.[21] Although the concentration of DMF was determined without interference from monitoring ion on the mass chromatogram by these purification processes, the ions causing interference for DBF and DBM could not be removed completely.

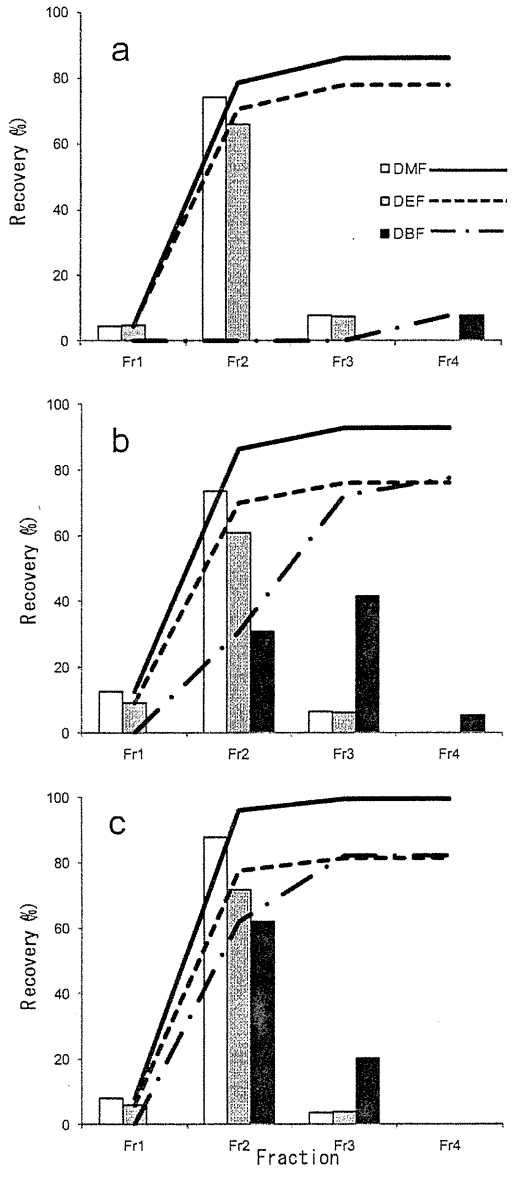


Fig. 1. Effects of Ethyl Acetate Content in Methanol on Elution Pattern of DMF, DEF, and DBF from Graphite Carbon Cartridge

a: 100% methanol, b: methanol containing 25% ethyl acetate, c: methanol containing 50% ethyl acetate. Fr1: eluted sample solution, Fr2: eluted solution 0–2 ml, Fr3: eluted solution 2–4 ml, Fr4: eluted solution 4–6 ml; the lines on the graph represent the accumulated amount of target compound eluted.

Recoveries, Limits of Detection (LOD), and Limits of Quantification (LOQ)

Recovery tests were performed by adding every compound into the samples. For the desiccant samples, 0.05 μg of every compound was added to 0.5 g of a blank sample (n = 3, 0.1 mg/kg). For the product samples, 0.05 μg of every compound was added

to 0.5 g of a blank sample that did not contain the chemicals being studied (n = 3, 0.1 mg/kg), except for DBF and DBM because of the reasons described above (0.5 μg of DBF and DBM were added to the blank samples, 1.0 mg/kg, n = 3). The results of the recovery tests are shown in Table 3. The recoveries of DMF were 77% (C.V. = 1.1%) from the desiccant samples and 54% (C.V. = 1.3%) from the product samples. The DMF recovery from the product samples was lower than that from desiccant samples due to evaporation during the several concentration processes involved in purification. The recoveries of the other compounds ranged from 62% to 91% (C.V. = 0.21–2.6%) from desiccant samples and 40–87% (C.V. = 2.3–3.8%) from product samples. Although the recoveries were slightly low, all the C.V. values were below 4%, and it was thought that the reproducibility of the methods in this study was sufficient to determine the concentrations of DMF and other chemicals in the desiccant and product samples. The data obtained in this study were not corrected by the obtained recovery results.

The LOD and LOQ were calculated from the results of recovery tests that involved 0.025 μg being added to 0.5 g of the samples (n = 3, 0.05 mg/kg). In the case of DBF and DBM in the product samples, 0.25 μg of every compound was added. LOD[22] and LOQ[23] were calculated as follows:

$$\mathrm{LOD} = 3.3 \times \rho/ar \quad (1)$$

$$\mathrm{LOQ} = 10\rho \quad (2)$$

where ρ is the standard deviation obtained from the results of a low-concentration analysis, a is the slope of the calibration curve, and r is the relative sensitivity. The resulting LOD and LOQ values are listed in Table 3. The LOD and LOQ of DMF were 0.0032 and 0.012 mg/kg in the desiccant samples and 0.0058 and 0.012 mg/kg in the product samples, respectively. The LOQs of DMF obtained in this study were significantly lower than the value mandated by the EU (below 0.1 mg/kg).

