

Table 1 (Continued)

Product name	Principal drug	Concentration for medical use	Additives	Medication	Color
Minomycin® intravenous for drip use	Minocycline Hydrochloride	1 mg/mL		Instillation	Clear
Perdipine®	Nicardipine Hydrochloride	0.1 mg/mL	D-Sorbitol, pH adjuster	Instillation	Clear
Bisolvon® injection	Bromhexine Hydrochloride	2 mg/mL	Glucose	Intravenous injection	Clear
Modacin® injection	Ceftazidime	10 mg/mL	Sodium carbonate	Instillation	Clear
Diflucan® intravenous solution	Fluconazole	1 mg/mL		Instillation	Clear
Doyle® for injection	Aspoxicillin	50 mg/mL	Sodium chloride	Instillation	Clear
Adona® (AC-17) injection	Carbazochrome sodium sulfonate	0.05 mg/mL	Sodium hydrogensulfite, D-sorbitol, propylene glycol	Instillation	Clear
Group 4 ^a					
Atonin®-O	Oxytocin	0.01 units/mL	Chlorobutanol	Instillation	Clear
Atarax®-P Parenteral solution	Hydroxyzine Hydrochloride	0.05 mg/mL	Benzyl alcohol, pH adjuster	Instillation	Clear
Zantac® injection	Ranitidine hydrochloride	0.1 mg/mL	pH adjuster, phenol	Instillation	Achroma yellow (clear)
Kenketsu venoglobulin®-IH YOSHITOMI	Human immunoglobulin G	50 mg/mL	D-Sorbitol, pH adjuster	Intravenous injection	Clear
Pantol® injection	Panthenol	250 mg/mL	Benzyl alcohol	Intravenous injection	Clear
Buminat® 25%	Human serum albumin	250 mg/mL	Sodium N-acetyl tryptophan, sodium caprylate, sodium hydrogen carbonate	Intravenous injection	Clear
Neuart®	Human antithrombin III	25 units/mL	Sodium chloride, sodium citrate, D-mannitol	Instillation	Achroma yellow (barely opacity)
Millisrol® injection	Nitroglycerin	0.5 mg/mL	D-Mannitol, pH adjuster	Instillation	Clear
Metilon®	Sulpyrine	2.5 mg/mL	Benzyl alcohol	Instillation	Clear
Erythrocin®	Erythromycin Lactobionate	2.5 mg/mL	Benzyl alcohol	Instillation	Clear
Dalacin® S injection	Clindamycin phosphate	3 mg/mL	Benzyl alcohol	Instillation	Clear
Group 5 ^a					
Tienam® for intravenous drip infusion	Imipenem Cilastatin sodium	5 mg/mL	Sodium hydrogencarbonate	Instillation	Achroma yellow (clear)
Glucose® injection	5% glucose			Instillation	Clear
Fesin®	Ferric oxide, saccharated	0.4 mg/mL		Instillation	Clear
Actit® injection	Maltose, sodium chloride, potassium chloride, magnesium chloride, potassium dihydrogen phosphate, sodium acetate			Instillation	Clear
Atropine sulfate injection	Atropine sulfate	0.5 mg/mL		Intravenous injection	Clear
Vicillin® for injection	Ampicillin sodium	10 mg/mL		Instillation	Clear
Neophyllin®	Aminophylline	0.5 mg/mL	Ethylenediamine	Instillation	Clear
Fosmisin®-S Bag 2g for intravenous drip infusion	Fosfomicin sodium	20 mg/mL	Glucose solution	Instillation	Clear
Calcicol®	Calcium gluconate	85 mg/mL		Instillation	Clear
Cefamezin® α	Cefazolon sodium hydrate	10 mg/mL		Instillation	Clear
PN-Twin® No.2	Amino acids, electrolytes		Sodium hydrogen sulfite	Instillation	Clear
Succin®	Suxamethonium chloride	2 mg/mL		Instillation	Clear
Optiray®	Ioversol	320 mg/ml as iodine		Intravenous injection	Clear
Protermol®-L injection	l-Isoprenaline hydrochloride	1 μg/mL	Sodium hydrogen sulfite L-cysteine hydrochloride	Instillation	Clear

^a A detailed information on this classification was described in the part of Section 2.

transferred to a 96-well plate, and absorbance of the sample was measured by μ Quant (BIO-TEK Instruments, Inc., Vermont, USA) at 450 nm for methyl yellow, 530 nm for Sudan III, and 590 nm for 1,4-diaminoanthraquinone.

2.4. Measurement of static contact angle and electrical conductivity

Ten microlitre of each surfactant solution and pharmaceutical injection was dropped on PVC sheets. After 120 s, the width and height of the drops were measured with a G-1-1000 instrument (ERMA, Tokyo, Japan). The static contact angle was computed by the following formulas

$$r^2 = (w/2)^2 + (r - h)^2, \quad \sin \delta = (w/2)/r$$

where, r is the radius of drop (mm), w the width of drop (mm), h the height of drop (mm), δ the static angle of contact.

Electrical conductivity of each test solution was measured by COS conductivity analyzer (CEH-12, Horiba, Tokyo, Tokyo).

2.5. Elution test of DEHP and determination of DEHP content

PVC sheet (1 cm \times 3 cm, thickness: 0.4 mm) was put in a screw-capped glass tube, and 5 ml of pretest solutions (Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS) were added to the respective tubes. After shaking for 2 h at room temperature, an aliquot (0.1 ml) of the solution was taken into another glass tube, and distilled water (2 ml), sodium chloride (10 mg), and 5 ml of diethyl ether containing 50 ng/ml DEHP- d_4 were added to the tube. After shaking for 30 min followed by centrifugation at 3000 rpm for 10 min at room temperature, the organic phase was collected and dehydrated with anhydrous sodium sulfate followed by GC-MS analysis described below.

Pharmaceutical injections including Sandimmun® and Prograf® adjusted to the concentration used for medical treatment were enclosed in PVC tubing (inner diameter, 2.13 mm) cut to 10 cm length. The length and volume of the enclosed injection were 8 cm and 0.285 ml, respectively, and the surface area in contact with the enclosed injection was 5.35 cm². After shaking the tube for 1 h at room temperature, the enclosed test solution was transferred to a screw-capped glass tube,

and the sample for GC-MS analysis was prepared by the same method as that described above.

To determine DEHP content, PVC sheet and tubing (20 mg) were dissolved in 20 ml of THF by soaking overnight at room temperature. An aliquot (0.1 ml) of the solution was diluted 10,000 times with diethyl ether containing 50 ng/ml DEHP- d_4 , and then analyzed by GC-MS. DEHP contents of the PVC sheet and tubing used in this study were 36.2 and 32.9% (w/w), respectively.

2.6. GC-MS analysis

A JMS700 instrument (JEOL, Tokyo, Japan) equipped with a Hewlett-Packard HP6890 series GC system and an auto-injector (Agilent Technologies, Palo Alto, CA) were used for GC-MS analysis (resolution = 5000). Chromatographic separation was performed with BPX-5 fused silica capillary column (25 m \times 0.22 mm I.D., film thickness: 0.25 μ m, SGE, Melbourne, Australia).

The sample (2 μ l) was injected in the pulsed splitless mode. The injector temperature was 260 °C. Flow rate of helium carrier gas was 1 ml/min. Column temperature was programmed as initial temperature to 120 °C for 2 min then increasing to 300 °C at 10 °C/min. Electron impact (EI)-mass spectrum was recorded at 70 eV, and the ions of m/z 149.024 for DEHP and 153.049 for DEHP- d_4 were selected as the quantitative ions in the selective ion mode (SIM) analysis 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 lines and three times for the other samples. Preparation of calibration curves and calculation of quantitative data were performed by the computer software TOCO (Total Optimization of Chemical Operations), Version 2.0, practicing the function of mutual information (FUMI) theory (Hayashi and Matsuda, 1994; Hayashi et al., 1996, 2002; Haishima et al., 2001, 2004).

3. Results and discussion

3.1. Precision of quantitative GC-MS analysis and release profile of DEHP from PVC sheet

Background analyses of DEHP originating from each reagent and GC-MS instrument showed that

