Determination of Benzo[*a*]pyrene, Benz[*a*]anthracene and Dibenz[*a*,*h*]anthracene in Creosotes and Creosote-Treated Woods

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The amount of benzo[*a*]pyrene (BaP), benz[*a*]-anthracene (BaA), and dibenz[*a*,*h*]anthracene (DBA) has been restricted to a concentration of 10 μg/g each in creosotes, and 3 μg/g each in creosote-treated woods, respectively, because of the possibility of the risk of skin cancer in consumers, and creosotes can otherwise contain high concentrations of each chemical. We already reported the content of 16 polycyclic aromatic hydrocarbons (PAHs) and phenols in creosotes and creosote-treated wood as determined by gas chromatography-mass spectrometry (GC-MS) and absorptiometry [*Chemosphere*, 60, 1279–1287 (2005)]. However, the limit of determination of each PAH per sample was > 40 μg/g according to that method, the sensitivity of which was insufficient for determining the allowable levels of these 3 compounds. Moreover, a substantial amount of time was needed for GC-MS analysis. In the present study, we improved upon our previous analytical method in order to increase the sensitivity of the test and to reduce the duration of GC-MS analysis. Creosote was extracted from treated wood samples by dichloromethane-soak incubation, and was placed on a Sep-Pak silica cartridge and eluted with dichloromethane. The eluates were evaporated and dissolved in dichloromethane. The sample solution spiked with the internal standard solution was injected into the GC-MS system. The limit of determination of each chemical in the test solution was approximately 0.2 μg/ml, which corresponded to 1–2 μg/g in each sample. The duration of GC-MS analysis was approximately 17 min. A collaborative study was also carried out in order to evaluate the reproducibility of the method for determining low levels of BaP and related compounds in creosotes. The present method was applied for the analysis of certain commercially available creosotes and creosote-treated wood samples in Japan. It was confirmed that some creosotes and railway sleepers contained these compounds in high concentrations, thus exceeding the allowed control value.

Key words —— polycyclic aromatic hydrocarbon, creosote, benzo[*a*]pyrene, GC-MS, wood preservative

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INTRODUCTION

Creosote is a mid-heavy distillate of coal tar.[1)] The majority of the creosote produced to date has been used as raw material for carbon black, while much of the remainder has been used as a wood preservative [The Japan Aromatic Industry Association, Inc., http://www.jaia-aroma.com/, Japan Wood Preserving Association (JWPA), http://wwwsoc.nii.ac.jp/jwpa/]. Wood treated with creosote was formerly used for railway sleepers and poles for the transport of electricity, but creosote-treated wood is now commonly used for the foundations of buildings, fences, and stakes for agricultural use, and also for the manufacture of garden furniture and outdoor recreational facilities in parks. However, direct contact with creosote can lead to skin irritation and disease,[2,3)] and is likely to be carcinogenic in humans; creosote is therefore classified as belonging to Group 2A among potential human carcinogens, as established by the International Agency for Research on Cancer (IARC).[4)] Creosote contains high quantities (up to 85%) of polycyclic aromatic hydrocarbons (PAHs),[1)] and the U.S. Environment Protection Agency (U.S. EPA) has defined 16 PAHs as priority pollutants.[5)] Benzo[*a*]pyrene (BaP) is one of the most thoroughly investigated PAHs, and is classified as belonging to Group 2A among potential human carcinogens.[6)] Therefore, BaP has been chosen as a marker for creosote treatment and is taken as an indicator for the toxicity of creosote. The Scientific Committee on Toxicity, Ecotoxicity and the Environment reported that the cancer risk from exposure to creosote is greater than previously thought.[7)] Such concerns have led to a new Directive (2001/90/EC) that was adopted by the European Council,[8)] according to which creosote that contains BaP at a concentration of higher than 0.005% (50 μg/g) by mass, as well as water-extractable phenols at a concentration

of higher than 3% by mass, may not be used in the treatment of wood; moreover, wood treated in such a manner may no longer be placed on the market. Each country in the European Union (EU) has thus been restricting the use of creosote since 2003.

