2.2.2. Heat treatment

The PVC sheets were kept at temperatures of 4, 37 and 60 °C for 1 week, 2 weeks, 1 month, 2 months and 3 months. The positive control was a PVC sheet kept at 100 °C for 25 days.

2.2.3. Optical irradiation

The embossed side is the outer surface of a blood bag. Some PVC sheets were irradiated with visible light using fluorescent lamp placed at a distance of 75 cm. On the other hand, the other PVC sheets were irradiated UV-ray using UV germicidal lamp placed at distance of 60 cm (UV intensity: 52.5 $\mu W/cm^2$) in clean-bench. These PVC sheets were irradiated for 1 week, 2 weeks, 1 month, 2 months or 3 months. After irradiation, the samples were stored in the shade. The positive control for visible light irradiation was a PVC sheet exposed to sunlight for approximately 1 year. The positive control for UV irradiation was a PVC sheet irradiated with a 254-nm UV lamp at a distance of 3 cm for 25 days. PVC tubing cut to a length of 10 cm was irradiated with a 254-nm UV lamp at a distance of 3 cm for 14 days.

2.3. GC–MS

A Hewlett-Packard HP 6890 Series GC system equipped with an auto-injector (Agilent Technologies, Palo Alto, CA) and a JMS700 spectrometer (JEOL, Tokyo, Japan) were used for gas chromatography-mass spectrometry (GC–MS). Chromatographic separations were performed with a BPX-5 fused silica capillary column (25 m × 0.22 mm i.d., film thickness: 0.25 μm, SGE Japan, Kanagawa, Japan).

A sample (2 μl) was injected in the pulsed splitless mode. The injector temperature was 260 °C. Helium was used as the carrier gas at a flow rate of 1 ml/min. The column temperature was programmed from 120 to 300 °C (held for 2 min) at a rate of 10 °C/min. The electron impact (EI)-mass spectrum was recorded at 70 eV for qualitative analysis, and ions of *m/z* 149.024 (DEHP) and 153.049 (DEHP-d_4) were selected as quantitative ions in selective ion monitoring (SIM) analysis (resolution = 5000) using the lock and check method of calibrating standard ions (*m/z* 168.989 of PFK). Quantitative analysis of each sample was repeated five times for calibration curves and twice for the other samples. The preparation of calibration curves and the calculation of quantitative data were performed using computer software TOCO, version 2.0 (total optimization of chemical operations), applying the function of mutual information (FUMI) theory (Hayashi and Matsuda, 1994; Hayashi et al., 1996, 2002; Haishima et al., 2001).

2.4. Migration test

The migration of DEHP from PVC sheets was examined in 5 ml of Sandimmun® prepared according to the instructions on the package insert. PVC sheets, which were irradiated or heat-treated were kept in test tubes and extraction was carried out by shaking at room temperature for 1 h. A 0.1 ml aliquot of the extract was pipetted into another test tube, and 2 ml of distilled water and 5 ml of diethyl ether containing 50 ng/ml DEHP-d_4 were added. The mixture was then subjected to extraction with shaking for 10 min. After centrifugation at 3000 rpm for 10 min, the organic phase was collected and dehydrated with anhydrous sodium sulfate, and subjected to GC–MS analysis.

PVC tubing cut to 10 cm length was used in the DEHP migration test, and filled with Sandimmun® (tube length, 8 cm; capacity, 0.285 cm^3; and surface area, 5.35 cm^2). The tubing was subjected to extraction with shaking at room temperature for 1 h. The extract was transferred into another test tube and treated in the same manner as that for PVC sheets.

2.5. Determination of DEHP compounds in PVC sheet by GC–MS

A PVC sheet sample (0.02 g) was dissolved in 20 ml of THF by soaking overnight at room temperature. A 0.1 ml aliquot of the solution was pipetted out and diluted with 2.0 ml of diethyl ether. A 0.1 ml aliquot was obtained, mixed with 50 ng/ml DEHP-d_4 (1 ml) and diethyl ether (8.9 ml), and then analyzed by GC–MS.

2.6. Analysis of surface structure

2.6.1. Infrared spectrometry

A JIR-SPX 200 (JEOL, Tokyo, Japan) was used for FT-IR spectroscopy coupled with attenuated total reflection (ATR) analysis. To analyze the PVC sheets, we used a germanium crystal, and the incidence angle was set at 45°.

2.6.2. Electron spectroscopy for chemical analysis

ESCA measurements were performed using an ESCA-3200 (Shimadzu, Kyoto, Japan). Only the inner side of the blood bag was measured for the heat treatment group and the visible light irradiation group, whereas both sides of the blood bag were measured for the UV irradiation group.

2.6.3. Static angle of contact

A solution of Sandimmun®, prepared according to the instructions on the package insert, was added dropwise to PVC sheets. After 120 s, the width and height of the droplet were measured with a G-1-1000 (ERMA, Tokyo, Japan). The static angle of contact with Sandimmun® was computed as follows:

$$L^2 = \left(\frac{w}{2}\right)^2 + (L-h)^2$$

$$\sin\delta = \left(\frac{w/2}{L}\right)$$

L is radius of droplet (mm); w is width of droplet (mm); h is height of droplet (mm); and (δ) static angle of contact.

Only the inner side of the blood bag was measured for the heat treatment group and the visible light irradiation group, whereas both sides of the blood bag were measured for the UV irradiation group.

2.7. Tensile test

A PVC sheet (0.7 cm × 3 cm, center width: 0.4 cm, thickness: 0.04 cm) was used as the sample (Fig. 1). Measurements were performed using an Autograph AG-20 kNG (Shimadzu, Kyoto, Japan) at a speed of 40 mm/min.

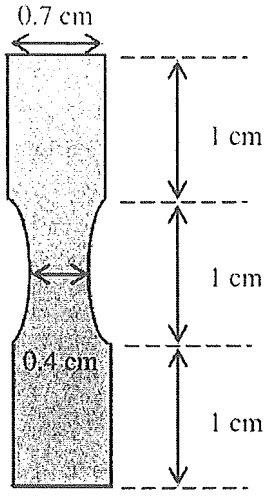

Fig. 1. The PVC sheet used in the tensile test A PVC sheet: 0.7 × 3 cm, center width: 0.4 cm, thickness: 0.04 cm.