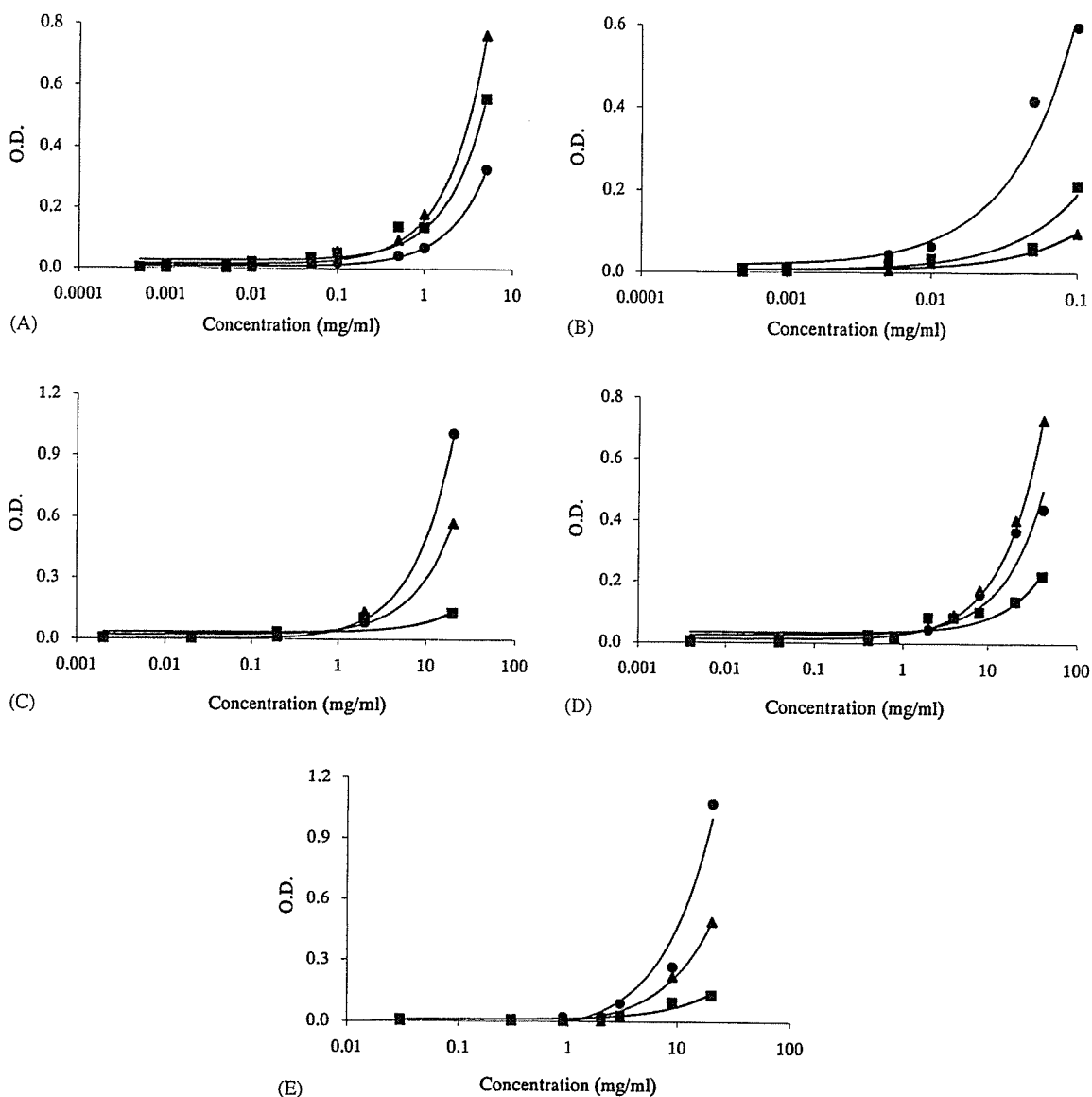


Fig. 1. Lipophilic pigment solubility against various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS. Methyl yellow(●), Sudan III (■), and 1,4-diamino-anthraquinone (▲) were used as the pigments. Absorbance of methyl yellow dissolved in Sandimmun® and Tween® 80 was measured after five times dilution with distilled water.

0.93 ± 0.31 ng/ml DEHP ($n=5$) was detected as background contamination when 50 ng of the internal standard (DEHP- d_4) was used in the quantitative analyses. On the basis of the background value, the experimental LOD and LOQ were calculated as 1.85 and 4.01 ppb, respectively. A calibration curve was obtained for the peak ratio of DEHP to DEHP- d_4 versus

DEHP concentration level. The response was found to be linear in the validated range (5–200 ppb) with correlation coefficient (r) exceeding 0.999. Further, the 95% confidence interval calculated by TOCO was sufficiently narrow, indicating that the present GC-MS method could be used for DEHP analysis with high accuracy.

Table 2
DEHP release capacity and physicochemical properties of lipophilic injections and surfactants

Solution (mg/ml)	Release amount of DEHP		Lipophilic pigments' solubility ^a						Electrical conductivity		Contact angle to PVC sheet	
			Methyl yellow ^b		Sudan III		1,4-diamino anthraquinone					
	ppm	S.D.	O.D. at 450 nm	S.D.	O.D. at 530 nm	S.D.	O.D. at 590 nm	S.D.	μS/cm	S.D.	°	S.D.
Sandimmun®												
0.0005	0.22	0.003	0.001	0.002	0.001	0.001	0.001	0.001	12.13	0.56	84.69	1.35
0.001	0.35	0.01	0.003	0.003	0.009	0.001	0.001	0.001	11.93	0.82	nt	nt
0.005	0.77	0.01	0.003	0.001	0.006	0.002	0.003	0.001	12.55	0.46	78.17	1.77
0.01	1.16	0.01	0.004	0.001	0.020	0.001	0.005	0.001	12.02	0.61	72.36	0.21
0.05	2.84	0.01	0.019	0.001	0.036	0.000	0.019	0.001	12.46	0.31	64.72	0.55
0.1	4.22	0.03	0.018	0.001	0.051	0.001	0.059	0.001	11.66	0.55	60.39	0.97
0.5	9.01	0.05	0.042	0.001	0.137	0.001	0.094	0.001	18.91	0.36	50.47	1.48
1	10.90	0.15	0.069	0.001	0.136	0.001	0.180	0.004	26.90	0.78	46.65	1.98
5	22.19	0.26	0.325	0.001	0.555	0.002	0.762	0.005	104.80	1.32	42.05	1.62
Prograf®												
0.0005	0.25	0.01	0.006	0.001	0.009	0.001	0.002	0.002	8.11	0.26	81.07	0.26
0.001	0.34	0.02	0.010	0.001	0.009	0.005	0.004	0.001	8.09	0.32	79.38	1.01
0.005	0.99	0.01	0.043	0.001	0.022	0.002	0.006	0.001	8.53	0.15	75.06	0.66
0.01	1.71	0.003	0.063	0.001	0.033	0.005	0.025	0.001	8.61	0.22	74.66	1.52
0.05	5.31	0.05	0.418	0.005	0.062	0.002	0.057	0.001	10.52	0.45	67.54	0.88
0.1	8.95	0.04	0.597	0.004	0.211	0.005	0.097	0.001	11.51	0.38	65.07	0.87
0.5	42.26	1.64	nt	nt	nt	nt	nt	nt	nt	nt	55.67	0.83
HCO-60												
0.002	0.09	0.01	0.003	0.003	0.005	0.001	0.001	0.001	13.27	0.52	84.22	1.92
0.02	0.28	0.01	0.003	0.003	0.010	0.001	0.001	0.001	16.07	0.66	80.79	1.39
0.2	1.15	0.01	0.011	0.001	0.033	0.001	0.012	0.001	16.51	0.43	76.54	2.48
2	5.72	0.04	0.083	0.001	0.106	0.002	0.135	0.001	16.39	0.59	66.23	0.34
20	22.32	0.25	1.006	0.005	0.130	0.013	0.571	0.007	18.36	0.64	63.31	5.18
40	28.90	0.22	nt	nt	nt	nt	nt	nt	26.80	0.80	61.02	0.70
Tween® 80												
0.004	0.38	0.01	0.001	0.001	0.005	0.001	0.002	0.002	15.93	0.38	84.01	1.28
0.04	0.49	0.01	0.001	0.001	0.009	0.001	0.002	0.003	14.82	0.29	77.91	0.40
0.4	2.77	0.02	0.011	0.001	0.027	0.001	0.010	0.003	15.60	0.41	70.28	0.87
0.8	4.30	0.03	0.015	0.002	0.018	0.001	0.017	0.001	16.49	0.35	68.78	1.23
2	6.58	0.03	0.045	0.001	0.083	0.002	0.055	0.001	15.20	0.47	64.43	6.80
4	9.26	0.15	0.083	0.001	0.083	0.004	0.094	0.003	13.49	0.33	58.70	1.03
8	13.17	0.17	0.159	0.002	0.101	0.001	0.175	0.003	18.50	0.50	56.05	0.33
20	20.07	0.32	0.365	0.007	0.136	0.001	0.403	0.002	31.40	0.82	54.21	0.53
40	25.56	0.20	0.438	0.004	0.219	0.002	0.728	0.004	57.70	0.91	51.89	0.61
SDS												
0.03	0.44	0.005	0.001	0.001	0.009	0.001	0.001	0.001	20.90	0.59	82.48	1.29
0.3	1.10	0.02	0.002	0.001	0.006	0.002	0.001	0.001	41.90	0.72	77.65	0.57
0.9	2.25	0.01	0.021	0.019	0.007	0.001	0.001	0.001	102.20	1.33	63.15	0.93
2	3.70	0.01	0.022	0.001	0.018	0.001	0.002	0.001	373.00	1.56	41.51	0.63
3	6.67	0.03	0.088	0.001	0.027	0.001	0.024	0.001	533.00	1.96	40.03	1.21
9	14.75	0.09	0.268	0.003	0.094	0.001	0.220	0.003	1120.00	2.42	40.23	0.64
20	18.05	0.18	1.071	0.014	0.129	0.003	0.491	0.004	3220.00	2.68	33.94	3.09

nt, not tested.

^a Values after subtracting blank value.

^b O.D. of Sandimmun and Tween 80 was measured after five times dilution with distilled water.

Release test of DEHP from medical grade PVC sheet was performed using GC-MS analysis. Two kinds of pharmaceuticals and three kinds of surfactants were used as the test solutions for DEHP extraction. Qualitative analysis of DEHP was performed by scan mode EI-MS (Haishima et al., 2004), and the release profile of DEHP from the sheet is shown in Table 2. Sandimmun® and Prograf®, typical lipophilic injections containing polyoxyethylene castor oil or HCO-60, and ethanol as additives, were found to release DEHP from the sheet concentration-dependently. Significant release of DEHP was observed at concentrations higher than 0.05 mg/ml, and the released amounts reached 22.19 ± 0.26 ppm by Sandimmun® (5 mg/ml) and 42.26 ± 1.64 ppm by Prograf® (0.5 mg/ml). Three kinds of surfactant, including HCO-60, Tween® 80, and SDS, were also found to release DEHP from the PVC sheet in a concentration-dependent manner. In particular, the release was significantly increased more than the concentration of approximately 1 mg/ml that is critical micelle concentration (CMC) of each surfactant, and the released amounts reached 28.90 ± 0.22 , 25.56 ± 0.20 , and 18.05 ± 0.18 ppm by the extraction with 40 mg/ml of HCO-60, Tween® 80, and 20 mg/ml of SDS, respectively (Table 2).