In Japan, the recycling of disused railway sleepers as exterior wood for use in gardens has recently become popular. Disused railway sleepers imported from other countries and/or new exterior wood products have been sold at retail stores that deal in carpenter's tools and gardening supplies. Consequently, the opportunity to come into contact with creosote is increasing among the general public in Japan. In our monitoring study, more than 50 μg/g of BaP was found in creosote and creosote-treated wood products available on the market in Japan.[9] To reduce the health risk posed by creosote, the Ministry of Health, Labour and Welfare decided to restrict the use of creosotes containing elevated amounts of carcinogenic PAHs. The health risks were estimated on the basis of the opportunity and time that a child could come into contact with creosote containing BaP in Japan. Based on these considerations, the amount of BaP was restricted to the following concentrations: 10 μg/g in creosotes and 3 μg/g in creosote-treated woods. Both benz[*a*]anthracene (BaA) and dibenz[*a*,*h*]anthracene (DBA) are also classified as belonging to Group 2A, due to their carcinogenic potential. The amount of DBA is lower than that of BaP in creosote, but DBA has a similar toxic equivalency factor relative to that of BaP.[10–12] The toxicity of BaA is not as great as that of BaP, but the amount of BaA in creosotes has been reported to be several times higher than that of BaP.[9] Therefore, the amount of these 2 PAHs are also restricted to the same level as that of BaP in creosotes. Creosotes that are commercially available in Japan undergo alkaline treatment after distillation (personal communication with a manufacturer), such that the content of water-extractable phenols is slight, relative to the EU control value.[9] It is therefore not considered important to measure and restrict the content of the phenols in creosotes manufactured in Japan.

The purpose of this study was to improve our previous analytical method[9] for the simultaneous determination of low levels of various PAHs (primarily BaP, BaA, and DBA) in creosotes and creosote-treated woods. Bestari *et al*. investigated the PAH content in wood products and the leaching behavior of creosote-treated wood by high-performance liquid chromatography (HPLC).[13] Anklam *et al*. used a HPLC system equipped with a fluorescence detector for the determination of the BaP content in creosotes.[14] HPLC determination is sensitive to PAHs, but the identification of individual PAHs by comparison of their retention time is less accurate than the use of gas chromatography (GC); furthermore, it remains difficult to detect 3 PAHs simultaneously when using fluorescence HPLC. A GC system equipped with a capillary column and mass selective-ion-monitoring (SIM) is useful for detecting each chemical selectively and provides sufficient separation for the quantification of the PAHs in a complex.[15–22] Therefore, we used GC-mass spectrometry (GC-MS) for the determination of PAHs in creosotes. With our previous method, the sensitivity of detection was low with respect to the determination of 3–10 μg/g of PAHs in creosote and creosote-treated wood products,[9] and a long period of time was required for GC-MS analysis, *i.e.*, more than 30 min per sample. Therefore, we adopted an evaporation-concentration step and changed the column temperature conditions in order to increase the sensitivity of testing and to reduce the amount of time needed to perform GC-MS.

MATERIALS AND METHODS

Samples ——— Four commercially available creosotes (Nos. 1–4) and non-creosote type (creosote-alternative) oil-based wood preservative paints were purchased from stores in Tokyo Metropolis, and in Gumma and Kagawa prefectures. Four creosotes (Nos. 5–8) were provided by the Japan Aromatic Industry Association, Inc. (JAIA). The three trial products used for the collaborative study (codes A–C) were supplied by a manufacturer. Three new creosote-treated wood products were supplied by the Japan Wood Preservers Industry Association (JWPIA). The JWPIA reported that two of the products (samples B and C) were Kempas, those were used for railway sleepers. The other sample was Japanese hemlock, which was used for building foundations (sample A). Two previously used railway sleepers (samples D and E) that had been treated by creosote penetration and were imported for use in gardening were also purchased. One of these samples was pine wood. Sample F was a brand-new wooden stake made with Japanese cedar, the surface of which was painted with creosote.

Chemicals ——— An EPA PAH mixture containing acenaphthene, acenaphtylene, anthracene, BaA,

benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, BaP, chrysene, DBA, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene at a concentration of 2000 μg/ml in dichloromethane : benzene (1 : 1) (Spelco, U.S.A.) 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 (Spelco, or Wako Pure Chemical Industries, Ltd., Japan) were obtained as an internal standard for the determination of PAH levels. 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.

