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Skin analysis to determine causative agent in dermal exposure to petroleum products

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Abstract

This study evaluates the usefulness of skin analysis to determine the causative agent in cases of dermal exposure. The study consists of an animal experiment and two human cases. The petroleum components detected at high concentrations in skin samples resembled the composition of those in the corresponding petroleum products. However, the petroleum components in blood were detected at low concentrations and were a different composition. Skin is considered to be an advantageous sample to estimate the petroleum product in clinical and forensic cases of dermal exposure.

Keywords: Skin; Dermal exposure; Petroleum; Hydrocarbons; GC–MS

1. Introduction

Blood or urine samples are commonly used specimens for diagnosis in the medical field. In cases of intoxication, estimating the causative agent is the first requirement for treatment in the clinical field. If the cases are involved in accidents or crimes, identifying the chemical is essential for forensic investigation. Except for several medicines or illicit drugs that can be analyzed by an automated drug monitoring system or commercially available screening kit, rapid determination of the causative chemical in biological samples is generally laborious.

Our recent animal studies involving kerosene commonly involved in crimes or accidents have suggested that skin analysis is much more efficient than blood analysis to identify kerosene components in dermal exposure cases. This is because (1) aliphatic hydrocarbons, essential components to discriminate petroleum products, tend to remain in skin [1,2], (2) aromatic hydrocarbons, relatively common components in most petroleum products, are easily absorbed via skin into blood-circulation [3], and (3) to analyze aliphatic components, a small amount of skin sample is adequate as compared to blood sample [1,2].

In addition to kerosene, gasoline and light oil are also commonly used petroleum products in daily life and are often involved in accidents or crimes due to their easy accessibility, inflammable and inflammatory properties [4,5]. These products consist of thousands of hydrocarbons and they are roughly discriminated from each other by their constitution of aliphatic hydrocarbons, which are a result of different boiling points of distillates. The purpose of this study was to evaluate whether skin samples would be useful to identify a dermally exposed petroleum product through an animal experiment and two firsthand practical human cases.

2. Materials and methods

2.1. Reagents

Regular gasoline, kerosene and light oil were obtained from Shell Petroleum Ind. (Tokyo, Japan) and were used as standard petroleum products. Analytical grade *o*-xylene-

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d_{10} (used as internal standard; IS), saturated aliphatic hydrocarbons (AHCs) (C_{10}–C_{20}), three kinds of trimethylbenzene (TMB)(1,3,5-, 1,2,4- and 1,2,3-TMB) and naphthalene were purchased from Wako Pure Chemical Ind. (Osaka, Japan). The standard solutions were dissolved in ethanol. In this experiment, ethanol and *n*-pentane were distilled three times before being used.

2.2. Biological samples

The samples were obtained from an animal experiment using rats and two human cases.

2.2.1. Animal experiment

The experimental protocols were approved by the Animal Experimental Committee at Shimane University School of Medicine (the protocol number #02-100). Six male Sprague–Dawley rats (B.W. 300–350 g)(Charles River Breeding Labs, Yokohama, Japan) were used for this experiment and were under anesthesia (2 ml/kg i.m. of a mix of droperidol 1.25 mg/ml and fentanyl 0.025 mg/ml) during the whole experiment. Dermal exposure and sampling were performed utilizing our previously reported method [1]. Briefly, the abdominal fur was closely clipped and a square piece of cotton (4 cm × 4 cm) soaked with 4 ml of either standard gasoline, kerosene or light oil was applied for 2 h ($n = 2$ of each). The cotton was covered with impermeable stretch film to prevent vaporization and immobilized by adhesive bandage (10 cm × 20 cm) during the 2 h exposure period. The exposed skin was thoroughly washed with soap at 2 h, and then the rats were sacrificed by decapitation. Trunk blood was collected and the exposed skin was excised for GC–MS assay. The skin samples were put into a small plastic bag with the air eliminated. To keep the petroleum components stable, the samples were stored at −80 °C until assay.

2.2.2. Human cases

Case 1. A 73-year-old woman visited our hospital with complaining of extensive erythema over her abdomen, which had developed from the previous evening. The lesions seemed to be caused by dermal exposure to some chemical. The lesions were first washed thoroughly with saline. The detached roof of bulla (mainly epidermis) over the lesions was removed by scissors, which is a routine painless procedure in clinical treatments before applying ointment. Though the removed bulla was generally discarded, the part of the bulla (total of about 0.1 g) was saved as a skin sample for the diagnostic analysis in this case. Five milliliter of blood was collected for routine hematological and biochemical analyses and for the diagnostic analysis. These materials were collected with the written informed consent of the patient. A part of the skin sample (0.01 g) and blood sample (0.5 ml) was extracted after the sampling and the rest of the samples were stored at −80 °C.