3. Results and discussion

3.1. Determination of DEHP released from PVC products by GC–MS

First, the background was analyzed in order to examine the accuracy of the GC–MS method. When 50 ng/ml DEHP-d_4 with diethyl ether solution was used as the internal standard, 0.93 ± 0.31 ng/ml DEHP (n = 5) was detected in the internal standard. The DEHP background peaks in the internal standard solution were trace levels (S_0). In addition, the precision (SD) was expressed as SD_0. The limit of detection (LOD) and the limit of quantification (LOQ) of DEHP were calculated using S_0 and SD_0; LOD ($S_0 + 3 \times SD_0$) and LOQ ($S_0 + 10 \times SD_0$) were 1.9 and 4.0 ng/ml, respectively. A calibration curve was obtained for the peak ratio of DEHP to DEHP-d_4 versus the DEHP concentration level. The response was found to be linear in the validated range with a correlation coefficient (r) exceeding 0.999. Furthermore, the 95% confidence interval calculated by TOCO was sufficiently narrow to determine the amount of DEHP released from the PVC products. We found that this GC–MS method could be used for DEHP analysis with high accuracy.

The levels of DEHP that migrated from the PVC sheets were then determined, and the time course is shown in Fig. 2. Heat treatment and optical irradiation were each performed for 1 week, 2 weeks, 1 month, 2 months, and 3 months. At 2 months, the levels of DEHP migrating into Sandimmun® were slightly decreased by heat treatment and visible light irradiation, however no remarkable change was observed between the treatments, or between those treatments and their respective positive controls. The level of DEHP migration from the heat-treated PVC sheets has decreased the temperature-dependent. The most possible factor for the temperature-dependent, the sublimation/vaporization was occurred by heat treatment in

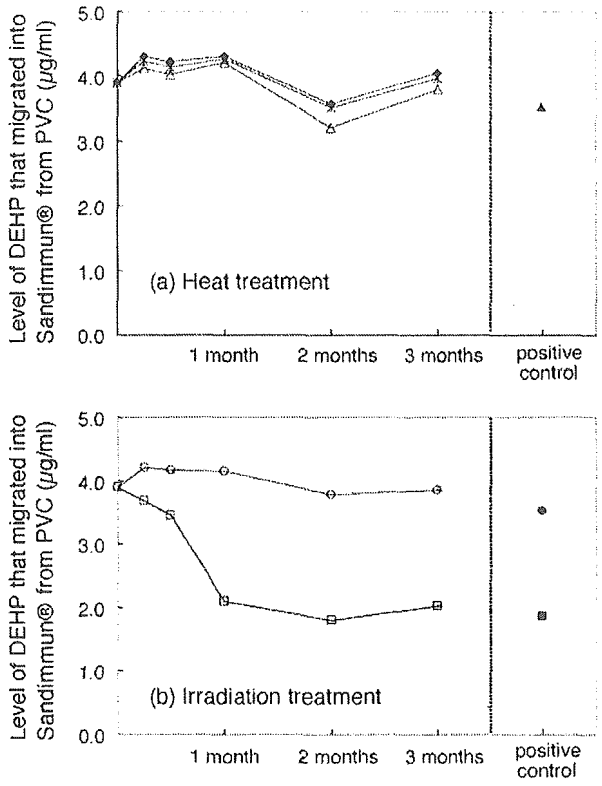

Fig. 2. Level of DEHP migrating into Sandimmun® injection from PVC sheet samples. (a) Heat treatment: PVC sheets were kept at 4 °C (♦); 37 °C (*) and 60 °C (△). Heated positive control: PVC sheets were kept at 100 °C for 25 days (▲). (b) Irradiation treatment: PVC sheets were irradiated with visible light (○) and UV (□). The visible irradiated positive control (●) was irradiated with sunlight for approximately 1 year. The UV-irradiated positive control (■) was irradiated with a UV lamp at 254 nm (3 cm, 25 days). The quantitative data were performed using computer software TOCO, Version 2.0 (Total Optimization of Chemical Operations), applying the function of mutual information (FUMI) theory.

PVC sheet. On the other hand, the significant change on migration was observed in UV-irradiated PVC sheets. The levels of DEHP migrating from the PVC sheets showed a time-dependent decrease in the UV irradiation group. At 1 month after UV irradiation, the level of DEHP migrating from the PVC sheet was reduced to approximately half that of the negative control. No significant change was observed thereafter, even if irradiation was continued. In addition, the level of DEHP migrating from the PVC sheet after UV irradiation over 3 months was not different from that of the positive control. We hypothesized that the reduction by half of the DEHP level has caused by UV-irradiated sides. We thought the UV-irradiated side (outer surface) of PVC sheet induces suppression in DEHP migration, and inner surface of PVC sheet dose not influence in migration. In order to confirm this hypothesis, we examined the PVC tubing that was able to distinguish outer and inner surface. The outer surface of the PVC tubing was subjected to strong UV irradiation. Subsequently, the level of DEHP migrating from the inner surface of the PVC tubing was determined. As a result, it was found that the level of DEHP released from the inner surface of the PVC tubing was almost the same as that of the negative control PVC tubing. It was concluded that the inner surface of the PVC tubing was not influenced by UV irradiation from the outside, since there was no change in the levels of DEHP released when compared with the control.

3.2. DEHP content examination

The DEHP content in the PVC sheets subjected to heat treatment or optical irradiation was determined. No significant difference in the DEHP content was found between the heat treatment groups and the visible light irradiation group. The positive controls of the two groups had almost the same DEHP content. On the other hand, the DEHP content in the UV-irradiated PVC sheets decreased slightly with time (Table 1). The most possible factor for the time-dependent, the sublimation/vaporization was occurred by UV-irradiated PVC sheet.

The rate of decrease in the DEHP content of the UV-irradiated PVC sheet was not equivalent to that of the level of migration. Therefore, the level of the suppression of DEHP migration was more remarkable than that of decreasing-content of DEHP.

3.3. Surface analysis

3.3.1. Surface analysis by FT-IR

FT-IR with ATR spectra was obtained from PVC sheets subjected to optical irradiation or heat treatment. Fig. 3a shows a characteristic absorption band at 635 cm^{-1}, due to C–Cl stretching vibration from PVC. We also observed absorption due to C–H from the aromatic compound and the carbonyl group from DEHP at 742 and 1720 cm^{-1}, respectively. Furthermore, an absorption band due to the alkane C–H bond from PVC and DEHP was found at nearly 1250 cm^{-1}.