Table 4. Concentrations of Fumaric and Maleic Acid Diesters in the Samples

Sample name	DMF	DEF	DBF	DMM	DEM	DBM
S1	—	—	—	—	—	—
S2	—	—	—	—	—	—
S3	—	—	—	—	—	—
S4	—	—	—	—	—	—
S5	—	—	—	—	—	—
S6	2.3	—	—	—	—	—
S7	—	—	—	—	—	—
S8	—	—	—	—	—	—
S9	—	—	—	—	—	—
S10	—	—	8.4	—	—	720
S11-1	—	—	—	—	—	—
S11-2	—	—	—	—	—	—
S12	—	—	—	—	—	—
S13	—	—	—	—	—	—
S14	—	—	—	—	—	—
S15	—	—	—	—	—	—
S16	—	—	—	—	—	—
S17	—	—	—	—	—	—
S18	—	—	—	—	—	—
S19	—	—	—	—	—	—
S20-1	—	—	—	—	—	—
S20-2	0.60	—	—	—	—	—
P6-1	0.21	—	—	—	—	—
P6-2	0.11	—	—	—	—	—
P6-3	0.14	—	—	—	—	—
P10-1	—	—	—	—	—	29
P10-2	—	—	9.6	—	—	340
P10-3	—	—	12	—	—	440
P11-1	—	—	—	—	—	—
P11-2	—	—	—	—	—	—
P16-1	—	—	—	—	—	—
P16-2	—	—	—	—	—	—
P17-1	—	—	—	—	—	—
P17-2	—	—	—	—	—	—
P17-3	—	—	—	—	—	—
P18	—	—	—	—	—	—
P19-1	—	—	—	—	—	—
P19-2	—	—	—	—	—	—
P20-1	—	—	—	—	—	—
P20-2	—	—	—	—	—	—

unit: mg/kg. — Not detected.

Concentrations of DMF in the Samples

The concentrations of DMF in the samples are shown in Table 4. DMF was detected in two desiccant samples, S6 and S20-2, and the corresponding concentrations were 2.3 and 0.60 mg/kg, respectively. The mass chromatogram (m/z = 113) of S6 and the mass spectrum (scan mode: m/z = 50–350) of the DMF detected in S6 are shown in Fig. 2. The S6 and S20-2 sachets were enclosed with sandals (P6) and sports shoes (P20), and these footwear products were manufactured in China. In the case of S6, the notes were printed on the sachet in Chinese, English, and Japanese (Fig. 3a). The notes state that the sachet contains a desiccant with an anti-mold agent that is inedible. Although the same notes were printed on the sachets of S11-1 and S11-2, DMF was not detected in these desiccant samples. In contrast, the notes on the sachet of S20-2 were printed in four languages as follows: "DO NOT EAT" (En-

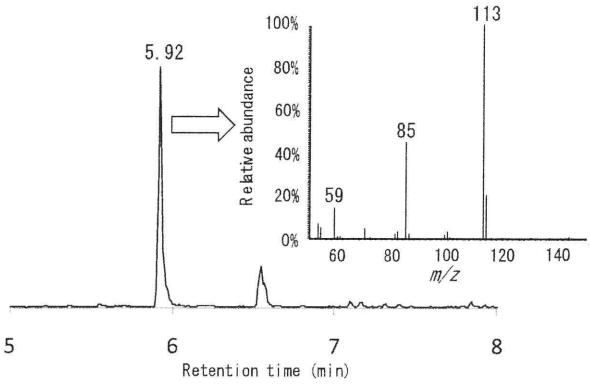
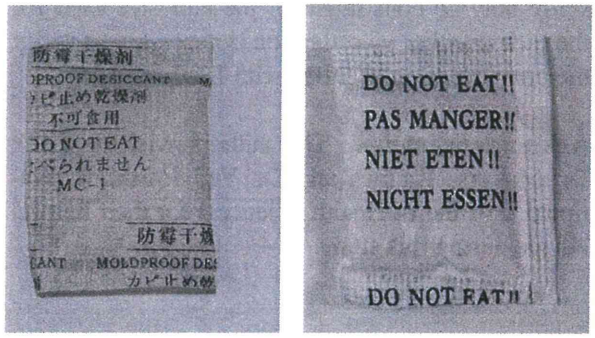


Fig. 2. Mass Chromatogram of DMF Obtained from the Desiccant Sample (S6, selected ion monitoring mode: m/z = 113) and Mass Spectrum of DMF Obtained by Scan Analysis (scan mode: m/z = 50–350).