Instruments —— A gas chromatograph (Hewlett-Packard GC5890 Series II Plus) connected to a JEOL JMS-AM II 20 mass spectrometer was used for the analysis of the individual PAHs. System control and data processing were performed using the JEOL Automass II program (JEOL Datum). The column was a PTE™-5 fused silica capillary column (30 m in length and 0.25 mm i.d. coated with a 0.2 μm thickness of 5% diphenyl-95% dimethylpolysiloxane film, Spelco). Sep-Pak Plus silica-cartridges (Part No. WAT020520) for solid phase extraction were obtained from the Waters Corporation (U.S.A.).

Extraction —— For the creosote oils, the weight (g) of a 0.5 ml sample was measured in advance. The Sep-Pak Plus silica-cartridge was conditioned using 10 ml of dichloromethane. Then, 0.5 ml of the sample was loaded onto the cartridge and was eluted with 10 ml of dichloromethane into a round-bottomed flask. The eluate was evaporated to approximately 2 ml under reduced pressure.

For extraction from the wood products, a section of approximately 2 cm in depth from the surface of the sample was removed, and that section was further cut into small pieces of *ca.* 2–3 mm wide and 2 cm long. A portion of the 1.0 g fragment was placed into a screw-capped glass tube. After the addition of 20 ml of dichloromethane, the tube was incubated for 24 hr at 37°C. The extract was filtered off and collected into a round-bottomed flask. The wood sample and tube were washed with a small amount of dichloromethane. The combined eluate was evaporated under reduced pressure. The concentrated eluate was loaded onto a Sep-Pak Plus silica-cartridge and eluted with 10 ml of dichloromethane from the cartridge into a flask. The eluate was then concentrated to a volume of approximately 2 ml by evaporation.

GC-MS Determination —— The total volume of the extract solution was adjusted to 5 ml with dichloromethane. For the determination of a high quantity of PAHs over the range of a calibration curve, a series of diluted sample solutions (1 ×, 20 ×, 100 ×, 500 ×, and 1000 ×) was prepared with dichloromethane. Two ml of each (diluted) extraction solution was obtained and spiked with 0.5 ml of the internal standard solution (*e.g.*, chrysene-d12 20 μg in dichloromethane), and the sample solution (1 μl) was injected into the GC-MS system in a splitless manner. The GC column temperature was programmed as follows: the column was first maintained at 60°C for 2 min and was then heated at a rate of 25°C/min to 300°C, after which it was held at this temperature for 6 min. The injection temperature was maintained at 280°C. The GC-MS transfer line temperature was 280°C, and the ion source temperature was 180°C. The carrier gas was helium and the column flow was maintained at 1.0 ml/min. The MS electron impact ionization energy was 70 eV. Detection was carried out using a total scan, (TIC) m/z = 45–500, and SIM. The individual m/z values for each compound are shown in Table 1. The PAH standard solutions (*e.g.*, 0.5, 1, 2, 5, and 10 μg/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 were established. Compounds in the sample solutions were identified based on their retention times and on the basis of agreement with the mass chromatograms of the PAH standard solutions using a BenchTop/PBM Mass Spectral Identification system (Palisade Co., U.S.A.) with the Wiley Registry of Mass Spectral Data (John Wiley & Sons. Inc., U.S.A.). Each PAH concentration (μg/ml) in an appropriate diluted sample solution was derived from the calibration curves, and then the levels of the PAHs (μg) per 1 g of sample were derived.

RESULTS AND DISCUSSION

GC Conditions

GC-MS is the method of choice for conducting analyses of water and soil in the U.S. EPA.[16] A column with a film of 5% diphenyl-95% dimethylpolysiloxane gives excellent separation of 16 PAHs, so we used a PTE™-5 column of this type. In our previous study, the column was heated at a rate of