3.2. Determination of physicochemical property of test solution

Three kinds of physicochemical properties of Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS were measured to determine whether the properties could be used as markers to predict the level of DEHP released by these solutions from medical grade PVC sheet as described above. As shown in Fig. 1 and Table 2, the absorbance of each lipophilic pigment, including methyl yellow, Sudan III, and 1,4-diaminoanthraquinone, which have different absorption maximums, dissolved in each solution was increased in proportion to the rise of the solution concentration. Of the three kinds of lipophilic pigment, methyl yellow exhibited the highest response regarding the increase of absorbance, and the response of Sudan III was the lowest.

In order to evaluate the affinity of the test solutions against PVC sheet, static contact angle to the surface of PVC sheet was measured. As shown in Table 2, the angle of each solution was decreased in a concentration-

dependent manner, indicating that the affinity was increased according to the rise of solution concentration. The electrical conductivity of each test solution was also measured as a marker predicting DEHP release level. As shown in Table 2, electrical conductivity of all the solutions except Prograf® was increased in a concentration-dependent manner. In particular, the value of SDS, an ionic surfactant, was remarkably increased according to the increase of concentration. On the other hand, no significant change was observed in the electrical conductivity of Prograf®.

As shown in Figs. 2–4, the profiles of these physicochemical properties appear to significantly relate to the release behaviors of DEHP from medical grade PVC sheet by the extraction with the solutions. However, some pharmaceuticals may exhibit very low electrical conductivity, similar to that of Prograf® (Fig. 4 and Table 2), and the value is greatly influenced by the amounts of electrolytes present in solution rather than by the lipotropy of the solution, which is not the case for other two physicochemical properties. Taking the above findings into consideration, electrical conductivity may be not useful as a marker to predict the level of DEHP released from PVC medical devices. On the other hand, no such disadvantage was recognized in the lipophilic pigment solubility test, in which good correlation to the release behavior of DEHP was observed (Fig. 2), indicating that the DEHP release level from PVC medical devices could be predicted by the test. Although static contact angle value appears to change linearly according to the concentration of the test solution, the value suggests that this property may also be useful as a marker (Fig. 3).

3.3. Detailed evaluation of the relationship between release potency of DEHP and physicochemical properties of pharmaceuticals

A detailed investigation was performed to evaluate the relationship between release behavior of DEHP from medical grade PVC tubing used as a transfusion set and the physicochemical properties, namely lipophilic pigment solubility and static contact angle, of pharmaceuticals. For this investigation, 53 pharmaceutical injections including Sandimmun® and Prograf® as positive control were scientifically selected from 180 injections used in the department of Obstetrics

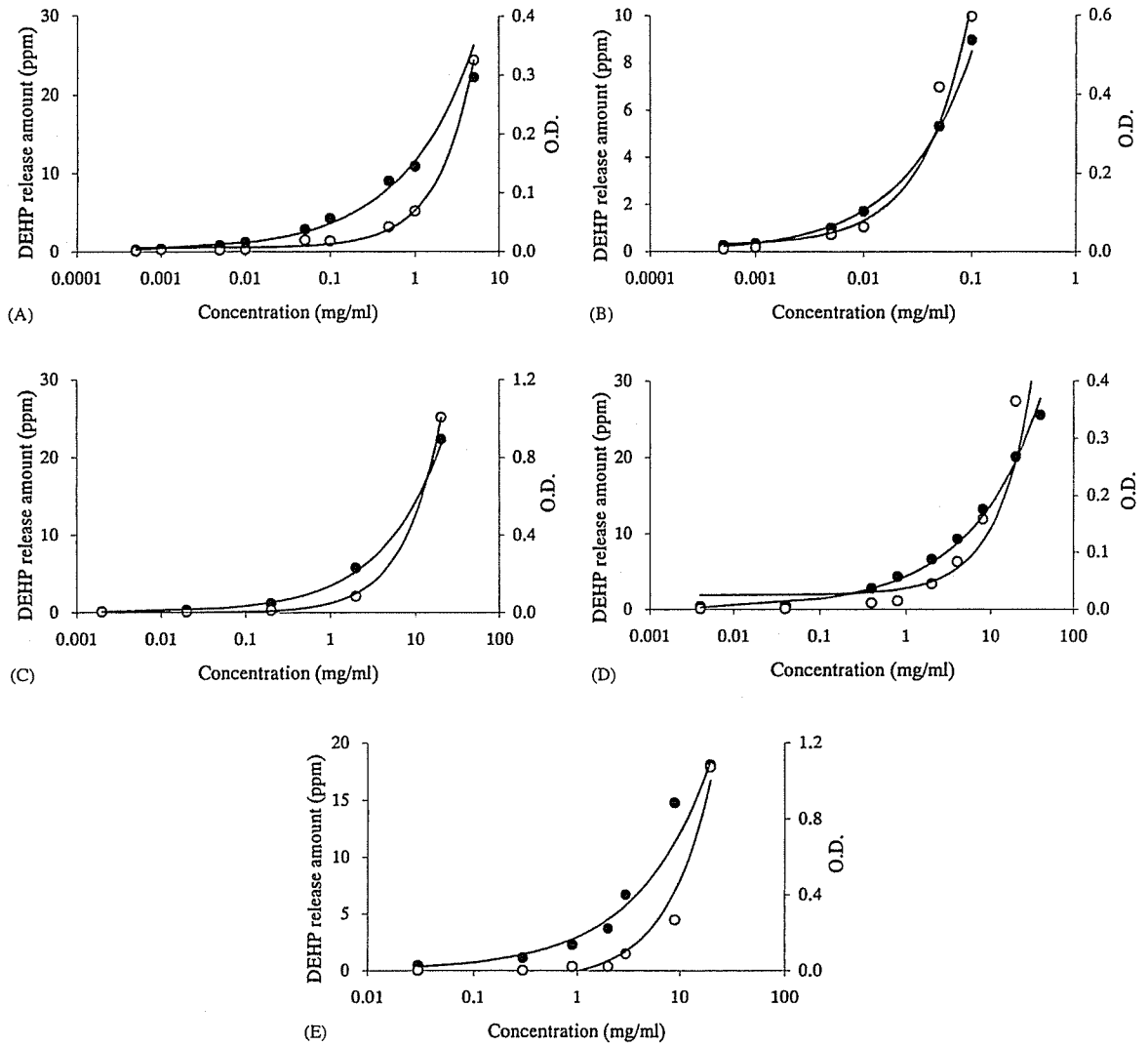


Fig. 2. Relationship between DEHP release potency (●) and methyl yellow solubility (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS. Absorbance of Sandimmun® and Tween® 80 was measured after five times dilution with distilled water.

and Gynecology, School of Medicine, Tokai University (Kanagawa, Japan). Based on the properties of drugs and additives contained in each pharmaceutical, these injections were divided into five groups, as follows: lipophilic injections (group 1), pH-dependent pharmaceuticals for solubilization (group 2), low solubility pharmaceuticals (group 3), pharmaceuticals suspected to induce DEHP migration (group 4), and hydrophilic injections as negative control (group 5), as shown in Table 1.

The release potency of DEHP from the PVC tubing was estimated by using 53 injections adjusted to the concentration used for medical treatment (Table 1). As shown in Table 3, Sandimmun®, Diprivan®, Ropion®, and Florid®-F, assigned to group 1, released large amounts of DEHP, and significant release was also observed by Prograf®, Sohvita®, Kaytwo® N, and Horizon®. In the other injections assigned to group 1, Predonine® (10 mg/ml) showed relatively low release of DEHP, and no remarkable release was recognized by