(a) (b)

Fig. 3. Photographs of Sachets that DMF Was Detected a: S6, b: S20–2.

glish), "PAS MANGER" (French), "NIET ETEN" (Dutch), and "NICHT ESSEN" (German) (Fig. 3b); however, no note was printed in Japanese. The same notes were also printed on the sachet of S20-1; however, the font type of the note was different from that used for S20-2, and DMF was not detected in S20-1.

On the other hand, in the case of the sandal product sample (P6), for which DMF was detected in the enclosed desiccant sample, DMF was detected in the three product part samples which were the sole surface (P6-1), the mesh cloth strap inside (P6-2), and the inside of the synthetic leather strap (P6-3) (Fig. 4a). The concentrations of DMF in P6-1, 2, and 3 were 0.21, 0.11, and 0.14 mg/kg, respectively (Table 4). Although DMF was detected in the desiccant sample (S20-2), it was not detected in the product samples of sports shoes (P20-1 and 2) enclosed with the sachet (S20-2). Furthermore, DMF was not detected in any other samples. The DMF concentrations detected in this study exceeded

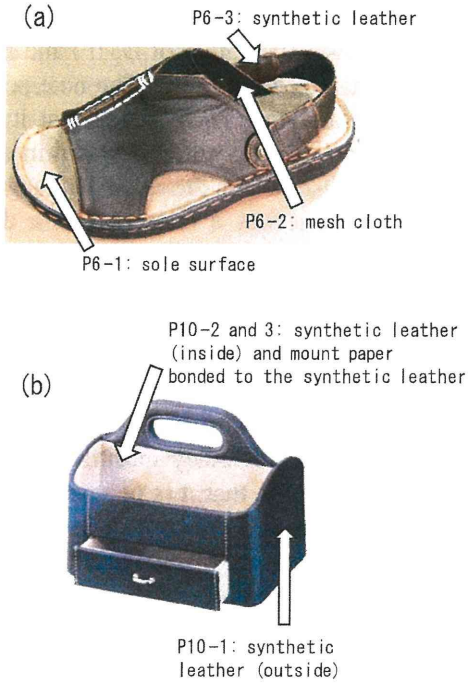


Fig. 4. Photographs of Sandal (a: P6) and Rack (b: P10) that DMF Was Detected

the EU regulated value (0.1 mg/kg), and the concentration of one desiccant sample (S6) was exceeded 1.0 mg/kg which showed a strong reaction in the patch tests.[7]

The detection of DMF was expected in S11-1, S11-2, S20-1, P20-1, and P20-2; however, DMF was not detected in these samples. It has been reported that DMF concentration decreases to around one-tenth after two weeks of storage at room temperature without any wrapping.[24] It was indicated that DMF was released from such products via evaporation, and it adsorbed on other product.[10] Thus, the low concentrations of DMF observed in this study may be attributed to the evaporation of DMF because the desiccant and/or product were produced a long time ago. Furthermore, a small amount of DMF may be used in the desiccants and/or products distributed in the Japanese market. Information regarding the samples investigated in this study was insufficient; hence, we cloud not conclude whether the DMF detected in this study was used intentionally.

In Spain, 37 sachets enclosed with clothes, accessories, footwear, and furniture were collected, and the desiccants contained in the sachets were analyzed by GC/MS.[19] It was reported that the concentrations of DMF detected in 27 samples were

0.239 to 2640 mg/kg (more than half of these samples contained over 100 mg/kg of DMF) and a certain sachet contained DMF only. The note printed on the sachet containing DMF stated that it only contains silica gel.[19] "Do not eat" was printed on the sachet of S20-2 in non-Japanese languages in this study. Thus, it was a concern that a desiccant sachet containing DMF enclosed with products distributed in the Japanese market may not have a label stating "mold-proof agent." Furthermore, in EU countries, the detection frequencies of DMF increase in the winter[13] and contact dermatitis related to DMF is induced at very low concentrations if a person has been previously sensitized to DMF. Therefore, we conclude that it is necessary to analyze more samples to prevent contact dermatitis related to DMF in Japan.