Table 1
DEHP content in PVC sheet samples (w/w, %)

	4 °C	37 °C	60 °C	Visible light	UV light
1 week	31.2 ± 0.09	31.9 ± 0.61	33.2 ± 0.35	34.1 ± 1.65	36.2 ± 2.14
2 weeks	32.6 ± 0.44	33.3 ± 0.25	31.7 ± 0.03	34.8 ± 1.36	34.7 ± 3.32
1 month	32.9 ± 0.39	34.2 ± 0.45	35.0 ± 1.11	34.1 ± 0.85	33.7 ± 5.11
2 months	33.2 ± 0.12	33.9 ± 0.25	33.3 ± 0.43	32.8 ± 0.18	29.4 ± 0.63
3 months	33.8 ± 0.04	32.9 ± 0.26	30.9 ± 0.34	29.5 ± 4.05	27.1 ± 0.37

Negative control samples: 36.0 ± 2.60%; positive control samples subjected to heat treatment: 32.4 ± 0.45%; positive control samples irradiated with visible light: 32.6 ± 0.70%; positive control samples irradiated with UV: 30.8 ± 0.53%.

The FT-IR spectra of the heated-treated and visible light-irradiated PVC sheets were almost the same as that of the negative control. The spectrum was rectified using software because the ATR spectrum depended on the wavelength to calculate the areas of the characteristic absorption bands for DEHP or PVC. When the area ratios for the heat-treated or visible light-irradiated PVC sheets were compared with those of the negative control, no clear change was seen. On the other hand, as shown in Fig. 3b–f, the UV-irradiated PVC sheets were found to exhibit broadened absorption bands with time. These results led us to hypothesize that UV irradiation caused a change in the surface structure. The FT-IR spectrum of the non-UV-irradiated side was the same as that of the negative control, indicating that there was no change in the surface structure.

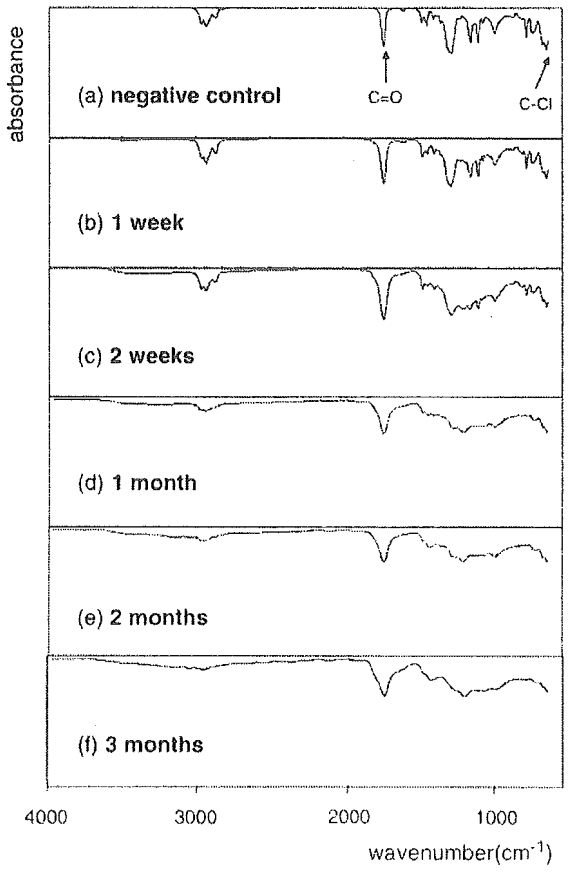

Fig. 3. FT-IR spectra of UV-irradiated and negative control PVC sheets FT-IR spectra of PVC sheets: negative control (a); and those irradiated with UV light for 1 week (b); 2 weeks (c); 1 month (d); 2 months (e) and 3 months (f).

3.3.2. *Surface analysis by ESCA*

Surface analysis of the PVC sheets was carried out and carbon, oxygen, chlorine and silicon were found on the sheet surface. As shown in Fig. 4a, the surface structure of the PVC sheets was not influenced by heat treatment or visible light irradiation because the composition ratio was maintained. On the other hand, in the UV-irradiated PVC sheets (Fig. 4b), the chlorine content was decreased and the oxygen content was increased with time. For the inner surface of the UV-irradiated PVC sheets, the composition ratio was hardly changed compared to the negative control in the period of 1 week to 1 month. However, after 2 months, the composition ratio was not changed at all compared with the negative control.

3.3.3. *Surface analysis by static angle of contact measurement*

In order to evaluate the affinity of the PVC sheets and the actual concentration of the Sandimmun® injection, we measured the static angle of contact. The static angle of contact was 37.1 ± 0.84 and 53.4 ± 0.93° for the outer and inner surfaces of the non-treated PVC

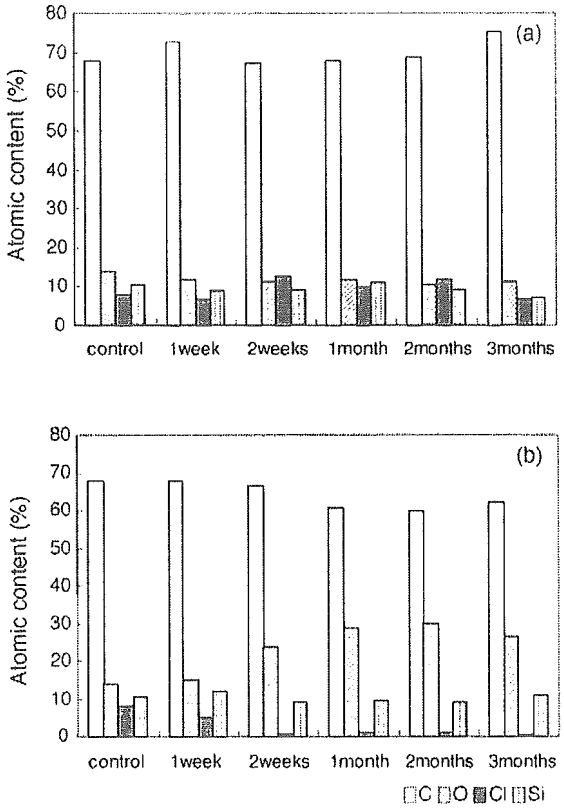

Fig. 4. Elemental analysis of PVC sheets by ESCA: (a) typical bar graph of PVC sheet samples subjected to heat treatment or visible light irradiation, and negative control and (b) bar graph of PVC sheet sample subjected to UV irradiation.