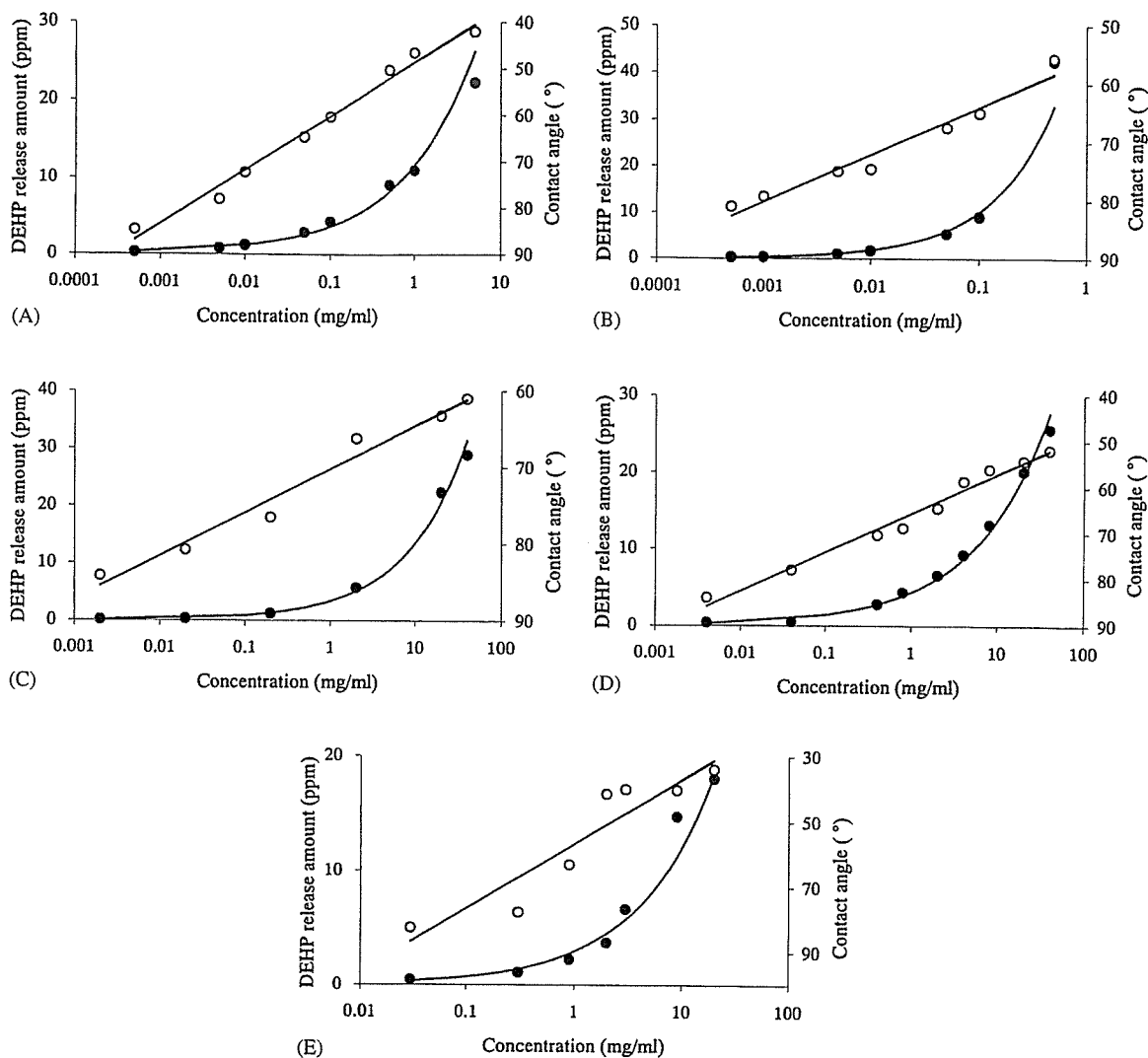


Fig. 3. Relationship between DEHP release potency (●) and static contact angle to PVC sheet (○) of various concentration of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

Humulin® R, Prostaton®, or Predonine® (1 mg/ml). On the other hand, no significant DEHP migration was observed by most of the other injections assigned to groups 2 through 5, and the concentration range of DEHP released into each injection was approximately 100–400 ppb. Exceptionally, Aleviatin® containing propylene glycol and ethanol (group 2) and Buminate® and Neuart®, which are human serum preparations (group 4), released relatively high amounts of DEHP, and Elaspol® (group 2) released a relatively low amount of DEHP.

The amount of methyl yellow, which exhibited the highest response regarding the increase of absorbance described above, dissolved in each pharmaceutical is listed in Table 3 as the absorbance at 450 nm. In this solubility test using lipophilic pigment, Sandimmun®, Buminate®, Florid®-F, Aleviatin®, Horizon®, Kaytwo® N, Diprivan®, and Ropion®, all of which showed potent DEHP release, showed high absorbance (over 0.8). However, absorbance of Prograf®, Neuart®, Sohvita®, and Elaspol® were lower than approximately 0.05. On the other hand, the

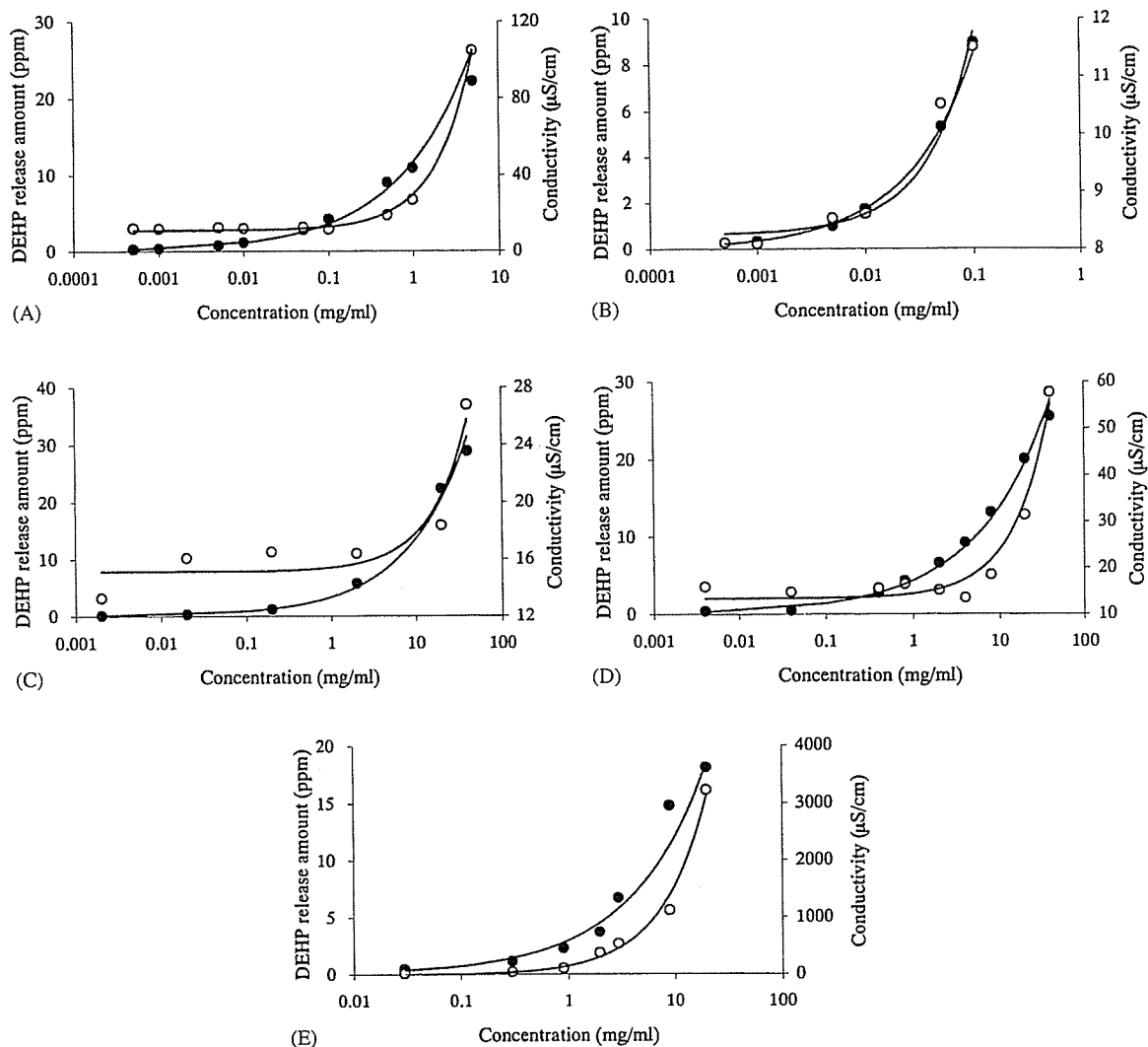


Fig. 4. Relationship between DEHP release potency (●) and electrical conductivity (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

values of other injections that demonstrated low potency of DEHP release were lower than 0.026. Exceptionally, absorbance of Optiray® and of Pantol® was approximately 0.1.

Static contact angle values of 53 pharmaceuticals to PVC sheet are listed in Table 3. All pharmaceuticals that did not exhibit remarkable release of DEHP from medical grade PVC tubing showed relatively large contact angles ranging from approximately 70°–90°. On the other hand, among the injections showing high potency of

DEHP release, Florid®-F, Horizon®, Sandimmun®, and Aleviatin® exhibited low contact angles of $36.68^\circ \pm 2.81^\circ$, $48.74^\circ \pm 2.66^\circ$, $52.73^\circ \pm 0.93^\circ$, and $58.30^\circ \pm 2.53^\circ$, respectively. However, static contact angle of Predonine® (10 mg/ml), Diprivan®, Prograf®, Sohvita®, Ropion®, Buminate®, Kaytwo® N, Elaspol®, and Neuart®, all of which also released DEHP from PVC sheet, were relatively high, with values ranging from 72.83° to 88.61°.

The relationship between the released amount of DEHP and the value of the physicochemical properties

Table 3
DEHP release capacity and physicochemical properties of pharmaceutical injections used in this study

Product name	DEHP amount migrated into injections		Contact angle to PVC sheet		Solubility of methyl yellow ^a	
	ppb	S.D.	°	S.D.	O.D. at 450 nm	S.D.
Group 1						
Sandimmun®	27363.9	384.8	52.73	0.925	0.989	0.000
Prograf®	4091.9	31.9	78.11	1.418	0.041	0.001
Diprivan®	19451.2	852.5	78.17	0.961	5.983 ^b	0.103
Ropion®	17838.5	821.6	81.31	1.778	19.500 ^b	0.007
Sohvita®	1157.1	5.1	81.32	1.362	0.008	0.001
Kaytwo® N	8457.5	62.9	82.20	1.102	4.105 ^c	0.007
Humulin® R	281.6	6.0	76.11	2.338	0.003	0.001
Prostarmon®-F	185.8	17.3	88.41	0.451	0.001	0.000
Florid®-F	30098.3	423.3	38.68	2.810	1.366	0.028
Horizon®	2008.8	257.6	48.74	2.656	2.596	0.150
Predonine® 10 mg/ml	915.6	182.3	72.83	2.122	0.022	0.001
Predonine® 1 mg/ml	407.1	2.4	87.46	0.445	0.002	0.000
Group 2						
Gaster®	166.0	0.9	87.83	0.445	0.003	0.001
Droleptan® 2.5 mg/ml	171.0	0.6	77.74	0.880	0.008	0.001
Droleptan® 50 µg/ml	167.4	24.6	89.55	0.521	0.002	0.001
Elaspol®	885.7	10.6	86.59	1.871	0.002	0.000
Aleviatin®	5009.0	288.1	58.30	2.534	1.872	0.015
Methotrexate®	372.8	6.8	88.64	0.926	0.001	0.001
Serenace®	50.6	2.5	77.59	1.881	0.005	0.000
Bosmin®	290.3	24.6	86.63	0.819	0.006	0.000
Group 3						
Partan M	462.7	4.2	88.52	0.898	0.007	0.000
Musculax®	192.7	1.5	87.60	2.737	0.001	0.001
Carbenin®	237.0	1.2	87.14	1.205	0.001	0.001
Minomycin®	150.0	8.9	88.65	0.900	0.012	0.001
Perdipine®	211.6	24.0	87.28	1.961	0.002	0.001
Bisolvon®	174.9	23.7	85.38	0.629	0.017	0.000
Modacin®	301.0	0.5	88.86	0.870	0.002	0.001
Diflucan®	210.5	1.2	88.08	0.610	0.002	0.001
Doyle®	296.7	2.6	86.16	1.814	0.002	0.001
Adona®	246.1	3.0	88.00	2.189	0.001	0.001
Group 4						
Atonin®-O	423.1	0.8	87.48	1.170	0.002	0.001
Atarax®-P	430.8	144.4	88.53	1.242	0.002	0.001
Zantac®	197.9	29.5	88.85	0.468	0.002	0.001
Kenketsu Venoglobulin®-IH	243.9	14.3	83.98	1.888	0.018	0.001
Pantol®	412.1	18.2	69.78	1.093	0.087	0.000
Buminate®	10080.8	84.1	81.68	1.915	1.130	0.057
Neuart®	2008.2	21.8	88.61	0.930	0.003	0.001
Millisrol®	267.6	8.9	87.74	0.630	0.002	0.000
Metilon®	302.8	3.8	86.80	1.745	0.001	0.001
Erythrocin®	92.2	0.7	81.49	3.162	0.003	0.000
Dalacin® S	274.9	4.0	84.56	1.232	0.002	0.001
Group 5						
Tienam®	205.1	1.6	88.64	0.909	0.002	0.000
Glucose®	284.6	4.8	87.38	1.333	0.002	0.001
Fesin®	244.5	5.5	87.97	1.859	0.026	0.011