Concentrations of Other Compounds in the Samples

DBM was detected in the desiccant sample (S10), and its concentration was very high (720 mg/kg). Furthermore, DBM was detected in the three product samples that were parts of synthetic leather located on the outside and inside surfaces of rack (P10-1 and 2) and mount paper (P10-3) bonded to the synthetic leather inside the rack (Fig. 4b). DBM concentrations of P10-1, 2, and 3 were 29, 340, and 440 mg/kg, respectively. DBF was also detected in S10, P10-2, and 3, respectively. An adhesive containing DBM may be used for bonding synthetic leather and mount paper; hence, DBM may evaporate from the adhesive, and it may be adsorbed on the surface of the synthetic leather and the desiccant. Although DBF may be generated from DBM, the generation process has not been determined thus far. DEF, DMM, and DEM were not detected in any of the samples; this result is similar to the results of a previous study on dermatitis related to furniture containing DMF.[25] Occupational contact dermatitis related to DBM at a factory using adhesives was reported,[16] and the cross-reaction of DBM and DMF is unknown; hence, it is necessary to investigate the cross-reaction of DMF with DBM.

In conclusion, desiccants in the sachets and products (footwear and rack) enclosed with the desiccant sachets were analyzed to determine the concentrations of DMF and several fumaric and maleic acid diesters (DEF, DBF, DMM, DEM, and DBM). The product samples were sorted by material. A total of 21 desiccant samples and 18 product samples (seven footwear products and one rack product) were analyzed. DMF was detected in the range of 0.11–2.3 mg/kg in the two desiccant samples and three product samples (from one product). The DMF concentrations detected in this study exceeded the EU regulated value (0.1 mg/kg), and the concentration of one desiccant sample (S6) was exceeded 1.0 mg/kg which showed a strong reaction in the patch tests in a previous study. The note printed on one of the sachet containing DMF read "mold-proof desiccant, do not eat"; in contrast, the note on the other sachet merely read "do not eat." DMF has strong sensitization and irritation activities; hence, it is necessary to analyze more samples to prevent contact dermatitis related to DMF in Japan. DBM was detected in the rack product and the desiccant enclosed with this rack; its concentration ranged from 29 to 720 mg/kg. The DBM detected in this study may be constituent of the adhesive used for the rack. Further investigation is necessary to verify the cross-reaction of DBM with DMF.

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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

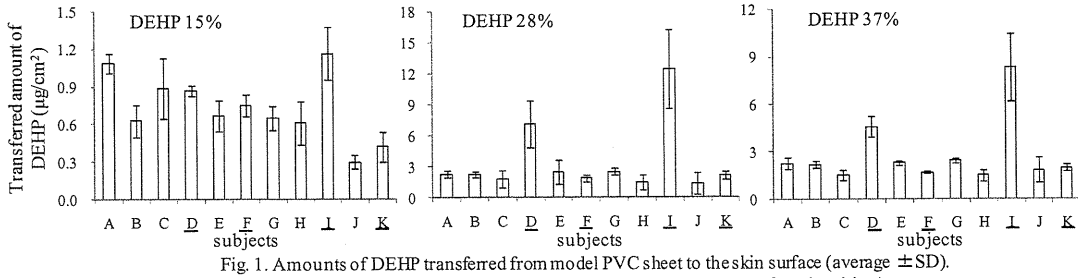

Fig. 1. Amounts of DEHP transferred from model PVC sheet to the skin surface (average ±SD).
(Alphabetical order means in order of age and underline means female subject)

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.

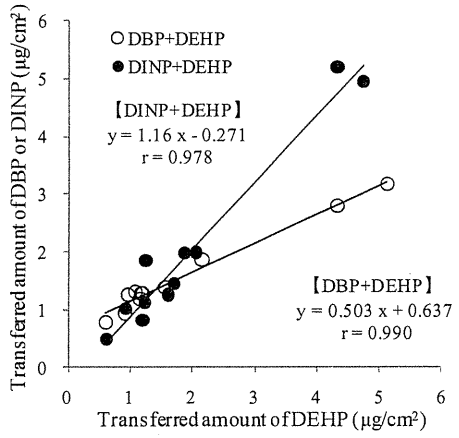

Fig. 2. Relationships between amount of DEHP and DBP or DINP transferred from mixture sheet.

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Osteoblast Compatibility of Calcium-Incorporated Ti-Zr-Nb Alloys

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Summary

Ca was incorporated into the surface of the Ti-Zr-Nb alloys in the $CaCl_2$ treatment and $Ca(OH)_2$ treatment following the NaOH treatment. According to the XRF analysis, the larger amount of Ca was incorporated in the $Ca(OH)_2$ treatment than in the $CaCl_2$ treatment. The Ca-incorporated Ti-Zr-Nb alloys were evaluated in the cytotoxicity and osteoblast compatibility *in vitro*. The untreated Ti-6Al-4V showed cytotoxicity, but Ti-Zr-Nb alloys did not show cytotoxicity with or without treatment. The Ca-incorporated Ti-Zr-Nb alloys showed good osteoblast compatibility. In Ti-Zr, Ti-Zr-4Nb, pure Ti and Ti-6Al-4V, the $Ca(OH)_2$ treatment enhanced the osteoblastic differentiation than the $CaCl_2$ treatment.