sheets, respectively. We hypothesize that the difference in the contact angle is due to differences in the embossed processing of the outer and inner surfaces (Fig. 5c (control) and Fig. 5b (control)). As shown in Fig. 5, the static angle of contact of the PVC sheets using the Sandimmun® injection as the wetting agent was not affected by either heat treatment or visible light irradiation (Fig. 5a). On the other hand, the static angle of contact of the inner surface of the UV-irradiated PVC sheets was decreased with time (Fig. 5b). In addition, the static angle of contact of the inner surface of the UV-irradiated positive control PVC sheets was almost the same as that of the inner surface of the PVC sheets UV-irradiated for 3 months. On the other hand, the static angle of contact of the outer surface of the UV-irradiated PVC sheets was increased markedly from the control to 3 months (Fig. 5c).

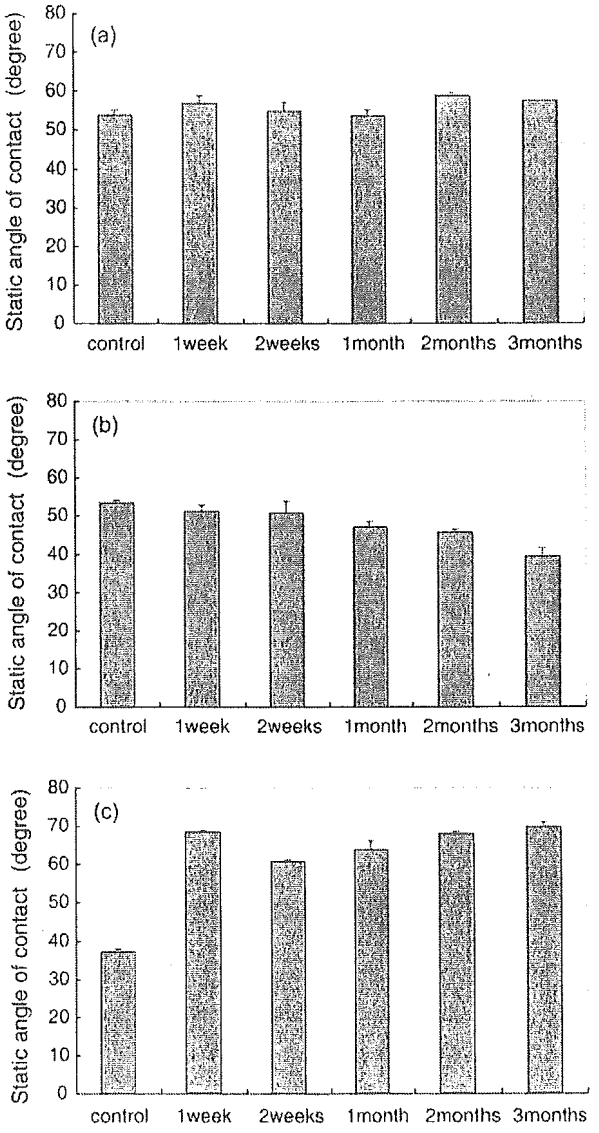

Fig. 5. Static angle of contact of PVC sheet samples Sandimmun® injection was used to determine the static angle of contact of PVC sheets subjected to heat treatment or optical irraditation. Typical bar graph of PVC sheet samples subjected to heat treatment or visible light irradiation. Bar graph of PVC sheet samples subjected to UV irradiation. The static angle of contact obtained by the inner surface of PVC sheet. Bar graph of PVC sheet samples subjected to UV irradiation. The static angle of contact obtained by the outer surface of PVC sheet.

Therefore, surface structure of the heat-treated PVC sheets and visible light irradiated PVC sheets did not change with time. On the other hand, surface structure of the UV-irradiated PVC sheets was markedly changed.

Table 2
Maximum force to break the PVC sheets for tensile test

	4 °C	37 °C	60 °C	Visible light	UV light
1 week	37.03 ± 1.68	36.54 ± 1.15	37.64 ± 2.01	36.63 ± 1.32	37.59 ± 0.86
2 weeks	36.12 ± 1.34	36.92 ± 0.52	36.20 ± 0.80	36.97 ± 1.12	36.28 ± 0.67
1 month	36.12 ± 1.07	36.86 ± 2.13	36.46 ± 1.39	36.70 ± 1.69	35.73 ± 0.76
2 months	37.84 ± 1.93	36.43 ± 2.14	36.08 ± 1.56	37.03 ± 0.39	36.43 ± 0.52
3 months	36.76 ± 1.48	36.28 ± 2.04	36.52 ± 0.81	36.86 ± 1.77	36.76 ± 1.05

($n = 4$) Negative control samples: 36.37 ± 0.78 N; positive control samples subjected to heat treatment: 37.28 ± 0.92 N; positive control samples irradiated with visible light: 37.11 ± 1.33 N; positive control samples irradiated with UV: 33.07 ± 2.88 N.

3.4. Tensile test

Flexibility and stability are some of the reasons why PVC products are used widely. In order to examine the deterioration of PVC products subjected to heat treatment or optical irradiation, the tensile strength and elasticity were measured. The maximum force to break the PVC sheets ranged from 33.1 ± 2.9 to 37.8 ± 1.9 N regardless of treatment (Table 2).

Therefore, there were no notable changes in the tensile strength and elasticity of the PVC sheets when heat treatment or optical irradiation was applied.

4. Conclusions

The DEHP content and the surface structure of the PVC products, and the levels of DEHP migrating from them were measured in order to determine the influence of external factors on PVC products during storage. In addition, a tensile test was carried out to determine the tensile strength and elasticity of the PVC products. It was hypothesized that UV irradiation led to changes in the surface structure, and that change was responsible for the decreased levels of DEHP migrating from the PVC products using a drug solvent diluted according to the package insert.