Case 2. A 37-year-old man was found dead at the scene of a fire. The next day (about 24 h after the fire), a medicolegal autopsy was performed to elucidate the cause of death and the situation caused death. The body surface was covered with variety of first to fourth degree burns. Small skin samples (approximately 1 cm × 1 cm) were collected as forensic specimens from different parts of the body (left shoulder, left chest, right and left arms, abdomen) together with regular specimens (blood, urine, tissue samples) at autopsy. The incisions were sutured after the sampling. Subcutaneous fat and most of the dermis were immediately removed from the upper part of the skin (mainly epidermis) by scissors, and then the upper layer of skin was stored at −80 °C until assay.

2.3. Sample preparation

Petroleum components in skin (0.01–0.03 g) and whole blood (0.5 ml) were extracted by liquid-liquid extraction utilizing our previously reported method [1,2]. Briefly, the skin sample was quickly minced by scissors and immediately put into distilled water (2 ml) in a glass tube that was previously weighed. The tube was then weighed again to calculate the skin weight. IS (10 ng) and *n*-pentane (7 ml) were sequentially added. Petroleum components were extracted with *n*-pentane for 20 min using a shaker (SA-31, Yamato Scientific, Kyoto Japan). The tube was centrifuged at 3000 rpm for 10 min and put into a freezer at −80 °C to freeze the aqueous phase (bottom layer). One hour later, the pentane layer was decanted into a new glass tube and concentrated under a N_2 stream into about 100–150 μl. The 1 μl of the extract was injected onto a GC–MS system. For the blood sample, 0.5 ml of whole blood was put into distilled water (1.5 ml), and then the skin sample procedures were followed exactly.

2.4. GC–MS condition

GC–MS analysis was carried out using a HP-5972 GC–MS system. The separation was achieved by a capillary column (HP-5MS, 0.25 mm × 30 m i.d., 0.25 μm thickness). The column temperature was set at 50 °C for 1 min, increased at 10 °C/min up to 280 °C and held for 10 min. The temperature of injection port and ion source was set at 270 and 280 °C, respectively. Scan mode (*m*/*z* 40–300) and selected ion monitoring (SIM) mode analyses were performed for qualitative and quantitative purposes, respectively. *m*/*z* 98 (IS), 120 and 105 (TMBs), 128 (naphthalene), and 57 and 71 (aliphatics) were monitored for three kinds of TMBs, naphthalene and aliphatics (C_{10}–C_{20}) to be quantified.

3. Results

3.1. Determination of gasoline, kerosene and light oil

Fig. 1 shows typical mass chromatograms of the major components of extracted standard petroleum products (1 μl)