Introduction

Ti alloys are used as materials of bone fixations and artificial joints in orthopedics. Ti-6Al-4V is used generally, but it is known that vanadium ions have strong cytotoxicity. We reported Nb ions promoted the differentiation of osteoblasts[1-3] and Ti-Zr-Nb alloys had good mechanical property and osteocompatibility *in vitro* and *in vivo*.[4,5] On the other hand, the materials with a high apatite-forming ability in a simulated body fluid are expected to bond directly with the bone *in vivo*.[6] Ti alloys which have been alkali- and heat-treated to improve the apatite-forming ability have been applied clinically. Furthermore, the Ca incorporation into the alkali-treated Ti alloys have been attempted to achieve an even higher apatite-forming ability. We examined the Ca incorporation into the Ti-Zr-Nb alloys and confirmed Ca-incorporated Ti-Zr-Nb alloys had a high apatite-forming ability. In this study, we evaluated the cytotoxicity and osteoblast compatibility of the Ca-incorporated Ti-Zr-Nb alloys.

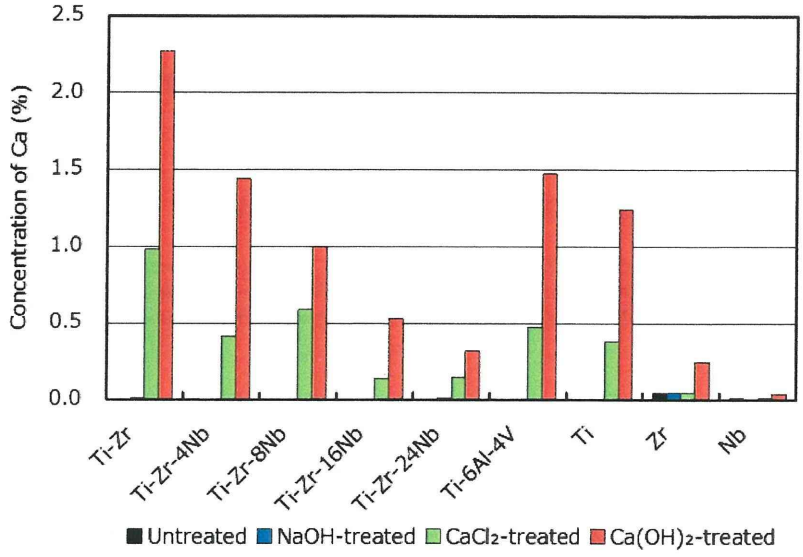

Fig. 1 Calcium amount of the surface of the samples by XRF using the fundamental parameter method.

Materials and Methods

Ti-Zr. Ti-Zr-4Nb. Ti-Zr-8Nb. Ti-Zr-16Nb and Ti-Zr-24Nb were used in this experiment. In addition. pure Ti. Zr and Nb were used as structural elements of the Ti-Zr-Nb alloys and Ti-6Al-4V was used as a comparison. All samples were 14.0 mm in diameter and 1.0 mm in thickness.

The samples were soaked in NaOH aqueous solution at 60°C for 24 hr (NaOH treatment). Subsequently. the NaOH-treated samples were soaked in $CaCl_2$ aqueous solution ($CaCl_2$ treatment) or $Ca(OH)_2$ aqueous solution ($Ca(OH)_2$ treatment) at 60°C for 24 hr. The Ca amount of the $CaCl_2$-treated and $Ca(OH)_2$-treated surfaces was measured by X-ray fluorescence spectrometry (XRF) using the fundamental parameter method.

The cytotoxicity of the samples was evaluated by the colony formation assay with direct contact using Chinese hamster lung fibroblast V79 cells.[7] The osteoblast compatibility of the samples was evaluated with normal human osteoblasts (Cambrex Bio Science Walkersville. Inc.) cultured in α-modified minimum essential medium supplemented with 10% fetal bovine serum and 5 mM disodium β-glycerophosphate. The osteoblast proliferation was estimated with the cell number measured by WST-8 assay.[7] The osteoblastic differentiation was estimated with the alkaline phosphatase (ALP) activity measured using p-nitrophenylphosphate as a substrate.[7]

Results and Discussion

All Ti alloys and pure Ti incorporated almost double amount of Ca in the $Ca(OH)_2$ treatment compared with the $CaCl_2$ treatment. Particularly. Ti-Zr. Ti-Zr-4Nb. pure Ti