Table 3 (Continued)

Product name	DEHP amount migrated into injections		Contact angle to PVC sheet		Solubility of methyl yellow ^a	
	ppb	S.D.	°	S.D.	O.D. at 450 nm	S.D.
Actit®	262.8	5.0	86.88	2.117	0.002	0.001
Atropine sulfate	200.7	5.1	87.99	1.065	0.001	0.001
Vicillin® for injection	262.3	6.8	88.85	0.886	0.003	0.000
Neophyllin®	301.1	4.0	89.77	0.466	0.001	0.005
Fosmisen®-S	289.6	6.7	88.39	0.462	0.001	0.000
Calcicol®	179.4	4.3	88.20	1.259	0.001	0.001
Cefamezin® α	215.1	0.9	87.93	1.171	0.003	0.001
PN-Twin® No.2	328.5	5.0	88.37	0.941	0.001	0.000
Succin®	228.6	2.1	89.20	0.226	0.002	0.001
Optiray®	404.0	79.5	85.49	0.761	0.162	0.002
Proterol®-L	326.3	8.6	87.75	1.425	0.002	0.001

^a Values after subtracting blank value.

^b Measured after 50 times dilution.

^c Measured after five times dilution.

is shown in Figs. 5 and 6. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection and instillation through transfusion set was assumed to be 5 min and 1 h, respectively. Although it is known that the released amount of DEHP from PVC tubing is influenced by drip rate (Hanawa et al., 2000; Hanawa et al., 2003), this factor was not considered in this risk assessment. When body

weights of adult and neonate patients were assumed to be 50 and 3 kg, respectively, the absolute amounts of DEHP corresponding to the lower limit (40 µg/kg/day) of TDI value restricted by JMHLW represented 2000 and 120 µg per day, respectively. As shown in Fig. 5, a good proportional correlation was recognized between the DEHP release potency and methyl yellow solubility of each pharmaceutical. The response was found to be linear with correlation coefficient exceeding 0.707 for the pharmaceuticals administered by instillation and

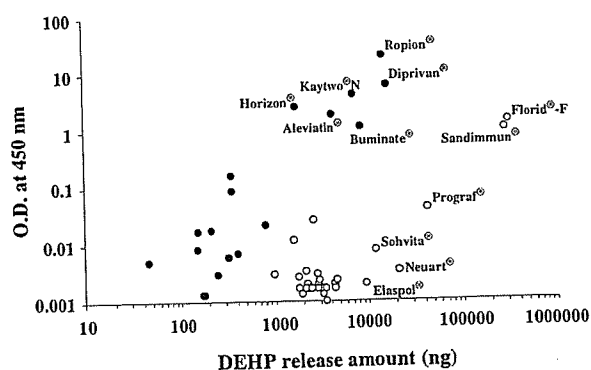


Fig. 5. Relationship between the released amount of DEHP and methyl yellow solubility of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection (●) and instillation (○) through transfusion set were assumed to be 5 min and 1 h, respectively.

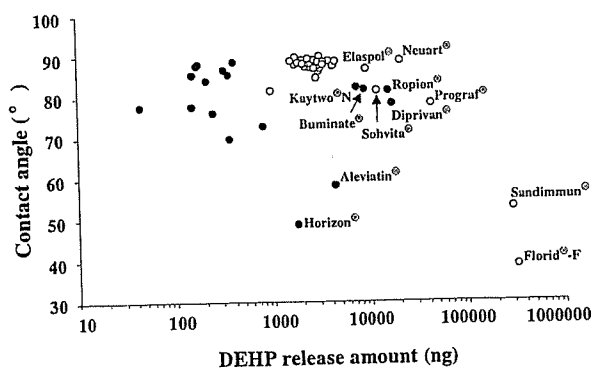


Fig. 6. Relationship between the released amount of DEHP and static contact angle of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection (●) and instillation (○) through transfusion set were assumed to be 5 min and 1 h, respectively.

0.819 for the pharmaceuticals by intravenous injection. Most of the pharmaceuticals administered by instillation did not cause DEHP exposure to patients over the lower limit of the TDI value. It was noted, however, that Sandimmun® and Florid®-F exhibited release of DEHP over the lower limit (120 µg) for neonates. When the threshold of DEHP exposure in medical treatment using transfusion set to neonate patients was set at 0.8 as absorbance of methyl yellow, only Sandimmun® and Florid®-F of all the pharmaceuticals administered by instillation showed high absorbance (i.e., over the threshold). Although Prograf®, Neuart®, Sohvita®, and Elaspol® could release relatively large amounts of DEHP, the exposure amounts to neonate patients were under the lower limit of TDI value and the absorbance of each pharmaceutical was lower than 0.8 in methyl yellow solubility test. On the other hand, none of the pharmaceuticals demonstrating significant release potency of DEHP from PVC tubing (Table 3) when administered to the patients by intravenous injection through transfusion set, including Diprivan®, Ropion®, Buminate®, Kaytwo® N, Aleviatin®, and Horizon®, caused DEHP exposure over the lower limit of TDI value, largely because of the short time required for administration. It was demonstrated, however, that methyl yellow solubility test could reflect the real potency of DEHP release, by which Diprivan®, Ropion®, Buminate®, Kaytwo® N, Aleviatin®, and Horizon® showed high absorbance (more than 0.8). These results clearly indicate that the risk of DEHP exposure to the patients could be predicted by methyl yellow solubility test.

Similar risk assessment was performed with static contact angle to PVC sheet of pharmaceuticals as a marker, the results of which are shown in Fig. 6. The risk of DEHP release caused by Sandimmun® and Florid®-F could be predicted by creating a borderline at an angle of 60°. All other injections, with the exception of Horizon® and Aleviatin®, exhibited a large angle more than the set value. It was suggested that the pairing of propylene glycol and ethanol, contained only in Horizon® and Aleviatin® as additives, may be responsible for DEHP release and low value of static contact angle, and that the angle was not influenced by the concentrations of soy bean oil, glycerin, and lecithin contained in Kaytwo® N, Ropion®, and Diprivan®. The concentration of HCO-60 must be very significant regarding DEHP release and low contact angle, because

although Prograf® contains the same or similar surfactant as Florid®-F and Sandimmun®, the medical use concentration of Prograf® is relatively low compared to those of Sandimmun® and Florid®-F; hence, Prograf® shows a high contact angle on this test. From these results, it was suggested that static contact angle to PVC sheet of pharmaceuticals could be a useful marker to predict the risk of DEHP exposure to neonate patients. It seems, however, that in contrast with the results of the methyl yellow solubility test, the contact angle to PVC sheet of pharmaceuticals does not always reflect the real potency of DEHP release, based on the findings that Kaytwo® N, Ropion®, Buminate®, and Diprivan® showed relatively high contact angles despite their high potency of DEHP release (Table 3).

4. Conclusions

In the present study, the DEHP release behavior of pharmaceutical injections was compared with the potency of physicochemical properties of the injections in order to develop a simple method for predicting the level of DEHP migrating from PVC medical devices into the injections. It was shown that although some pharmaceuticals had high release potency of DEHP from PVC products, most of the pharmaceuticals tested did not cause significant DEHP exposure to patients in the form applied for medical use. However, neonate patients may be exposed to DEHP over the lower limit of TDI value when Sandimmun® and Florid®-F are administered by instillation through transfusion set. The risk could be predicted by methyl yellow solubility test, the results of which were closely related to DEHP release potency of pharmaceuticals. Some pharmaceuticals possess their own color characteristic, and the measurement of absorbance of methyl yellow may be inhibited by a color having a λ_{\max} similar to that of methyl yellow. In this case, however, it appears that Sudan III and 1,4-diamino-anthraquinone, which have different λ_{\max} , can be used instead of methyl yellow as marker pigments. Thus, the solubility test of lipophilic pigments is very simple and rapid in comparison with the typical and complicated elution tests of DEHP using GC-MS and LC-MS, and it may be applicable in the medical field, particularly in hospital, as one of the methods for the safety and risk assessment of DEHP exposure originating from the use of PVC products.

Acknowledgement

This work was supported by grant H14-Iyaku-005 and H15-Risk-017 from the Ministry of Health, Labor, and Welfare of Japan. We greatly appreciate cooperation of pharmaceutical companies that have given us Sandimmun® and Prograf® injections.