In order to clarify the change in the surface structure, we examined the surface by ESCA. In UV-irradiated PVC sheets, we observed that the hydrogen chloride level was decreased and oxidation proceeded with time. Similarly, in the FT-IR spectra, we observed that the absorption band characteristic of C–Cl stretching vibration from PVC and the C–H band from the aromatic compound were decreased with time. In addition, the absorption bands in the FT-IR spectra were found to broaden with time. PVC oxidation and crosslinking were surmised to explain these events. Based on these results, the UV irradiation of PVC products induced a decrease in the levels of DEHP migration, and the PVC products maintained their features, such as flexibility and stability. Some studies have reported the decreased DEHP release from PVC products by modifying the surface structure of the products under various conditions such as UV-irradiation with sodium azide as an enhancer for absorbing UV-energy, gamma-ray irradiation, in an aqueous solution containing water-soluble compounds such as methacrylic acid, and gas plasma treatment under reduced pressure (Jayakrishnan et al., 1995; Krishnan et al., 1991). In comparison with these techniques, the simple UV-irradiation method described in this study seems to have a great advantage, because it can be performed easily under atmosphere conditions without reagents or special instruments.

Today, the medical device industry is searching for a substitute for DEHP as a plasticizer. Our results suggest that the levels of DEHP migrating from a PVC product can be reduced by easy surface treatment without changing the type of plasticizer. This could be useful method to develop novel PVC products, if other safety aspects are confirmed. Possible biological changes should not be ignored, since increased oxygen content on the surface could have an important impact on the activation of the clotting system and complements. A detailed investigation is in progress in our laboratory to develop novel PVC products.

Acknowledgement

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Effect of sterilization process on the formation of mono(2-ethylhexyl)phthalate from di(2-ethylhexyl)phthalate

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Abstract

The risk assessment of di(2-ethylhexyl)phthalate (DEHP) migrating from polyvinyl chloride (PVC) medical devices is an important issue. Many studies have been conducted to determine the level of DEHP migration. A recent report has indicated that DEHP in blood bags is hydrolyzed by esterase into mono(2-ethylhexyl)phthalate (MEHP). However, MEHP is thought to be even more toxic than the parent compound. Therefore, a method for the simultaneous determination of DEHP and MEHP was developed. The limits of quantification (LOQs) of DEHP and MEHP were 2.5 and 0.75 ng/ml, respectively. In this study, the effect of sterilization process on the levels of DEHP and MEHP migration was investigated. The level of migration of DEHP from gamma(γ)-ray sterilized PVC sheet was low compared with that of the unsterilized control. By contrast, the level of MEHP migration from the γ-ray sterilized PVC sheet was high compared with that of the unsterilized control. In addition, a high content of MEHP was found in the γ-ray sterilized PVC sheet.

Keywords: Sterilization; Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate; γ-Ray

1. Introduction

Polyvinyl chloride (PVC) is one of the most widely used polymer materials in the medical field. Flexible PVC is used in the manufacture of blood storage bags, blood tubing, and so on. Plasticizers are incorporated into PVC medical devices to increase their flexibility. The esters of phthalic acid, particularly di(2-ethylhexyl)phthalate (DEHP), are the most preferred plasticizers for medical grade PVC. However, the migration of DEHP from PVC medical devices has been reported [1–5]. DEHP in PVC products easily migrates into foods, drugs and body fluids [6–8]. The toxicity of DEHP has been evaluated [5,9–12], and a risk assessment study has suggested that it is relatively safe for humans.

However, it has been reported that DEHP is hydrolyzed enzymatically into mono(2-ethylhexyl)phthalate (MEHP) [13–15]. In vitro studies have indicated that MEHP inhibits FSH-stimulated cAMP accumulation in cultured Sertoli cells [16–20], in addition to reducing 17β-estradiol production and aromatase mRNA expression [21,22]. These results suggest that MEHP is an active metabolite of DEHP, and that any toxic effects of orally ingested DEHP are more likely to be due to the properties of the corresponding monoester rather than the intact DEHP, and that MEHP may be even more toxic than the parent compound. Medical devices are sterilized because they directly contact or are inserted into the human body. It has been reported that DEHP is hydrolyzed by such enzymes as lipases into MEHP in blood bags [13–15]. Some reports have indicated that the hydrolysis may have occurred during sterilization by autoclaving [23,24]. For medical devices, such sterilization processes as autoclaving, gamma(γ)-ray irradiation, and exposure of ethylene oxide gas (EOG), are usually performed.

Therefore, we investigated the effect of the sterilization process on the levels of migration of DEHP and MEHP from PVC medical devices. The PVC sheets that were subjected to various sterilization processes were extracted with purified water, 5% glucose solution or polyoxyethylated hydrogenated castor oil 60 (HCO-60). Moreover, the contents of DEHP and MEHP in the PVC sheets were examined. We have developed the column-

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switching (CS) liquid chromatography–tandem mass spectrometry (LC–MS/MS) as the method for determining DEHP and MEHP with high sensitivity and selectivity.

2. Experimental (materials and methods)

2.1. Chemicals and materials

Environmental analytical grade DEHP and DEHP-d_4 were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). MEHP and MEHP-d_4 were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). The structures of DEHP, MEHP and their surrogate standards are shown in Fig. 1. Phthalic acid esters, analytical grade acetonitrile and acetone were used in the experiments. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

The test material was PVC sheet that was subjected to γ-ray irradiation (^{60}Co: 24.2 kGy), autoclaving (115 °C × 40 min) or EOG (50 °C × 8 h) as the sterilization process. In addition, commercially available PVC tubing on which γ-ray sterilization (20–25 kGy) was performed was used. None of the sterilization processes were performed on the control sample that was kindly supplied by the manufacturer.

The extraction solvents were 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan), polyoxyethylated hydrogenated castor oil 60 (HCO-60) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and purified water.

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000™ (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray™ ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. A Shimadzu (Kyoto, Japan) LC-10 AS pump was used for providing flow through the extraction column to load and wash the sample and to equilibrate the extraction column. A Mightysil® RP-18 GP column (5 mm × 2.0 mm, 5 μm particle size) from Kanto Chemical was used for the separation. An Oasis® HLB extraction column (20 mm × 2.1 mm, 25 μm particle size) from Waters was used for the extraction and clean-up.