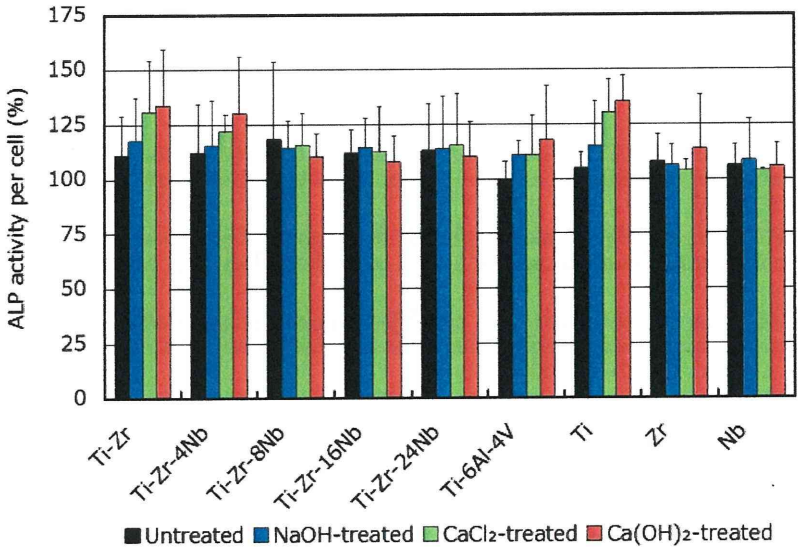

Fig. 2 Osteoblastic differentiation on the samples estimated with the ALP activity per cell measured using p-nitrophenylphosphate as a substrate.

and Ti-6Al-4V incorporated large amount of Ca. Pure Zr incorporated Ca only in the $Ca(OH)_2$ treatment. and pure Nb did not incorporate Ca in both treatments (Fig. 1).

The untreated Ti-6Al-4V showed cytotoxicity. but treated Ti-6Al-4V showed less cytotoxicity. Other samples did not show cytotoxicity with or without treatment. Compared with on the untreated Ti-6Al-4V. the cell number of osteoblasts increased on other samples. This result was supported by the cytotoxicity of the samples (data not shown).

The ALP activity of osteoblasts cultured on all other samples increased compared with on the untreated Ti-6Al-4V. Additionally. in Ti-Zr. Ti-Zr-4Nb. pure Ti and Ti-6Al-4V. the ALP activity of osteoblasts was promoted in order of by the NaOH. $CaCl_2$ and $Ca(OH)_2$ treatment (Fig. 2). In the previous study. the apatite-forming ability of Ti-Zr. Ti-Zr-4Nb. pure Ti and Ti-6Al-4V increased in order of by the NaOH. $CaCl_2$ and $Ca(OH)_2$ treatment. These results implied the osteoblastic differentiation was enhanced on the materials with a high apatite-forming ability.[8]

Conclusions

The Ca-incorporated Ti-Zr-Nb alloys showed good osteoblast compatibility. In Ti-Zr. Ti-Zr-4Nb. pure Ti and Ti-6Al-4V. the $Ca(OH)_2$ treatment enhanced the osteoblastic differentiation than the $CaCl_2$ treatment. The large amount of Ca incorporation would induce good osteoblastic differentiation cultured on the materials.

Acknowledgment

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Toxicological Studies of Nano-Suspensions of Silica, Silver and Zinc Oxide

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Summary

Nano-suspensions of silica, silver and zinc oxide were subjected to the cytotoxicity test, the chromosomal aberration test, and the 13-week repeated dose test for their safety evaluation. Silver showed the strongest cytotoxicity among the three. Only zinc oxide induced chromosome aberrations. In the *in vivo* test, zinc oxide caused inhibition of the normal body weight increase, increase in the relative lung weight, and pulmonary fibrosis. We propose the three tests as a candidate of a primary screening test battery for safety evaluation of nanomaterials (NMs).

Introduction

Development in the field of nanotechnology has brought NMs closer to us day by day and at the same time toxicological concerns of NMs have been growing. NMs are expected for a variety of applications not only in industry, but also in the field of medicine such as drug delivery system, gene transfer vectors, and scaffold for cell culture in regenerative medicine. NMs are new materials with unknown characteristics and we need to consider the safety of their use. In the present study, we performed the *in vitro* and *in vivo* toxicological studies of three NMs to evaluate their safety.