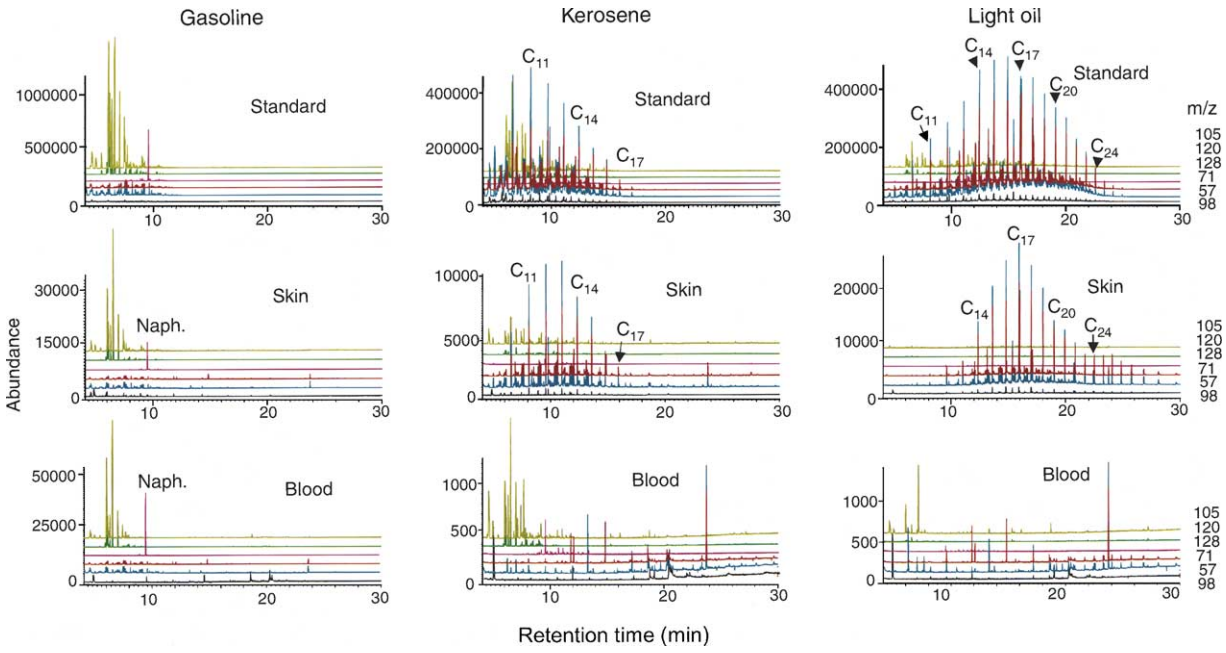

Fig. 1. The mass chromatograms monitoring IS (*m*/*z* 98), aliphatic hydrocarbons (*m*/*z* 57 and 71), naphthalene (Naph.) (*m*/*z* 128) and trimethylbenzenes (TMBs) (*m*/*z* 120 and 105) of extracted standard petroleum products (top), skin (middle) and blood (bottom) samples obtained from the animal experiment are shown. The units on the *Y*-axis are different among individual chromatograms.

(top). The individual detected peaks were identified by analyses of standard solution and/or library of mass spectra preinstalled in HP Chemistation™. The major hydrocarbons were detected in different spans of retention times depending on the products. Among the detected peaks, three kinds of TMBs and naphthalene (common components of three products) and C_{10}–C_{20} AHCs (different components depending on the product) were quantified by the peak-area ratios compared to IS of individual components. The detection limit was 20 pg for TMBs, 5 pg for naphthalene, and 5–13 pg for AHCs (C_{10}–C_{20}) on column (S/N = 3). The extraction recoveries of these standard components were 88–91% for TMBs, 95% for naphthalene and 78–94% for AHCs when 100 ng/ml of solution was extracted. However, these recoveries were

Table 1
The intra- and inter-day precision and accuracy of four components

	Spiked amount/sample (ng)	Intra-day		Inter-day	
		Precision (ng/g)	Accuracy (%)	Precision (ng/g)	Accuracy (%)
Skin					
1,2,4-TMB	50	46.5 ± 9.3	93.0	47.6 ± 10.5	95.2
	500	501.2 ± 70.2	100.2	486.9 ± 65.4	97.4
Naphthalene	50	51.3 ± 4.3	102.6	49.5 ± 7.3	99.0
	500	493.5 ± 26.7	98.7	476.9 ± 18.7	95.4
C_{11}	50	47.6 ± 8.5	95.2	43.8 ± 12.3	87.6
	500	521.0 ± 35.2	104.2	529.1 ± 80.3	105.8
C_{14}	50	44.3 ± 6.7	88.6	46.5 ± 7.0	93.0
	500	530.5 ± 21.6	106.1	510.8 ± 46.9	102.2
Blood					
1,2,4-TMB	50	47.5 ± 10.1	95.0	51.2 ± 9.6	102.4
	500	505.3 ± 43.2	100.6	508.1 ± 52.9	101.6
Naphthalene	50	50.6 ± 5.3	101.2	52.3 ± 4.6	104.6
	500	507.9 ± 18.9	101.6	490.5 ± 24.3	98.1
C_{11}	50	48.2 ± 7.6	96.4	46.6 ± 8.3	93.2
	500	532.0 ± 41.7	106.4	509.8 ± 64.2	102.0
C_{14}	50	48.7 ± 6.4	97.4	47.5 ± 6.9	95.0
	500	519.4 ± 38.6	103.9	497.7 ± 51.3	99.5

Data express mean ± SD (n = 6).

rough estimates since the final volume of solvent following evaporation under a N_2 stream was not able to exactly equal between samples.

For analyses of these components in skin and blood samples, the calibration curves, recoveries, intra- and inter day precision and accuracy were determined using several intact rats that were not exposed to any petroleum products by spiking with standard components. Good linearity was observed between 10 and 1000 ng/sample when individual components were spiked in skin and blood samples. If the recovery of standard solution was assumed to be 100%, the relative recoveries of individual components for skin and blood samples were 94–105% for skin samples and 89–102% for blood samples. The intra- and inter-day precision and accuracy are shown for four components (Table 1), and we found the coefficient variations to be greater at low concentrations in all components.