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Monitoring of polycyclic aromatic hydrocarbons and water-extractable phenols in creosotes and creosote-treated woods made and procurable in Japan

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Received 2 July 2004; received in revised form 21 December 2004; accepted 18 January 2005

Available online 2 March 2005

Abstract

The recycling of disused railway sleepers treated with wood preservatives such as creosote as exterior wood for use in gardens has recently become popular in Japan. Creosote contains high quantities of polycyclic aromatic hydrocarbons (PAHs), and can lead to skin irritation and disease. In this work we have determined the amount of PAHs and water-extractable phenols in creosote and creosote-treated wood products such as railway sleepers and stakes for agricultural use that are either made or are procurable in Japan. PAHs were extracted with dichloromethane and analyzed by gas chromatography–mass spectrometry. Among carcinogenic PAHs, benz(*a*)anthracene was detected in the highest concentration, varying between 228 and 6328 $\mu\text{g/g}$ in creosotes. Benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*a*)pyrene (BaP) were found in the range of 67–3541 $\mu\text{g/g}$. Almost all creosotes contained more than 50 $\mu\text{g/g}$ of BaP, which is the upper limit level that is permitted in the European Union (EU). Creosote-impregnated wood products, such as brand-new or secondhand railway sleepers and foundations, contained large amounts of BaP (58–749 $\mu\text{g/g}$) and benz(*a*)anthracene (250–1282 $\mu\text{g/g}$). Concentrations of between 692 and 2489 $\mu\text{g/g}$ of phenols were determined in the water extracts from creosotes, but the level was considerably less than the EU control value (3% by mass), and there was no correlation between the amount of water-extractable phenols and the amount of PAHs detected in each sample. The situation that consumers are free to use the creosotes containing a high concentration of carcinogens such as BaP may cause unacceptable damage to the health of persons handling these creosote products.

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Keywords: Polycyclic aromatic hydrocarbons; Creosote; GC–MS; Phenols; Wood preservative

1. Introduction

Creosote is a mid-heavy distillate of coal tar with a boiling point of between 200 and 400 °C (Gevao and

Jones, 1998). The annual production of creosote in Japan is about 70 million tons. The majority of this is used as raw material for carbon black, while much of the rest has been used as a wood preservative (market research of Journal of The Japan Aromatic Industry Association, Inc. and Japan Wood Preserving Association). Wood treated with creosote was formerly used for railway sleepers and poles for the transport of electricity. These items are now commonly used in the foundations

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of buildings, for building fences and as stakes for agricultural and fruit production, as well as in the manufacture of garden furniture and outdoor recreational facilities in parks.

One concern involved in the use of creosote is the long-term release into the environment of a wide variety of constituents that are present within creosote-treated wood (Bestari et al., 1998). Creosote can be toxic to animals, and direct contact with creosote can lead to skin irritation and disease (Agency for Toxic Substances and Disease Registry, 2002). The International Programme on Chemical Safety selected 33 polycyclic aromatic hydrocarbons (PAHs) for toxicological evaluation in Environmental Health Criteria (1998), and a variety of these PAHs are contained in high quantities (up to 85%) in creosote (Gevao and Jones, 1998). The US Environment Protection Agency (EPA, 1985) defined 16 unsubstituted PAHs as priority pollutants, which account for between 20% and 40% of the total weight of a typical creosote (Kohler et al., 2000). In the commission decision of European communities (2002), 12 PAHs were focused as the most important ones. Among the PAHs, benzo(*a*)pyrene (BaP) is one of the most thoroughly investigated and is classified in Group 2A of potential human carcinogens (IARC, 1983), so it was chosen as a marker for creosote treatment and is also taken as an indicator for the toxicity of creosote. Creosote was reported as being probably equally carcinogenic to humans (Group 2A) by the IARC (1987). The European Union (EU) has adopted limits to the marketing and use of creosote and creosote-treated wood according to a Council Directive in 1994. The Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 1999) related that the cancer risk from creosote is greater than previously thought, and exceeded the limits permissible under existing registration. Based on those concerns, a new Directive (2001/90/EC) was adopted by the European Council (Commission of the European Communities, 2001), and creosote that contains BaP at a concentration of higher than 0.005% (50 ppm) by mass and water-extractable phenols at a concentration of higher than 3% by mass may not be used in the treatment of wood and wood so-treated may not be placed on the market.

The recycling of disused railway sleepers as exterior wood for use in gardens has recently become popular in Japan. Disused railway sleepers imported from China and other countries or obtained from domestic railroad companies have been sold at retail stores that deal in carpenter's or gardening tools. New exterior wood products including sleepers treated with creosote can also be obtained in the shops. Consequently, the opportunity to come into contact with creosote is increasing for the general public. Though these railway sleepers had been exposed to the weather for several decades, large concentrations of PAHs have been reported to exist as res-

idues in this type of wood (Rotard and Mailahn, 1987; Kohler et al., 2000). Therefore, when the general public buys and handles such wood, which is even occasionally used for interior decoration, the numerous chemicals that are present as residues may impair their health. Each country in the EU has been restricting the use of creosote since 2003, but in Japan there is no control on the marketing and use of creosote and creosote-treated woods. To reduce the health risk from creosote in Switzerland, creosote with lowered BaP content has already been used (Kohler et al., 2000). In the present study, we determined the constituents (PAHs and water-extractable phenols) in creosote and creosote-treated wood (railway sleepers and stakes for agriculture) that are available on the market in preparation for regulation in Japan.

Creosote-treated wood has generally been extracted in a Soxhlet-apparatus (Rotard and Mailahn, 1987; Gevao and Jones, 1998; Kohler et al., 2000; Becker et al., 2001). Schwab et al. (1999) and Eriksson et al. (2001) reported that mechanical shaking and liquid extraction methods are also useful for analyzing petroleum hydrocarbons from soil, as well as Soxhlet extraction techniques. We examined whether soak extraction could be used as an alternative to Soxhlet extraction. A gas chromatography (GC) system equipped with a capillary column is generally used for the determination of PAHs in various fields, including wood products, water, air and the petro-chemical industry. Furthermore, mass selective-ion-monitoring is useful for detecting each chemical selectively and provides sufficient separation for quantification of the PAHs in a complex such as creosote. (Rotard and Mailahn, 1987; EPA, 1993; Woolgar and Jones, 1999; Kohler et al., 2000; Becker et al., 2001; Eriksson et al., 2001; Shu et al., 2003; Ou et al., 2004). Bestari et al. (1998) investigated the PAH content in wood products and the leaching behavior of creosote-treated wood by high-performance liquid chromatography (HPLC). Anklam et al. (1997) used an HPLC system equipped with a fluorescence detector for the determination of the BaP content in creosote. HPLC determination is sensitive to PAHs, but the identification of individual PAHs by comparison of their retention time is less accurate compared with GC. Therefore, in this study, we used GC-mass spectrometry (GC-MS) for the determination of PAHs in creosotes.

2. Experimental section

2.1. Samples

Four commercially-available creosotes (sample A–D) and five creosotes (E–I) provided by the Japan Aromatic Industry Association, Inc. (JAIA) were investigated. Three oil-based wood preservative paints (NA, NB

Table 1
List of wood products tested

No.	Use	Type of wood			
1	Foundation	Japanese hemlock	Creosote treated	New	Made in Japan ^a
2	Railway sleeper	Kempas	Creosote treated	New	Made in Japan ^a
3	Railway sleeper	Kempas	Creosote treated	New	Made in Japan ^a
4	Railway sleeper	Pine tree	Creosote treated	Used	Imported from China
5	Railway sleeper	Unknown	Creosote treated	Used	Imported from China
6	Railway sleeper	Pine tree	Non-treated	New	Made in Japan
7	Stake	Japanese cedar	Creosote treated	New	Made in Japan
8	Stake	Japanese cedar	Non-treated	New	Made in Japan

^a Supplied by the Japan Wood Preservers Industry Association (JWPIA).

and NC) that did not contain creosote were used for comparison. Used or new railway sleepers, foundation and wooden stakes were obtained and supplied by the Japan Wood Preservers Industry Association (JWPIA). The wood products tested were listed in Table 1.

2.2. Chemicals

An EPA polycyclic aromatic hydrocarbon (PAH) mixture containing acenaphthene, acenaphthylene, anthracene, benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*ghi*)perylene, benzo(*a*)pyrene (BaP), chrysene, dibenz(*a,h*)anthracene, fluoranthene, fluorene, indeno(1,2,3-*cd*)pyrene, naphthalene, phenanthrene and pyrene at a concentration of 2000 µg/ml in dichloromethane–benzene (1:1) (Supelco, USA) was used as a standard for the PAHs. The PAH standard mixture was diluted with dichloromethane to reach a final concentration of 10 µg/ml. Acenaphthene-d10, chrysene-d12 and phenanthrene-d10 (Supelco, USA or Wako Pure Chemical Industries, Ltd., Japan) were obtained as an internal standard for PAH determination. Each internal standard was dissolved in dichloromethane at concentrations of 5–20 µg/ml. All of the other chemicals were purchased from Wako Pure Chemical Industries, Ltd., Japan. A standard stock solution of phenol was prepared by dissolving phenol in water to a concentration of 100 µg/ml. A 4-aminoantipyrin reagent was prepared by dissolving 1.36 g of the reagent in 100 ml of water. A boric acid (H₃BO₃) buffer solution was used that consisted of a mixture of a 1 mol/l sodium hydroxide (NaOH) solution and a 3 mol/l H₃BO₃ solution at a ratio of 9:10. A potassium ferricyanide (K₃Fe(CN)₆) reagent was prepared by dissolving 8.6 g of K₃Fe(CN)₆ and 1.8 ml of an ammonium solution in water to a volume of 1000 ml.