Materials and Methods

A silica sol (amorphous SiO_2 17.8%, particle size 10-20 nm) and zinc oxide (40% in water, NanoTek® ZH1121W, Alfa Aesar) were commercially available. Silver powder (Sigma-Aldrich, particle size <100 nm) was suspended in water by ultrasonics, filtered through a 0.45 μm PVDF filter, and then the concentration of silver in the filtrate was determined by capillary electrophoresis. Cytotoxicity of the NMs was determined by

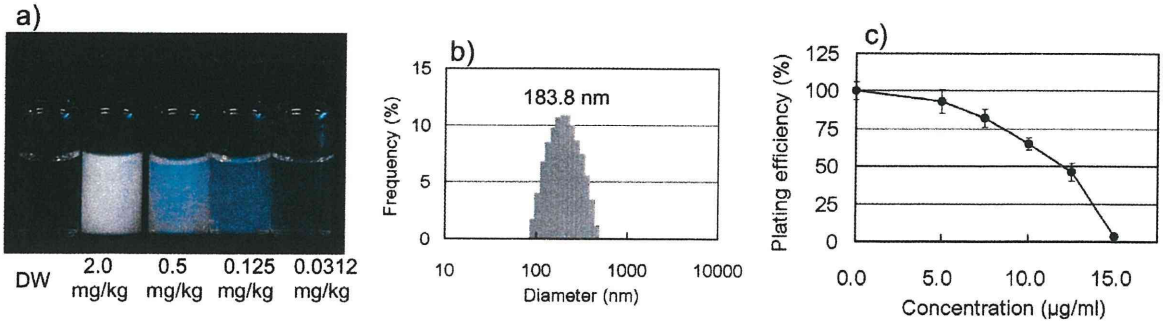

Fig. 1 Zinc oxide; a) Suspensions prepared for the each dose of the in vivo test, b) particle size distribution of the suspension, and c) the result of the cytotoxicity test in that CHL cells were treated with the suspension for six days. Values are expressed as mean ± SD for four wells.

Table 1. Chromosome aberration test of zinc oxide

Treat-ment (h)	Mass conc. (μg/ml)	Polyploid (%)	Cells with chromosome aberrations (%)*					
			ctg	ctb	cte	csb	cse	total
24	0	1	0	1	0	0	0	1
	2.5	2	0	1	0	0	0	1
	5	1	1	0	0	0	0	1
	10	1	2	1	5	0	0	7
	20	1	3	14	32	0	0	38
48	0	0	0	0	0	0	0	0
	2.5	1	0	0	0	1	0	1
	5	3	0	0	0	0	0	0
	10	2	0	0	0	1	0	1
	20	5	4	9	7	0	0	17

*Structural chromosome aberrations: ctg, chromatid gaps; ctb, chromatid breaks; cte, chromatid exchanges; csb, chromosome breaks; cse, chromosome exchanges.
Red figures indicate positive responses.

the colony formation assay using a Chinese hamster cell line CHL[1]. The chromosome aberrations (CAs) were investigated in CHL cells treated with the NMs for 24 or 48 h[1]. In the 13-week repeated dose test, six rats per group were intratracheally sprayed with the NMs in a 0.2 ml portion of test suspensions once a week for 13 weeks. One week after the last administration, rats were subjected to an autopsy and lungs were fixed for histopathological examinations. We used ANOVA to test the significance of differences between treated groups and their controls in data on body weight, relative organ weight, and hematology with post hoc comparisons made using the Dunnett test.

Results

The mean diameter of silica, silver, and zinc oxide in suspension was 54.2, 159.2, and 183.8 nm, respectively. The 50% growth inhibition concentra- tion of them was