2.3. Chromatographic and extraction conditions

The column-switching system was used for sample injection [25]. After 10 μl of the sample was injected with an autosampler, it was loaded onto the extraction column by flowing pure water at the rate of 1 ml/min using the LC-10 AS pump for 3 min. The matrices in the sample were eluted whereas DEHP and MEHP were retained on the extraction column. After the 3 min period, the switching valve was changed to configuration B (Fig. 2). The column oven was maintained at 40 °C. Separation was carried out with a mobile phase of acetonitrile/water (90/10, v/v) at a flow rate of 0.2 ml/min. The eluate from the analytical column was directed to the electrospray MS. After elution for 8 min, the switching valve was returned to the original position (configuration A in Fig. 2).

2.4. MS/MS conditions

The working parameters for turbo ionspray ionization MS/MS were as follows: declustering potentials, 81 V (DEHP and DEHP-d_4) and −60 V (MEHP and MEHP-d_4); curtain gas flow rates, 20 psi (DEHP and DEHP-d_4) and 30 psi (MEHP and MEHP-d_4); nebulizer gas (N_2) pressure, 30 psi; and turbo ionspray gas (N_2) pressure, 0 psi. The ion source temperature was maintained at 650 °C and the turbo ionspray voltages for DEHP (DEHP-d_4) and MEHP (MEHP-d_4) were 5500 and −4500 V, respectively. DEHP and DEHP-d_4 were detected in the positive mode, whereas MEHP and MEHP-d_4 were detected in the negative mode. The product ion mass spectra of DEHP, DEHP-d_4, MEHP and MEHP-d_4 obtained by the LC–MS/MS system

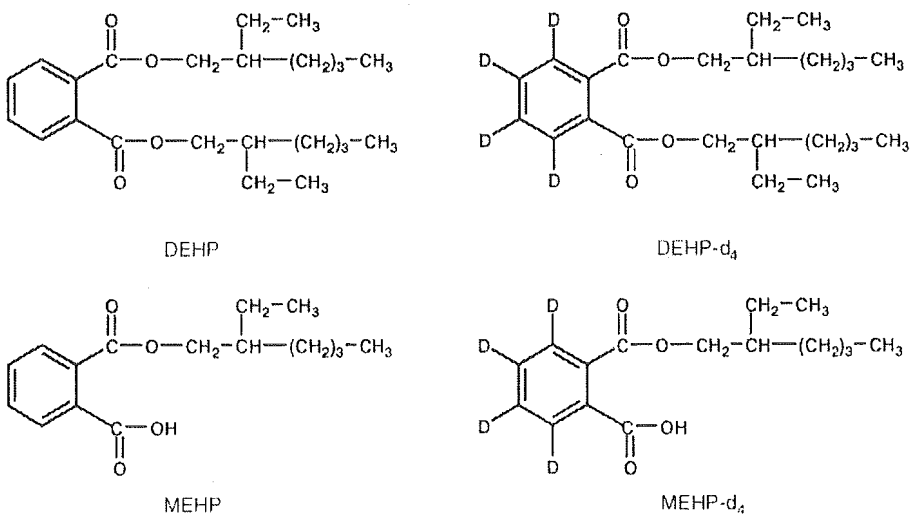

Fig. 1. Chemical structures of DEHP, MEHP and their internal standards.

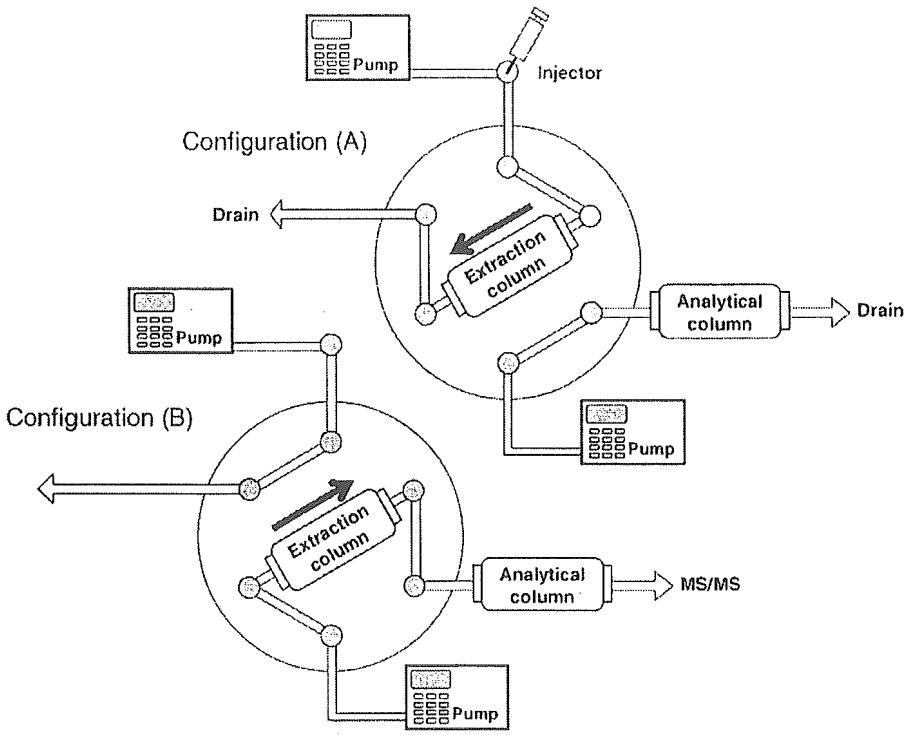

Fig. 2. Schematic representation of the column-switching LC–MS/MS system. (A) Configuration for sample loading and washing; (B) configuration for sample elution.

are shown in Fig. 3. The combinations of precursor ion and product ions were as follows: DEHP (precursor ion → product ion, *m/z* 391 → 149), DEHP-d_4 (*m/z* 395 → 153), MEHP (*m/z* 277 → 134), and MEHP-d_4 (*m/z* 281 → 138). The collision gas (N_2) pressures were set at 2 units (DEHP and DEHP-d_4) and 1 unit (MEHP and MEHP-d_4).