2.3. Instrument

An ultraviolet spectrometer (Shimadzu UV-160A) was used for the determination of the phenols. A gas chromatograph (GC) (Hewlett–Packard GC5890 Series

II Plus) connected to a JEOL JMS-AM II 20 mass spectrometer (MS) (GC–MS) was used for analysis of the individual PAHs. System control and data processing of the GC–MS were performed using the JEOL Auto-mass II program (JEOL Datum). The column was a PTE™-5 fused silica capillary column (30 m length and 0.25 mm i.d. coated with a 0.2 µm thickness of 5% diphenyl-95% dimethylpolysiloxane film, Supelco). Sep-Pak Plus silica-cartridges (Part No. WAT020520) for solid extraction were obtained from Waters Co. (Massachusetts, USA).

2.4. Analysis of PAH

2.4.1. Extraction

For creosote oils, the weight (g) of a 0.1 ml sample was measured in advance. The Sep-Pak Plus silica-cartridge was conditioned by rinsing with 10 ml of dichloromethane. 0.1 ml of the sample was loaded onto the cartridge, and then eluted with 10 ml of dichloromethane into the volumetric flask. Dichloromethane was added into the flask to a final volume of 20 ml.

For extraction from wood products, a section approximately 3 cm in depth from the surface of the samples was removed and cut into small pieces about 2–3 mm side and 3 cm long. In other analysis of stakes, only the surface (approximately 3 mm in depth) was shaved off. A portion of the 1.0 g fragment was weighed into a 15 ml screw-capped glass tube. After adding 10 ml of dichloromethane, the tube was incubated for 24 h at 37°C. The extract was filtrated and loaded onto a Sep-Pak Plus silica-cartridge that was conditioning described above. The wood sample and tube were washed with a small amount of dichloromethane and these washings were also added into the cartridge. The cartridge was further eluted with 10 ml of dichloromethane and the extraction solution was adjusted to a total of 25 ml.

2.4.2. GC–MS determination

The extraction solution was diluted with dichloromethane, and a series of diluted sample solution (1×,

20 \times , 100 \times , 500 \times and 1000 \times) was prepared. Two millilitres of each (diluted) extraction solution was spiked with 0.5 ml of the internal standard solution (e.g. acenaphthene-d10 20 μg in dichloromethane) and the sample solution (1 μl) was injected into the GC-MS system. The desorption time was 60 s in split-less mode. The GC column temperature was programmed as follows; it was first maintained at 60 $^{\circ}\text{C}$ for 3 min and was then heated at a rate of 10 $^{\circ}\text{C}/\text{min}$ up to 300 $^{\circ}\text{C}$, after which it was held at this temperature for 5 min. The injection temperature was maintained at 280 $^{\circ}\text{C}$. The GC-MS transfer line temperature was 280 $^{\circ}\text{C}$ and the ion source temperature was 180 $^{\circ}\text{C}$. The carrier gas was helium and column flow was maintained at 1.0 ml/min. The MS electron impact ionization energy was 70 eV. Detection was carried out using full scan (TIC, m/z = 45–500) and selective ion monitoring (SIM). Compounds in the sample solutions were identified from their retention times and from agreement with the mass chromatograms of the PAH standard solutions using a Bench-Top/PBM Mass Spectral Identification (Palisade Co., USA) with the Wiley Registry of Mass Spectral Data (John Wiley & Sons, Inc., USA). The PAH standard solutions (e.g. 0.5, 1, 2, 5 and 10 $\mu\text{g}/\text{ml}$) spiked with 0.5 ml of the internal standard solution were injected into the system and the calibration curves for the ratio of the peak area of each PAH to the internal standard for the respective mass of each ion was established. Each PAH concentration ($\mu\text{g}/\text{ml}$) in an appropriate diluted sample solution was derived from the calibration curves, and then the contents of PAHs (μg) per 1 g of sample were derived.

2.5. Analysis of water extractable phenols

2.5.1. Extraction

A creosote oil sample (0.1 ml) was shaken with 5 ml of water for 30 min, and a water layer containing the water-extractable phenols was obtained by centrifuging at 3000 rpm for 5 min.

For wood products, the following extraction conditions were used. Condition 1: About 1.0 g of the sample was cut into small pieces (described above) and was weighed into a 50 ml glass bottle and 10 ml of water was added. The bottle was stopped tightly and autoclaved at 121 $^{\circ}\text{C}$ for 15 min. Condition 2: The sample (1.0 g) and 10 ml of water were put into a glass bottle, shaken at room temperature for 1 h and heated at 37 $^{\circ}\text{C}$ for 24 h. The extract was obtained by filtration.

2.5.2. Determination

The content of the water-soluble phenols was determined by the 4-aminoantipyrin method (Pharmaceutical Society of Japan, 2000). The extract was reacted with 4-aminoantipyrin and potassium ferricyanide in boric acid buffer, and the absorbance of the solution was measured

at a wavelength of 510 nm. The phenol concentrations in 1 g of the samples were derived from the calibration curve.

3. Results and discussion

3.1. PAH in creosote

Column chromatography is generally used to extract PAHs from the various chemicals in creosote (Rotard and Mailahn, 1987; Shu et al., 2003; Ou et al., 2004). In this study, we separated the target PAHs by a solid phase extraction approach using Sep-Pak Plus silica-cartridges. Creosote (0.1 ml) was loaded on a Sep-Pak silica cartridge and eluted with 10 ml of dichloromethane. The concentrations of the PAHs in each fraction were determined and compared with those in the original creosote that had not been cleaned up. Each PAH was recovered at high yield (97–133%, data not shown). After washing with dichloromethane, a black-colored layer still remained at the top of the cartridge that could have contaminated the GC column and MS detector. The main object of the solid extraction was to remove these contaminants.

The analysis results for the nine creosotes and the three oil-based wood preservative paints are shown in Table 2. The limit of quantification (LOQ) of each PAH in the sample (not determined, ND) was estimated as about 40 $\mu\text{g}/\text{g}$, which was calculated from the lowest concentration (0.2 $\mu\text{g}/\text{ml}$) that can be determined in the diluted sample solution injected into the GC-MS. Among the 16 PAHs that we monitored, naphthalene, acenaphthene, fluorene, phenanthrene and anthracene were detected in high concentrations in commercially available creosote samples. Of the carcinogenic PAHs that were classified as cancer risk 2A or 2B (IARC 1983), benz(a)anthracene was detected in the highest concentration, varying between 228 and 6328 $\mu\text{g}/\text{g}$. Isomers of benzo(a)fluoranthene and BaP were detected at similar levels in the range of 67–3541 $\mu\text{g}/\text{g}$. The amount of indeno(1,2,3-*cd*)pyrene, dibenz(a,h)anthracene and benzo(ghi)perylene were ND–599 $\mu\text{g}/\text{g}$, ND–122 $\mu\text{g}/\text{g}$, and ND–620 $\mu\text{g}/\text{g}$, respectively. In contrast, the constituents of the wood preservative paints (not creosotes) were completely different, and these did not contain four- to six-ring PAHs above the LOQ. The PAH profiles (constituents and levels) of creosotes commercially available in Japan were similar to those of the old type of creosotes used in Europe (Woolgar and Jones, 1999; Kohler et al., 2000). Since 1994, improved creosotes have been used for wooden railroad ties in Switzerland, and these contain BaP at levels below 50 $\mu\text{g}/\text{g}$ by fractionating lower boiling components to reduce the amounts of carcinogenic four- to six-ring PAH (Kohler et al., 2000). Among the creosote samples tha

Table 2
PAH content in creosotes and non-creosote type wood preservative paints

Chemical	PAH content ($\mu\text{g/g}$)										Wood preservative (non-creosote)		
	Creosote										NA	NB	NC
	A	B	C	D	E	F	G	H	I				
Naphthalene	21349	24648	42174	54866	29462	13777	58857	25333	22261	423	31856	52031	
Acenaphthylene	4785	3186	1961	2261	1852	1895	7950	1480	2035	ND	ND	ND	
Acenaphthene	30899	26737	55628	69622	50065	55360	84230	80764	55143	ND	ND	ND	
Fluorene	30868	27547	17081	20627	25433	35433	83857	14462	30119	41	ND	ND	
Phenanthrene	27646	21059	55480	64249	90127	33830	13434	45240	113846	ND	ND	ND	
Anthracene	9771	7168	18391	15110	15489	8905	4751	11954	14220	ND	ND	ND	
Fluoranthene	9946	4292	23251	19766	28001	5221	589	25819	31856	ND	ND	ND	
Pyrene	5429	2240	12695	10323	15787	3410	220	17964	16763	ND	ND	ND	
Benz(<i>a</i>)anthracene	228	3637	2494	1706	3859	5616	ND	6328	4795	ND	ND	ND	
Chrysene	170	3035	1875	1454	3299	4033	ND	7153	4067	ND	ND	ND	
Benzo(<i>b</i>)fluoranthene	91	506	216	172	692	1183	ND	3541	664	ND	ND	ND	
Benzo(<i>k</i>)fluoranthene	79	358	165	132	468	876	ND	1532	449	ND	ND	ND	
Benzo(<i>a</i>)pyrene	67	260	116	108	465	882	ND	2514	433	ND	ND	ND	
Indeno(1,2,3- <i>cd</i>)pyrene	ND	ND	ND	ND	57	105	ND	599	ND	ND	ND	ND	
Dibenz(<i>a,h</i>)anthracene	51	ND	ND	ND	ND	ND	ND	122	ND	ND	ND	ND	
Benzo(<i>ghi</i>)perylene	ND	ND	ND	ND	ND	69	ND	620	ND	ND	ND	ND	

ND was $< 40 \mu\text{g/g}$.

we tested, only sample G contained BaP at less than $50 \mu\text{g/g}$. According to the manufacturer, this sample was produced for export to the EU. From these results, it became clear that creosote containing a high concentration of BaP is still sold and that the general public is still free to use such creosote for wood preservation in Japan. This situation may cause impermissible health damage to persons treating wood with these creosote products. The quality of creosote that may be used as a wood preservative under the Japanese Industrial Standard (JIS, 2004) has been revised.