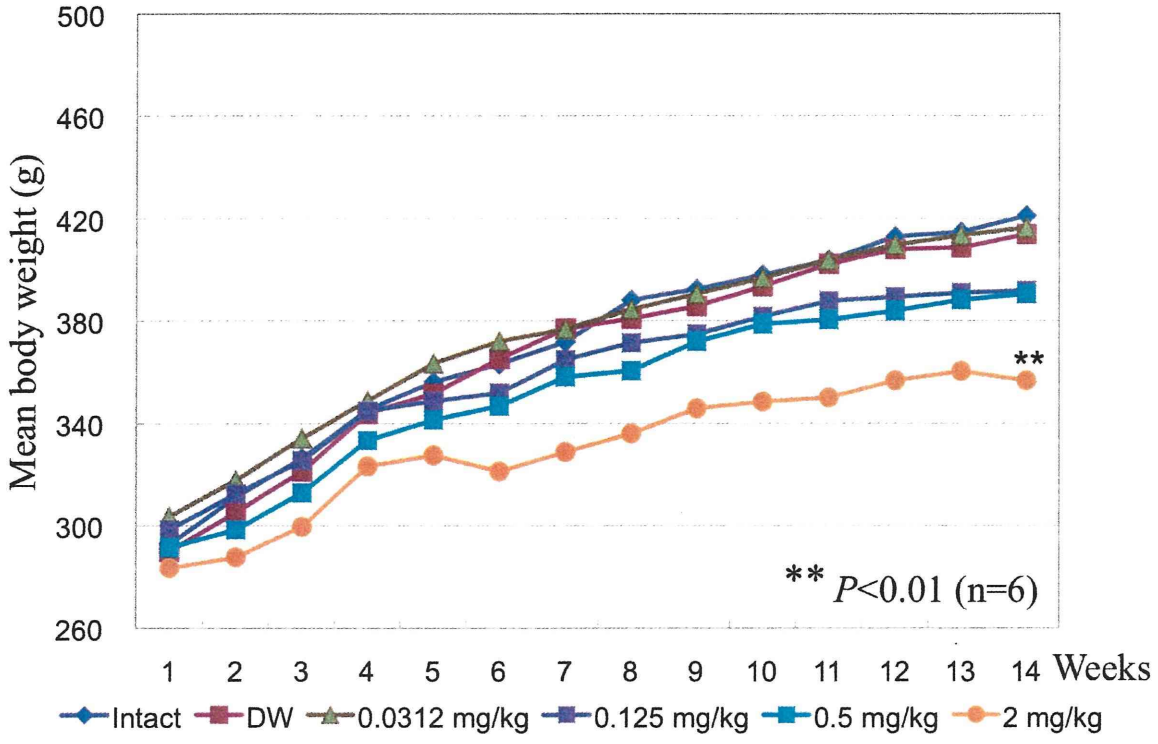

Fig. 2 Body weight curve of rats treated with zinc oxide in the 13-week intratracheal repeated dose test. A significant inhibition in the normal increase of body weight was observed at the highest dose of 2 mg/kg.

153.5, 1.25, and 12.0 μg/ml (Fig. 1), respectively, in the cytotoxicity test. Only zinc oxide induced structural CAs (Table 1).

In the 13-week repeated dose test, the normal increase in mean body weight was significantly inhibited at 2 mg/kg of zinc oxide (Fig. 2), although no clinical signs were found in rats treated with all test suspensions.

Histopathologically, granulomatous inflammation with foamy cells in the alveoli or surrounding the bronchiole and perivascular cell infiltration were detected as common lesions in rats treated with three test suspensions. The lesions increased in their intensities with dose, and similarly distributed in all the lobes indicating that they were evenly exposed to the NMs.

On the other hand, alveolar epithelial hyperplasia was prominent in rats treated with silver than with silica.

Microgranuloma and aggregation of foamy cells were found in the mediastinal lymph node at higher doses of silica and silver, indicating that macrophages with NMs phagocytosed moved to lymph node through lymphatic vessels. Zinc oxide markedly induced proliferation of alveolar/bronchial epithelium and mucinous cells in bronchus and fibrosis at two highest doses. NOEL of silica, silver, and zinc oxide was less than 0.06, 0.004, and less than 0.0312 mg/kg, respectively.

Conclusions

The three tests performed in the present study showed characteristic results of

each nano-suspension. It suggests that they may be a set of useful tools to screen NMs for safety evaluation, although further studies are needed to confirm the fact.

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打ち抜き試験による超高分子量ポリエチレンの機械特性評価

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Mechanical properties of UHMWPE evaluated by tensile punch test.

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Abstract

Tensile punch test has a wide range of applications due to the small specimen size required and the simplicity of the test method. Although there is an ASTM standard for testing UHMWPE, very strict requirements for jig and specimen dimensions makes the test method less than practical for common use. In this study, we investigated the effect of specimen thickness on the test results in order to develop a more practical and simpler test method.

Direct compression molded UHMWPE with a thickness ranging between 0.49 mm to 0.56 mm was fixed in a flat jig and punched with a hemispherical punch. From the obtained stress-strain curves, stiffness, initial peak load, ultimate displacement and work to failure were calculated.

Initially, excessive or insufficient compression force for fixation of the specimen was found to lead to unreliable results. Controlling the fixation torque of the fixation screws or using thickness gauges with the adequate thickness as a spacer effectively overcame this problem.

Secondly, calculated mechanical parameters were found to be affected by specimen thickness, but there were strong correlations between specimen thickness and each parameter. Therefore, it was considered that the influence of specimen thickness could be managed statistically, if multiple homogeneous specimens were available.

Finally, in order to confirm the effectiveness of this test method, specimens soaked in squalene were also tested. The results showed reduction of stiffness, which was consistent with a previous report. This study showed that the tensile property of UHMWPE can be estimated by the tensile punch test without strict control of specimen thickness as long as multiple homogeneous specimens are available.

Key words : UHMWPE, tensile property, punch test, tensile test.

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