Table 1. PAHs Covered in this Study

Peak No.	Compound	CAS No.	Molecular formula	Ion for quantification (m/e)	GC retention time (min)	IARC cancer risk	Determination	Regulation
1	Naphthalene	91-20-3	$C_{10}H_8$	128	5.59			
2	Acenaphtylene	208-96-8	$C_{12}H_8$	152	7.35			
IS1	Acenaphthene-d10		$C_{12}H_{10}$	164	7.44			
3	Acenaphthene	83-32-9	$C_{12}H_{10}$	154	7.45			
4	Fluorene	86-73-7	$C_{13}H_{10}$	166	8.16	3		
IS2	Phenanthrene-d10		$C_{14}H_{10}$	188	9.11			
5	Phenanthrene	85-01-8	$C_{14}H_{10}$	178	9.12	3		
6	Anthracene	120-12-7	$C_{14}H_{10}$	178	9.15	3		
7	Fluoranthene	206-44-0	$C_{16}H_{10}$	202	10.23	3		
8	Pyrene	129-00-0	$C_{16}H_{10}$	202	10.36	3		
9	Benz[*a*]anthracene	56-55-3	$C_{18}H_{12}$	228	11.47	2A	*a*)	*b*)
IS3	Chrysene-d12		$C_{18}H_{12}$	240	11.47			
10	Chrysene	218-01-9	$C_{18}H_{12}$	228	11.49	3	*a*)	
11	Benzo[*b*]fluoranthene	205-99-2	$C_{20}H_{12}$	252	13.02	2B	*a*)	
12	Benzo[*k*]fluoranthene	207-08-9	$C_{20}H_{12}$	252	13.04	2B	*a*)	
13	Benzo[*a*]pyrene	50-32-8	$C_{20}H_{12}$	252	13.30	2A	*a*)	*b*)
14	Indeno[1,2,3-*cd*]pyrene	193-39-5	$C_{22}H_{12}$	276	15.33	2B	*a*)	
15	Dibenz[*a*,*h*]anthracene	53-70-3	$C_{22}H_{14}$	278	15.36	2A	*a*)	*b*)
16	Benzo[*ghi*]perylene	191-24-2	$C_{22}H_{12}$	276	16.06	3	*a*)	

a) Compounds determined in this study. *b*) Concentration of the compounds in creosotes was regulated in Japan.

10°C/min, and the duration of analysis of 16 PAHs exceeded 30 min per sample.[9] In the present study, we changed the temperature rate to 25°C/min in order to reduce the duration of the analysis. The final temperature was set at 300°C, as based on the separation of indeno[1,2,3-*cd*]pyrene, DBA, and benzo[*ghi*]perylene. The retention time and quantitative ion mass of each PAH is shown in Table 1 under the present conditions. The GC-MS analytical time was reduced from > 30 to 17 min.

The calibration curve for the ratio of the peak area of each PAH to the internal standard (*e.g.*, chrysene-d12) for the respective mass of each ion was established, and each PAH concentration was derived from the calibration curve. The linear calibration curve for each PAH was obtained within the range of 0.2 and 5 μg/ml. The correlative coefficients of BaA, BaP, and DBA were 0.9984, 09961, and 0.9983, respectively. The limit of the quantity of these compounds in the injection solution was considered as 0.2 μg/ml, *i.e.*, the lowest concentration used for the linear calibration curve.

Solid Phase Extraction

Column chromatography is generally used to separate PAHs from various other chemicals in creosote.[15,18,21] Here, target PAHs were separated by a solid phase extraction approach using Sep-Pak Plus silica-cartridges. A PAH standard solution (10–20 μg in 0.1 ml) was loaded onto the cartridge, and then was eluted with 10 ml of dichloromethane. The recovery of PAHs in this fraction was 94.4–97.8%. In the next eluate with 10 ml dichloromethane, no PAH was detected. A similar recovery test was carried out using creosote, and a high yield of PAHs was eluted from the cartridge (data not shown). Solid phase extraction with dichloromethane was found to be efficient for separating the PAHs contained in creosote.

Extraction of PAHs

In this study, we first compared the efficiency of the solvents (*i.e.*, dichloromethane, methanol, hexane, and saline) on the extraction of PAHs from creosote-treated woods. A higher concentration of PAHs was detected in the extracts with dichloromethane than in those with hexane, methanol, and saline (Table 2). The dichloromethane extract could be loaded directly onto the Sep-Pak Plus silica cartridges, and was eluted with dichloromethane. Therefore, we found that dichloromethane was a suitable solvent for the extraction of PAHs from creosote-treated wood.

Soxhlet-extraction techniques have generally

Table 2. Comparison of Extraction Solvent on Recovery of PAHs from Creosote-Treated Wood Product

Compound	Amount detected (μg/g)			
	Dichloromethane	Methanol	Hexane	Saline
Naphtalene	295	81	9	0
Acenaphtylene	173	28	11	1
Acenaphtene	4854	1496	639	3
Fluorene	4385	1250	685	4
Phenanthrene	17578	5680	2749	6
Anthracene	4728	1590	1146	2
Fluoranthene	6406	2397	1097	4
Pyrene	4494	1613	762	4
Benz[*a*]anthracene	1019	315	131	4
Chrysene	976	291	131	3
Benzo[*b*]fluoranthene	558	106	38	2
Benzo[*k*]fluoranthene	382	71	26	2
Benzo[*a*]pyrene	380	82	25	0
Indeno[1,2,3-*cd*]pyrene	157	45	17	0
Dibenz[*a,h*]anthracene	134	24	18	0
Benzo[*ghi*]perylene	152	32	9	0