3.2. PAH in creosote-treated wood

Several solvents were used for the extraction of PAHs from creosote-treated woods. In our limited experiment, dichloromethane showed higher extraction efficiency than hexane, ethanol and methanol (data not shown). In addition, the dichloromethane-extract was loaded directly into the Sep-Pak silica cartridges and eluted with the solvent to prepare the sample solutions. Therefore, we found that dichloromethane was a suitable extraction solvent for PAHs from creosote-treated wood.

We examined whether soak extraction using dichloromethane could be used as an alternative to Soxhlet extraction. In the soak extraction, 1 g of creosote-treated wood was incubated in 10 ml of dichloromethane. In the Soxhlet-extraction method, 5.0 g of the sample was extracted with 100 ml of dichloromethane at 50°C for 24 h. The concentrations of the 13 PAHs extracted from samples of creosote-treated wood under both sets of conditions are shown in Table 3. These show that similar

levels of PAHs can be released by both dichloromethane soak extraction and by Soxhlet extraction. Although Schwab et al. (1999) stated that Soxhlet extraction has some potential for the loss of volatile compounds, such decrease in naphthalene, et al. was not observed in this study. However, the Soxhlet extraction technique requires special apparatus and long extraction periods. There was not a significant difference between soak extraction at room temperature and at 37°C over a 24 h period, but the total amounts extracted for 24 h incubation were higher than those were achieved by shake extraction for 1 h. Therefore, in this study, creosote-treated wood was soaked in dichloromethane at 37°C for 24 h and the amount of PAH in the extract was measured.

Sections taken approximately 3 cm in depth from the surface of the wood samples (railway sleepers, foundations, stakes) were extracted and the amount of PAHs was determined (Table 4). The LOQ of PAH in wood sample was about $4 \mu\text{g/g}$, which was derived as described above. In the brand-new creosote-treated products, the foundation (sample 1) and the railway sleepers (samples 2 and 3), phenanthrene were found to have the highest concentrations, and elevated amounts of acenaphthene, fluorene, anthracene and fluoranthene were also detected. The concentration of five- and six-ring PAHs in the used railway sleeper (sample 5) was higher than that in the new products. The level of BaP in foundations and in railway sleepers was $58\text{--}749 \mu\text{g/g}$, while for benz(*a*)anthracene it was $250\text{--}1282 \mu\text{g/g}$. Only small amounts of these compounds were detected in the untreated railway sleepers (sample 6). These results

Table 3
Effect of extraction condition on the recovery of PAH from creosote-treated wood samples

Chemical	Found ($\mu\text{g/g}$)			
	Shake (rt, 1 h)	Stand (rt, 24 h)	Stand (37 °C, 24 h)	Soxhlet-extraction (50 °C, 24 h)
Naphthalene	83	176	295	383
Acenaphthylene	85	114	173	158
Acenaphthene	2811	4388	4854	5740
Fluorene	3305	4178	4385	5090
Phenanthrene	11 841	17 020	17 578	12 620
Anthracene	4249	5150	4728	4790
Fluoranthene	4536	5900	6406	5590
Pyrene	3577	4360	4494	4740
Benz(<i>a</i>)anthracene	1013	1104	1019	989
Chrysene	987	1067	976	939
Benzo(<i>b</i>)fluoranthene	327	471	558	377
Benzo(<i>k</i>)fluoranthene	238	286	382	309
Benzo(<i>a</i>)pyrene	206	267	380	280

rt = room temperature.

Creosote-treated wood was cut in small pieces and 1.0 g was weighed into a tube. Dichloromethane 10 ml was added and the sample was extracted using each of the conditions listed. In the Soxhlet-extraction, 5.0 g of the sample was extracted with 100 ml of dichloromethane at 50 °C for 24 h. The eluate was taken by filtration and 1 ml of the eluate was loaded on a Sep-Pak silica cartridge and eluted with 10 ml dichloromethane. The eluate was adjusted to an appropriate volume with dichloromethane and injected into the GC-MS. The concentration of PAH in the sample was measured.

Table 4
Content of PAHs in wood products

Chemical	Amount ($\mu\text{g/g}$)							
	1	2	3	4	5	6	7	8
Naphthalene	374	1017	1064	439	338	7	303	6
Acenaphthylene	212	290	245	197	232	4	187	4
Acenaphthene	2000	4251	8043	2355	449	ND	266	4
Fluorene	1764	3131	2754	3207	390	ND	101	ND
Phenanthrene	6040	6069	1270	10619	7837	ND	105	ND
Anthracene	1831	1026	915	2573	1124	4	33	5
Fluoranthene	2447	1144	843	6013	7966	ND	24	ND
Pyrene	1411	813	731	3702	7278	ND	14	ND
Benz(<i>a</i>)anthracene	787	250	336	1282	1058	ND	ND	ND
Chrysene	715	226	401	875	1094	ND	ND	ND
Benzo(<i>b</i>)fluoranthene	256	88	192	194	973	ND	ND	ND
Benzo(<i>k</i>)fluoranthene	157	62	94	154	601	ND	ND	ND
Benzo(<i>a</i>)pyrene	134	58	130	125	749	ND	ND	ND
Indeno(1,2,3- <i>cd</i>)pyrene	14	ND	58	13	323	ND	ND	ND
Dibenz(<i>a,h</i>)anthracene	ND	ND	7	ND	35	ND	ND	ND
Benzo(<i>ghi</i>)perylene	8	ND	54	9	288	ND	ND	ND

ND means < 4 $\mu\text{g/g}$.

suggested that the PAHs detected were derived from the treatment with creosote. We guess that the difference in the PAH content in the samples was caused by the difference in the manufacturing method of creosote and the impregnation amount of creosote into the woods.

Kohler et al. (2000) reported that naphthalene and acenaphthene in the top layer of railroad ties had leached into the environment with aging, and carcinogenic

compounds such as benz(*a,h*)anthracene and BaP etc. remained at the same level in the products over an extended time, so the relative concentrations of these chemicals were significantly higher in the top layer than in the inner layers. It suggested that the determination of the PAH content in the outer layers is important for assessing the risk to human health following skin contact with creosote-treated products. The creosote con-

tent in the railway sleepers and foundations those are creosote-impregnated wood products is not significantly different between the outer layers and the center layers. In contrast, the amount of PAHs in a stake sample (No. 7) is small. Macroscopic studies of sections taken 1–2 mm from the surface showed a change in color, so creosote does not penetrate into the center of the wood but remains near to the surface. Accordingly, almost all of a layer cut approximately 3 cm in depth from the surface of a stake does not contain any creosote, and the total creosote content was low when a test solution was prepared. In a preliminary experiment, the dissipation pattern of PAHs in an extract prepared from a section taken 2 mm in depth from the surface of a stake was comparable to that observed in the extract from a railway sleeper or from the liquid creosote used in this study. It is believed that the creosote used to treat the stake was not a special grade product with decreased four- to six-ring PAH content. No significant occurrences of skin cancer have been reported in workers in creosote-impregnation plants or in consumers, but CSTEE (1999) warned of the cancer risk from creosote containing 50 µg/g BaP and from wood treated with such creosote.

3.3. Water-extractable phenols in creosote

The difference of phenols amount extracted from creosote by increased shaking time from 10 min to 2 h was small. In this study, a creosote sample was shaken with water for 30 min at room temperature, and the water layer was obtained by centrifuging. Table 5 presents the content of water-extractable phenols in creosote

Table 5
Content of water-extractable phenols in creosotes and non-creosote type wood preservatives

Sample		Water-extractable phenols (µg/g)
Creosote	A	1093
	B	1415
	C	692
	D	782
	E	2121
	F	2155
	G	1294
	H	2359
	I	2489
Wood preservative (non creosote)	NA	378
	NB	31
	NC	ND

The sample (0.1 g) was shaken with 5 ml water for 30 min and the water layer was obtained by centrifuging at 3000 rpm for 5 min. The content of water-extractable phenols was determined by the 4-aminoantipyrin method.

and wood preservative paints. Typical phenol concentrations of between 692 and 2489 µg/g were found in creosotes. In contrast, lower concentrations of phenols were found in non-creosote-type wood preservatives. There was no correlation between the amount of water-extractable phenols and the amount of PAHs in each sample. The levels of phenols determined in these creosotes were considerably lower than the maximum levels permitted in the EU (3% by mass) and were similar to the data (between 0.06% and 0.77%) reported by Kohler et al. (2000). Creosotes that are commercially available in Japan receive an alkaline treatment after distillation (personal communication from a manufacturer), so the content of phenols is believed to be low.

3.4. Water-extractable phenols in creosote-treated wood

There are a few reports on the investigation of water-extractable phenols in creosote-treated wood samples. In our preliminary examination, the detected phenol content increased with increasing extraction time and temperature. This means that migration of water into the sample for the extraction of phenols trapped inside the wood took a long time, and the quantity of water-extractable phenols detected varied with the extraction temperature and the incubation period.

Samples were extracted by autoclaving or incubation for 24 h at 37 °C. For all samples, the values obtained with the autoclave extraction process were approximately two to three times as large as those determined after the 24 h incubation process (Table 6). The phenol content was almost the same for railway sleepers and stakes that were treated or not treated with creosote under both conditions. It was thought that almost all of the water-extractable phenols determined in this study did not originate from the creosote-treatment but from phenol-structural compounds that were primarily contained in the wood or by the generation of oxidized compounds due to hydrolysis during the extraction process. The amount of phenols in the

Table 6
Content of water-extractable phenols in creosote-treated wood products

Sample	Creosote treatment	Water-extractable phenols (µg/g)	
		Autoclave (121 °C, 10 min)	Incubation (37 °C, 24 h)
1	Yes	706	319
2	Yes	363	181
3	Yes	392	176
4	Yes	533	50
5	Yes	360	74
6	No	425	113
7	Yes	324	158
8	No	529	193