3.2. Animal study

The chromatograms of Fig. 1 (middle and bottom) show skin and blood samples obtained from the animal experiment. The patterns of the chromatograms in blood appear different from standard kerosene and light oil once these products were absorbed via skin, while the chromatograms obtained from skin samples appear to be similar to the standard pattern. To characterize the difference of three petroleum products, the peak area ratio to IS of three kinds of TMBs and naphthalene were totalled as aromatics, and the peak area ratio to IS of saturated aliphatic hydrocarbons were totalled as aliphatics (AHCs), and then the percentage of AHCs (%AHCs) in the total of aromatics and AHCs were calculated [100 × AHCs/(AHCs + aromatics)] (Table 2). The peak area ratio, not concentration, was utilized for this calculation since (1) several aliphatic components ($<C_{10}$ and $>C_{20}$) were not quantified but detected and (2) peak area ratio can be easily calculated without calibration curves and may be convenient in practical examination to capture the rough pattern of detected components. In standard products, the %AHCs of gasoline was lower than those of kerosene and light oil. The %AHCs of skin samples were slightly greater than those of standard products, while those of blood samples were different in kerosene and light oil.

Table 2
The percentages of AHCs (%AHCs) calculated from peak area ratio to IS are shown [%AHCs = 100 × AHCs/(AHCs + aromatics)]

Standard product		Rat	Skin	Blood
Gasoline	12.6 ± 2.1	#1	14.3	68.0
		#2	15.1	74.6
Kerosene	85.4 ± 3.4	#3	93.3	16.0
		#4	88.4	5.0
Light oil	97.9 ± 0.3	#5	99.7	68.6
		#6	99.5	69.6

Aromatics represent the total of three kinds of trimethylbenzenes and naphthalene. Aliphatic hydrocarbons (AHCs) represent saturated aliphatic hydrocarbons. Data express mean ± SE for standard ($n = 5$) and actual data of individual animal ($n = 2$ of each).

Table 3
The concentrations (μg/g) of four components in human cases and the calculated %AHCs

	Aromatics		Aliphatics		%AHCs
	1,2,4-TMB	Naphthalene	C_{11}	C_{14}	
Case 1 (dermal exposure)					
Skin	1.70	1.91	71.95	143.36	99.3
Blood	0.02	n.d.	0.004	n.d.	17.5
Case 2 (dermal exposure + inhalation)					
Skin	1.67	1.50	10.39	14.15	96.8
Blood	0.24	0.08	1.09	n.d.	86.8

The concentrations in skin were greater in all components than those in blood. When the total concentrations of 3 TMBs or 11 AHCs (C_{10}–C_{20}) were compared between skin and blood, the concentration in skin was the greatest in light oil and the least in gasoline (Fig. 2).

3.3. Human cases

The mass chromatograms obtained from two human cases, and their concentrations of major components and %AHCs are shown in Fig. 3 and Table 3, respectively. In case 1, high amounts of aliphatics were detected in the skin sample with a similar pattern and similar components for kerosene (Fig. 3a)

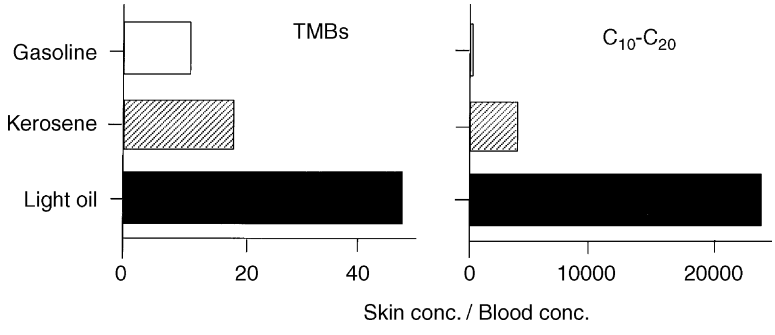

Fig. 2. The concentrations of trimethylbenzenes (TMBs) and C_{10}–C_{20} of aliphatic hydrocarbons were compared between skin and blood samples. The total amounts of three kinds of TMBs (1,3,5-, 1,2,4- and 1,2,3-TMBs) or total amounts of C_{10}–C_{20} of aliphatic hydrocarbons were calculated in blood and skin samples, and then the total amounts of skin samples were divided by those in blood samples.