Sample (1.0 g) was extracted with 20 ml of each solvent at 37°C for 24 hr. In the case of saline, the water layer was used, and liquid-liquid extraction was performed by shaking with dichloromethane. The extract was loaded onto a Sep-pak silica cartridge, and was eluted with 10 ml of dichloromethane. The fraction was diluted with dichloromethane to the appropriate concentration, and injected into GC-MS.

been used for the extraction of creosote-treated wood, but such an approach requires long extraction periods and is unsuitable for processing large numbers of samples. Some groups have reported that mechanical shaking and liquid extraction methods are also useful, as is Soxhlet extraction.[13,15,18,21,22] Our group also previously confirmed the usefulness of soak-extraction for the analysis of wood samples.[9] Sonication is thought to enable the rapid extraction of PAHs. Here, we compared the potential usefulness of sonication and soaking for extraction of PAHs from wood samples. Dichloromethane (20 ml) was added to 1 g of creosote-treated wood, and extraction was carried out with sonication for 30 min at room temperature or with incubation for 24 hr at 37°C. After extraction by either soaking or sonication, both samples were once again subjected to extraction by soaking them in 20 ml of dichloromethane for 24 hr at 37°C. The concentrations of 8 PAHs extracted under both sets of conditions are shown in Table 3. The amount of PAH released by the soaking approach to extraction was higher than that detected by sonication extraction. Upon the second cycle of soaking extraction following sonication, a considerable amount of PAH was detected. This finding indicated that a large quantity of PAH remained in the wood, even after sonication; moreover, most of the PAH was released after a single treatment by soaking extraction.

After the samples had been soaked, some amount of dichloromethane containing the PAHs remained in the wood, and thus complete extraction of PAHs was not considered possible. This issue may lead to a reduction in the recovery percentage, especially in cases involving a small quantity of PAHs. However, as noted above, the amount of extracted PAHs did not increase, even upon repetition of the soak extraction, and the PAHs not extracted from the wood under these conditions would not be expected to pose a health risk. We therefore chose the condition under which 1 g of sample would be incubated with 20 ml of dichloromethane for 24 hr at a temperature of 37°C for the extraction of PAHs.

In our previous study, the test solution that was extracted from the sample was not concentrated before injection into the GC-MS.[9] For creosotes, 0.1 g of sample was diluted with 20 ml of dichloromethane, and the extract from 1 g of wood sample was diluted to a total volume of 25 ml. In addition, the elution of 3 target PAHs from the GC column was slow. The limit of determination of each PAH per sample was > 40 μg/g according to that method. In the improved method, the eluate from the Sep-Pak Plus silica-cartridges was concentrated by evaporation before injection into the GC-MS. The eluate from 1 g of wood sample or 0.5 g of creosote was

Table 3. Amount of PAHs Extracted from Wood Product by Each Condition

Compound	Amount determined (μg/g)			
	Condition A		Condition B	
	(1) Incubation	(2) Incubation	(1) Sonication	(2) Incubation
Benz[*a*]anthracene	668	38	310	540
Chrysene	535	25	253	420
Benzo[*b*]fluoranthene	278	17	122	166
Benzo[*k*]fluoranthene	156	7	73	139
Benzo[*a*]pyrene	141	3	53	102
Indeno[1,2,3-*cd*]pyrene	41	ND	29	22
Dibenz[*a*,*h*]anthracene	12	ND	12	5
Benzo[*ghi*]perylene	31	ND	23	16

One gram of wood sample was extracted with 20 ml dichloromethane by incubation for 24 hr at 37°C for conditon A or sonication for 30 min at room temperature for conditon B (1). After obtaining the solution, each sample was again extracted with the same volume of dichloromethane by incubation for 24 hr for condition A or 48 hr for condition B (2). The amount of PAHs in each extract was determined. Data are the average values of 2–3 experiments. ND means < 1.0 μg/g.