2.5. Migration test

The migration of DEHP and MEHP from the PVC sheet (1 cm × 3 cm) was examined in 5 ml of each solvent. HCO-60 is a surfactant that is used in the formulation of such drugs as Prograf® and is involved in the migration of DEHP. In addition,

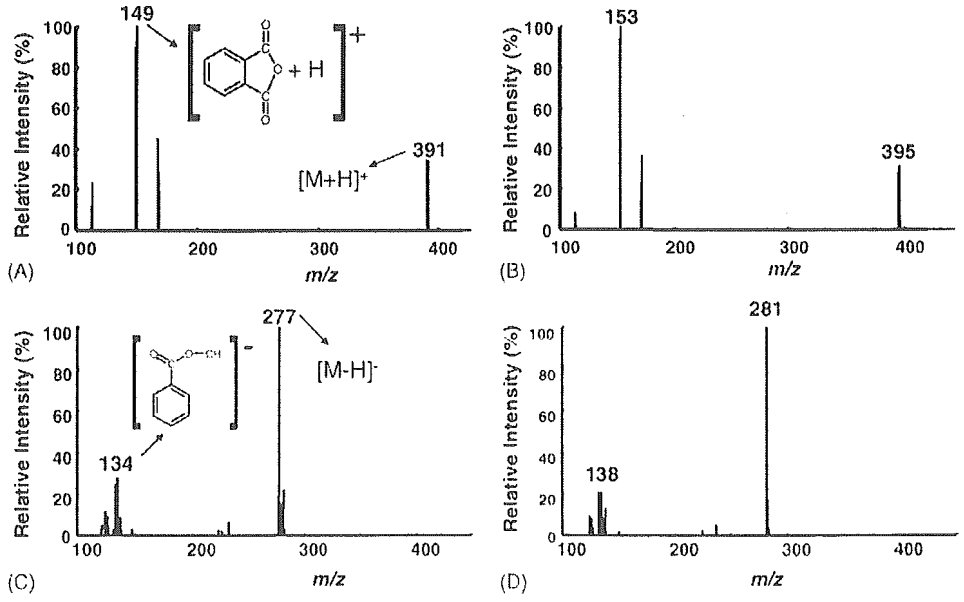

Fig. 3. Product ion mass spectra of DEHP, MEHP and their internal standards. (A) DEHP; (B) DEHP-d_4 (internal standard for DEHP); (C) MEHP; (D) MEHP-d_4 (internal standard for MEHP).

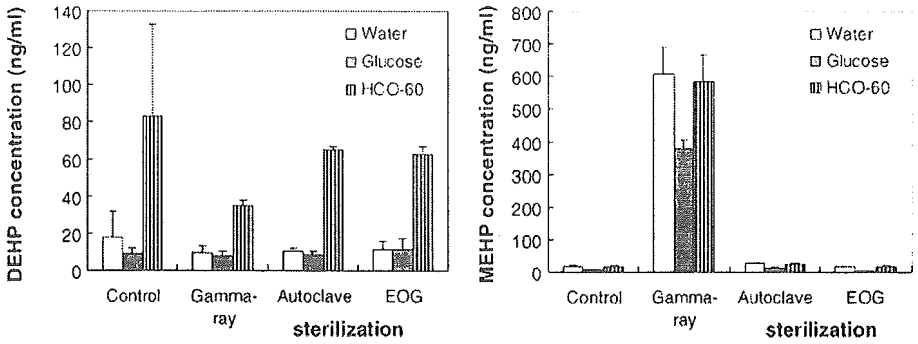

Fig. 4. Levels of DEHP and MEHP migration into various solutions from PVC sheet samples. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n = 3$). The error bar represents the standard deviation (S.D.).

it has reported that the level of DEHP migration was dependent on the concentration of HCO-60 [25]. We prepared 0.02 mg/ml HCO-60 for the migration test [26]. The samples were kept in test tubes and extraction was carried out with shaking at 37 °C for 1 h. A 1 ml aliquot of the extract was pipetted into another test tube, and DEHP-d_4 and MEHP-d_4 were added. Then, the sample solution was appropriate diluted prior to LC–MS/MS analysis.

The PVC tubing was cut to 10 cm length and filled with the solvents (tube length, 8 cm). The tubing was subjected to extraction with shaking at 37 °C for 1 h. The extracts were pipetted into another test tube containing DEHP-d_4 and MEHP-d_4. Then, all the samples were appropriate diluted prior to LC–MS/MS analysis.

2.6. Contents of DEHP and MEHP in PVC

A PVC sample (5 mg) was completely dissolved in 5 ml of THF. The solution was appropriate diluted with acetonitrile. Then, the internal standard was added prior to the analysis.

3. Results and discussion

3.1. Analysis of DEHP and MEHP by on-line SPE–LC–MS/MS

In the proposed method, the limits of quantification (LOQs) (signal-to-noise ratio >10) of DEHP and MEHP were 2.5 and 0.75 ng/ml with the standard solutions, respectively. For DEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (DEHP/DEHP-d_4) versus DEHP concentration, and was linear over the range of 2.5–500 ng/ml ($r = 0.998$). For MEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (MEHP/MEHP-d_4) versus MEHP concentration, and was linear over the range of 0.75–500 ng/ml ($r = 0.997$). We also examined the recovery using 5% glucose solution. For the glucose solution that was spiked with 50 ng/ml DEHP and MEHP, the average recoveries of DEHP and MEHP were 99.2% (R.S.D. = 3.2%, $n = 6$) and 109.0% (R.S.D. = 3.4%, $n = 6$), respectively.

3.2. Determination of DEHP and MEHP migration from PVC sheet and tubing

The developed method was applied to the determination of DEHP and MEHP migration from the PVC sheets that were subjected to various sterilization processes (Fig. 4). The migration of DEHP and MEHP from all the PVC sheets was observed. The level of DEHP migration had the following order: HCO-60 > water ≥ 5% glucose solution, similar to the report of Hanawa et al. [25]. Furthermore, when the PVC sheets were extracted with purified water and HCO-60, the levels of DEHP migration from all the PVC sheets that were subjected to the sterilization processes, particularly γ-ray sterilization, were low compared with the unsterilized control. On the other hand, the levels of MEHP migration from the unsterilized control, autoclaved and EOG sterilized PVC sheets were not different, whereas the γ-ray sterilized PVC sheet released a large amount of MEHP.