Table 4. Analytical Results of Trial Creosote Products Containing Low Concentrations of Regulated Compounds Performed in 4 Laboratories

Compound	Concentration (μg/g, mean $\pm$ S.D., n = 2–5)			
	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4
(a) Sample A				
Benz[*a*]anthracene	53.0 ± 6.9	52.0 ± 1.0	50.8 ± 0.6	NT[a)]
Benzo[*a*]pyrene	29.7 ± 5.4	29.0 ± 0.5	30.9 ± 0.3	36.4 ± 0.8
Dibenz[*a*,*h*]anthracene	6.5 ± 0.2	4.0 ± 0.2	5.2 ± 0.7	NT
(b) Sample B				
Benz[*a*]anthracene	18.0 ± 0.9	15.0 ± 0.4	14.5 ± 0.8	NT
Benzo[*a*]pyrene	12.5 ± 1.1	9.0 ± 0.3	11.0 ± 0.5	12.3 ± 1.3
Dibenz[*a*,*h*]anthracene	3.2 ± 1.0	2.3 ± 1.3	1.8 ± 0.2	NT
(c) Sample C				
Benz[*a*]anthracene	5.9 ± 0.2	6.0 ± 0.1	4.8 ± 0.2	NT
Benzo[*a*]pyrene	3.9 ± 0.3	3.0 ± 0.1	2.9 ± 0.4	3.6 ± 0.6
Dibenz[*a*,*h*]anthracene	ND[b)]	ND	1.1 ± 0.3	NT

a) NT = not tested. *b*) ND = not determined, < 1.5 μg/g.

adjusted to a total volume of 5 ml in solution before injection into the GC-MS. The peak height and area of each PAH observed in the GC chromatogram increased due to alteration of the column temperature. Therefore, the limit of determination of PAHs in the injection solution became 0.2 μg/ml. As regards the amount in the creosote and creosote-treated samples, the limit of determination of 3 PAHs was 1 and 2 μg/g, respectively.

Collaborative Study

In order to validate the method developed here, 3 creosotes containing different amounts of regulated chemicals were used, and these samples were analyzed by three different laboratories in terms of their concentrations of BaP, BaA, and DBA; a fourth laboratory (Laboratory 4) measured only the BaP content of these samples. The experiment was repeated 2–5 times at each laboratory (Table 4). Sample A contained large amounts of BaA, BaP, and DBA in these 3 creosotes. The value of BaA at a concentration of 50.8–53.0 μg/g was higher than that of BaP. The present concentrations of BaP (29.0–36.4 μg/g) were allowable according to the EU control (< 50 μg/g), but they remained above the allowed value in Japan. As regards sample B, all laboratories reported similar levels for each compound. Sample C contained BaA, BaP, and DBA at concen-

Table 5. Amount of 8 PAHs in Creosotes and Creosote-Like Wood Preservatives

Compound	Amount (μg/g)							
	Creosote							
	Commercially available product				Product supplied from manufacturer			
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Benz[*a*]anthracene	38	1763	959	857	2714	2005	18	6052
Chrysene	31	1500	848	800	2312	1410	15	6600
Benzo[*b*]fluoranthene	20	556	414	543	1481	1441	4	4918
Benzo[*k*]fluoranthene	13	456	304	379	874	803	5	2184
Benzo[*a*]pyrene	16	363	280	368	1018	1122	4	2591
Indeno[1,2,3-*cd*]pyrene	8	111	60	77	119	193	4	1257
Dibenz[*a*,*h*]anthracene	2	33	11	12	199	188	4	442
Benzo[*ghi*]perylene	8	106	55	70	72	113	4	1442

Compound	Amount (μg/g)			
	Creosote-like	Creosote		
	Commercially available product	Trial product		
	No. 9	Code A	Code B	Code C
Benz[*a*]anthracene	24	6	13	38
Chrysene	23	2	12	27
Benzo[*b*]fluoranthene	14	5	11	29
Benzo[*k*]fluoranthene	10	ND	6	14
Benzo[*a*]pyrene	13	2	8	28
Indeno[1,2,3-*cd*]pyrene	5	ND	3	10
Dibenz[*a*,*h*]anthracene	1	ND	2	4
Benzo[*ghi*]perylene	6	ND	3	10

ND means $< 1.5\mu$g/g.

trations of 4.8–6.0, 2.9–3.9, and 0 (*i.e.*, below the determination limit)–1.1 μg/g, respectively. All laboratories reported almost the same concentrations of PAHs in creosotes. Thus, the analytical method developed here appears to be sufficiently stable and can be used for the determination of low levels of BaA, BaP, and DBA.

Determination of PAHs in Creosote and Creosote-Treated Wood

The results of the analysis of 8 PAHs including BaA, BaP, and DBA in 8 creosotes (Nos. 1–8), 1 creosote-like (alternative) wood preservative (No. 9), and 3 creosotes used as trial products in the collaborative study (codes A–C) are shown in Table 5. These products were for sale and available to the general public for use in wood preservation prior to the introduction of the Japanese regulations. Among the carcinogenic PAHs classified as being associated with a cancer risk of 2A or 2B,[6] BaA was detected in the highest concentrations, varying between 18 and 6052 μg/g in these creosotes. Isomers of benzofluorancenes and BaP were detected at similar levels. The respective amounts of indeno[1,2,3-*cd*]pyrene, DBA, and benzo[*ghi*]perylene were low, compared to those of the other PAHs. The PAH profiles regarding the constituents and levels of creosotes in Japan were similar to those for the old type of creosotes used in the EU.[17,18] Improved creosotes, *i.e.*, those in which BaP amounts were reduced by the fractionation of lower boiling-temperature components, were used for the construction of wooden railroad ties in Switzerland.[18] Among the creosotes examined, both samples No. 1 and 7 contained BaP at levels of less than 50 μg/g, the allowable limit adopted for the EU regulations. The manufacturer reported that samples such as No. 7 have been produced for export to the EU by fractionating lower boiling components. Recently, the quality of the creosote that can be used as a wood preservative was revised for the Japanese Industrial Standard (JIS).[23]

Entirely different constituents were found in the wood preservative of the non-creosote type (No. 9), as shown in the total ion chromatogram of Fig. 1; in this sample, the amount of PAHs was low. The content of BaP was lower than the EU-sanctioned