Then, the levels of DEHP and MEHP migration from the commercially available PVC tubing sterilized by γ-ray were compared with those of the unsterilized one (Fig. 5). As expected, DEHP more easily migrated in HCO-60 than in water or glucose solution. In HCO-60, the level of DEHP migration from the γ-ray sterilized PVC tubing was low compared with that of the unsterilized one. Moreover, the unsterilized PVC tubing released little MEHP whereas the γ-ray sterilized PVC tubing released approximately 30–40 times more MEHP compared with the unsterilized one. In addition, MEHP was also released from the γ-ray sterilized PVC tubing that was extracted with water or glucose solution.

3.3. Contents of DEHP and MEHP in PVC sheet and tubing

The developed method was also applied to determine the contents of DEHP and MEHP in the PVC sheets (Table 1) and the PVC tubing (Table 2). We thought that the DEHP contents were almost the same in the various sterilized sheets because the DEHP contents were 26.8–27.8% (w/w) in the sterilized PVC sheets. By contrast, MEHP was detected in only the γ-ray sterilized PVC sheet. Therefore, PVC material might inherently contain MEHP, and the MEHP migrated directly into the solvent.

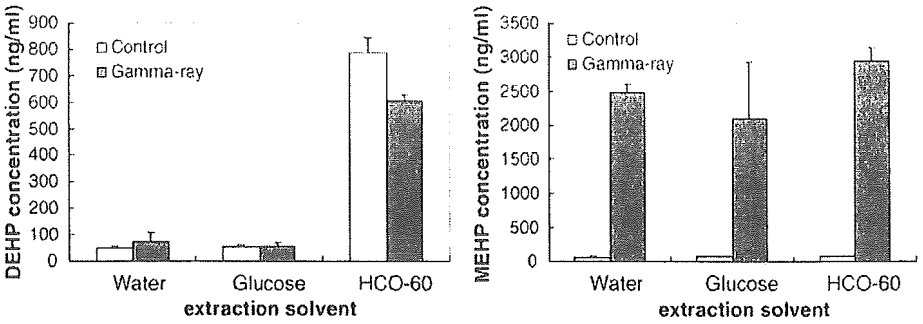

Fig. 5. Level of DEHP and MEHP migration into various solutions from commercially available PVC tubing. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n = 3$). The error bar represents the standard deviation (S.D.).

Table 1
Contents of DEHP and MEHP in PVC sheet samples treated with various sterilization

	Control	Gamma-ray	Autoclave	EOG
DEHP (%, w/w)	32.1 ± 5.7	27.8 ± 0.8	26.8 ± 1.6	26.8 ± 0.8
MEHP (mg/g)	<0.25	0.38 ± 0.05	<0.25	<0.25

Mean ± S.D., $n = 3$.

The commercially available PVC tubing which was sterilized by γ-ray was confirmed to contain MEHP, although the level was below the LOQ. A high dilution ratio was required because of the difference in level between DEHP and MEHP. Therefore, MEHP could not be determined in some of the sterilized PVC sheets.

In this study, the following phenomena were observed: (1) the level of DEHP migration from the γ-ray sterilized PVC sheet was low compared with that of the unsterilized sheet. Surface processing, an example of which is plasma treatment, is known to suppress DEHP migration [27,28]. We speculated that a similar surface processing occurred with γ-ray irradiation; (2) the level of MEHP migration from the γ-ray sterilized PVC sheet was significantly high compared with that of the unsterilized control. In addition, MEHP was released from the γ-ray sterilized PVC sheet regardless of the solvent used. We hypothesized that MEHP was inherently contained in the PVC sheet, and then directly migrated from it. To confirm this hypothesis, we determine the DEHP and MEHP contents in the PVC sheet; (3) MEHP was detected in the γ-ray sterilized PVC sheet. Although MEHP was also found to migrate from the PVC sheets sterilized by EOG or autoclaving, MEHP was not detected in them. We speculated that the MEHP contents in the other sterilized sheets were very low compared with that in the γ-ray sterilized PVC sheet. In addition, the most plausible reason for not detecting MEHP in the PVC sheets was the high dilution ratio of the samples; (4) although MEHP was also detected in PVC tubing, its amount could not be determined.

Table 2
Contents of DEHP and MEHP in commercially available PVC tubing

	Control	Gamma-ray
DEHP (%, w/w)	44.7 ± 2.9	53.9 ± 2.5
MEHP (mg/g)	<0.25	<0.25 (0.23)

Mean ± S.D., $n = 3$.

Taking these into consideration, we surmised that MEHP was inherently present in PVC and migrated directly from it, although MEHP was thought to be hydrolyzed by enzymes as lipases or by autoclave sterilization until now. In addition, we speculated that the sterilization by γ-ray was sufficient to decompose DEHP into MEHP in PVC materials. We conducted a risk assessment of MEHP migration. The level of MEHP migration was calculated as follows: we found that when γ-ray sterilized PVC sheet (1 cm × 3 cm) was extracted with 5 ml of solvent, approximately 600 ng/ml MEHP migrated from it. Therefore, the amount of MEHP migration was 3.0 μg (=600 ng/ml × 5 ml). As the superficial area involved in the migration was 6 cm^2, the level of MEHP migration per unit area was 0.5 μg/cm^2. The superficial area of the commercially available infusion set was 101.3 cm^2 at the maximum. Therefore, the amount of MEHP migration from the infusion set was 50.65 μg (=101.3 cm^2 × 0.5 μg/cm^2). When this infusion set was used for 2 days in a patient with 50 kg body weight, the patient was exposed to 0.51 μg/kg/day of MEHP. In 2001, the Center for Devices and Radiological Health of the U.S. Food and Drug Administration reported DEHP assessment as "Safety assessment of DEHP released from PVC medical devices." In Annex C of the report, "Aggregate safety assessment for coexposure to DEHP and MEHP," the relative potency of MEHP/DEHP was calculated to be 10. Therefore, an MEHP exposure of 0.51 μg/kg/day meant a DEHP exposure of 5.1 μg/kg/day. In addition, in this report, the tolerable intake (TI) of DEHP was 40 μg/kg/day by oral administration and 600 μg/kg/day by parenteral route. Although the level of MEHP that migrated from the γ-ray sterilized PVC did not exceed the TI, we must investigate the effect of formation of MEHP from DEHP by γ-ray sterilization for high risk patients such as infant and pregnant women.

Acknowledgements

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ilized by γ-ray, autoclaving or EOG were kindly supplied by the Terumo Corporation (Tokyo, Japan) and JMS Corporation (Hiroshima, Japan).

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