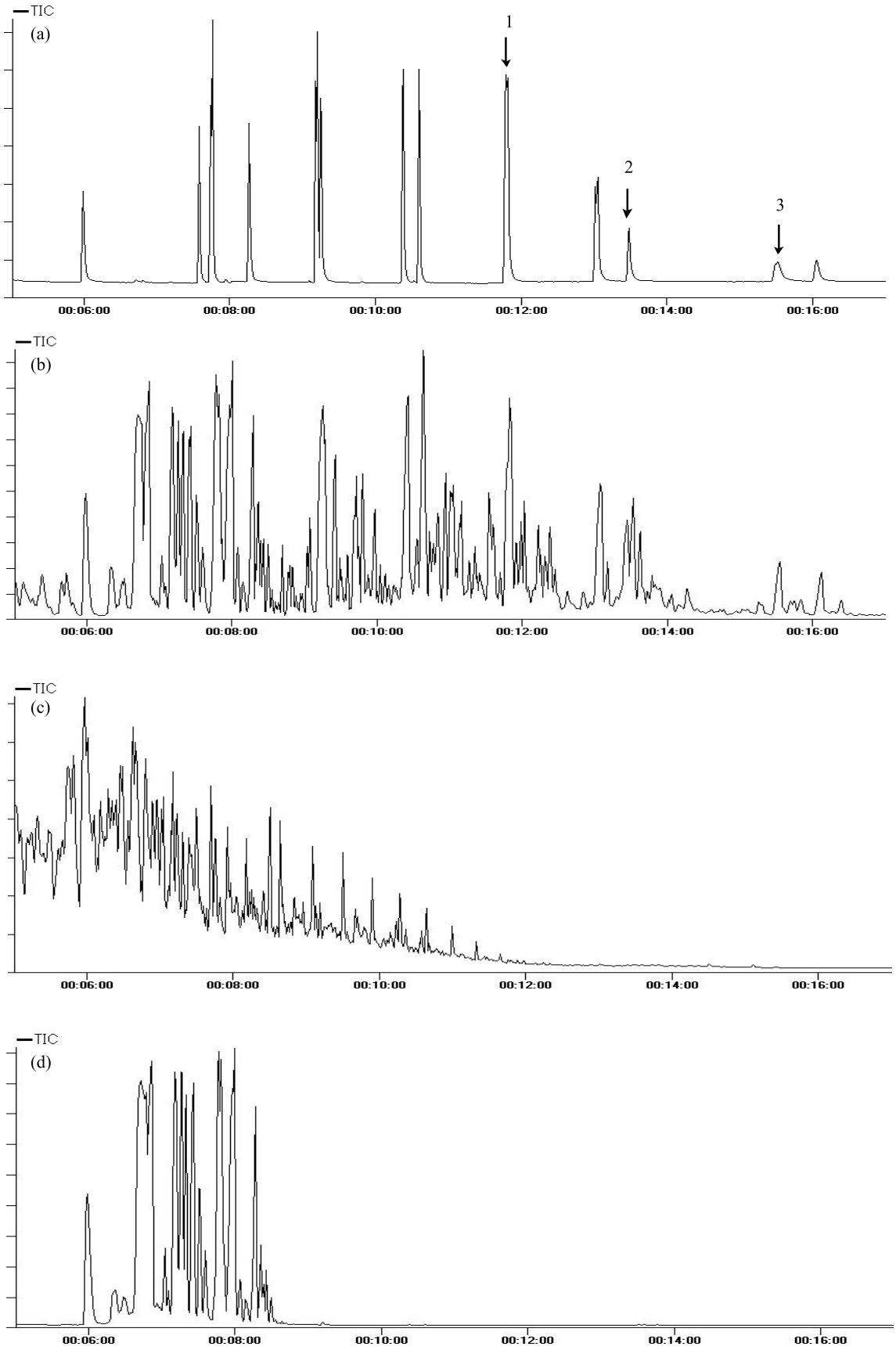

Fig. 1. Total Ion Chromatogram of Creosotes

(a) PAH standard solution (5 μg/ml), (b) sample No. 8, (c) sample No. 9, (d) sample code C. Peak 1: benz[*a*]anthracene, 2: benzo[*a*]pyrene, 3: dibenz[*a*,*h*]anthracene.

Table 6. PAHs Content in Creosote-Treated Wood Products

	Content (μg/g)					
	A	B	C	D	E	F
	Foundation	Railway Sleeper	Railway Sleeper	Railway Sleeper	Railway Sleeper	Stake
	New	New	New	Used	Used	New
Benz[*a*]anthracene	668	193	274	206	455	2
Chrysene	535	172	350	177	562	2
Benzo[*b*]fluoranthene	278	75	230	154	323	1
Benzo[*k*]fluoranthene	156	44	113	103	189	1
Benzo[*a*]pyrene	141	47	126	88	168	ND
Indeno[1,2,3-*cd*]pyrene	41	16	48	20	186	ND
Dibenz[*a*,*h*]anthracene	12	3	18	6	18	ND
Benzo[*ghi*]perylene	31	13	69	14	203	ND

ND means < 1.0 μg/g.

amount in all samples, but still exceeded 10 μg/g in No. 9 and in code C. This finding indicated that some wood preservatives, even those lacking creosote as a constituent, possibly contain high levels of PAHs, *i.e.*, those above the control amount. It is therefore necessary to be aware of the amount of PAHs, not only in cases involving creosote, but also in cases involving oil-type wood preservatives, in which creosote is not listed as a constituent. Sample code A was the only product tested here that conformed to the Japanese regulations.

Sample sections were taken approximately at 2 cm in depth from the surface of wood products manufactured in accord with the Japan agricultural standards (JAS) for the timber of the broadleaf tree. The samples were cut and treated with dichloromethane, and the amount of PAHs contained therein was determined (Table 6). In the commercially available samples A–E, amounts of PAH in excess of 3 μg/g were detected. These findings indicated that creosotes containing more than 10 μg/g each of BaA, BaP, and DBA had been used in the preservation of these samples. A small amount of PAHs per stake material was also detected. Macroscopic studies of sections taken 1 mm from the surface revealed a change in color, which indicated that the creosote had not penetrated to the center of the wood, and instead remained near the surface. Accordingly, almost all of the layers cut from the stake sample contained no creosote, and the total PAH content was low in the test solution prepared from this sample.

In conclusion, we improved upon our previous analytical method for determination of PAHs in creosotes and in creosote-treated woods. We adopted an evaporation-concentration step and changed the column temperature conditions in order to increase the sensitivity of testing and to reduce the amount of time needed to perform GC-MS. The result of a collaborative study indicated that the analytical method developed here appears to be sufficiently stable and can be used for the determination of low levels of BaA, BaP, and DBA. We found that these compounds in high concentrations, thus exceeding the allowed control value, were contained in some creosotes and railway sleepers.

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