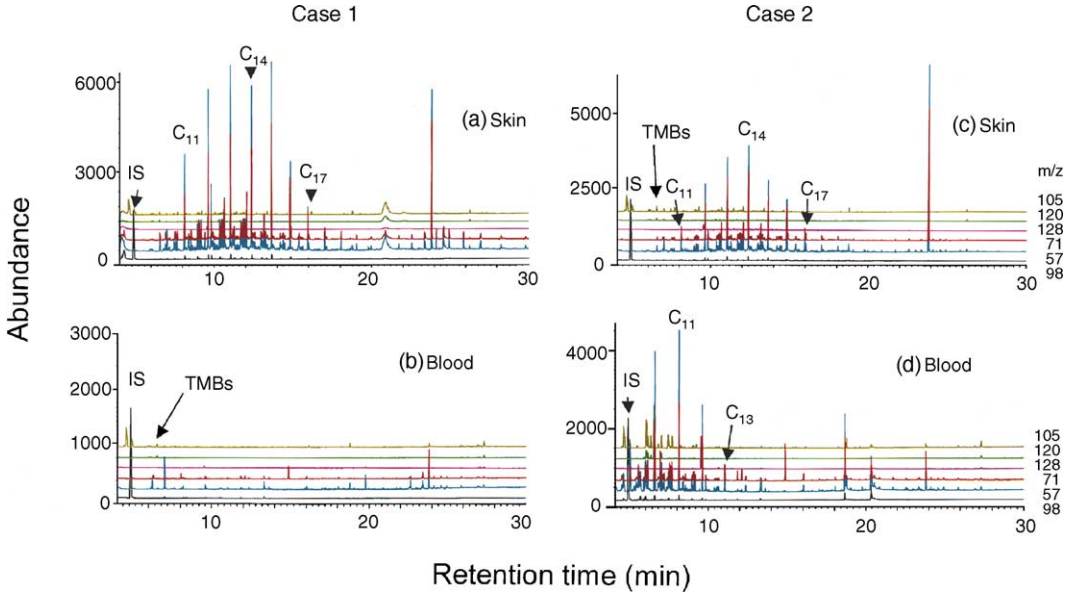

Fig. 3. Mass chromatograms obtained from human cases are shown.

and any other possible causative chemicals were not determined. Though trivial amounts of some kerosene components were detected in the blood, the causative agent could not be identified by blood analysis itself (Fig. 3b). The patient was diagnosed with kerosene dermatitis.

In case 2, aliphatics of C_{11}–C_{17} were detected with a similar pattern for standard kerosene in the skin sample (Fig. 3c). The left-arm skin sample, which consisted of mostly detached bulla, registered the highest concentration of kerosene components. In the blood, high concentrations of aliphatics of C_9–C_{13} were detected, and the components of detected AHCs were different from both standard kerosene and gasoline (Fig. 3d). Based on our overall forensic investigation, we consider the victim have committed suicide by self-burning using kerosene.

4. Discussion

Since GC–MS can easily detect hydrocarbons, numerous applications have been reported in various fields and there are many sensitive or specialized methods available depending on the purpose of study or target hydrocarbons [6–7]. A conventional method has been selected in our laboratory to detect a wide range of chemicals for screening purposes in forensic examinations [8]. This study focused on evaluating the usefulness of skin sample (as compared to blood), which has rarely been used as a biological specimen in forensic and clinical fields to identify the causative agent when dermal exposure was involved.

In the extraction procedures for skin samples, homogenization was not performed except for cutting skin into small pieces prior to weighing. This is because (1) homogenization lowers the recovery due to winding up into the shaft, which also results in bigger C.V. depending on how much skin sample is lost, (2) some components are easily evaporated during the homogenizing process due to their high volatilities and (3) pulverization of skin sample frozen in liquid nitrogen, which is sometimes utilized for the pretreatment of skin [9], has not worked well for small skin samples in our preliminary study. Quick performance in mincing skin, putting it into water and being capped was efficient and adequate for identifying the causative agent. The high recovery derived from skin samples was therefore expected because standard solution was spiked into the water together with the skin sample.

Though quantification from skin sample was carried out in this study, the quantitative data of skin samples are considered to be highly dependent on methodology due to skin's difficult physical properties. Equally degenerated skin sample is difficult to prepare unless some reliable pretreatment device is developed. There have been a few in vitro studies that analyzed the components of JP-8 jet fuel in skin samples, however, no study used homogenizer or a suitable device for preparation. Punched skin was directly put into a vial for headspace GC-FID in one study [10], and tape-stripped and cotton-swabbed methods were chosen in another study to determine radiolabeled components [11]. The concentrations obtained from these studies were generated by time-course changes within a same series of samples or by proportion among constituents within the sample. Unlike blood or urine analysis, concentrations obtained from skin analysis may not be comparable to other data obtained from different methodology. Skin analyses are more likely for qualitative purpose at this point, i.e. pattern analysis in a controlled condition.

The results of the animal experiment suggested that (1) skin sample chromatogram patterns were resembled those of standard petroleum products, while the patterns of blood samples differed (Fig. 1 and Table 2), (2) skin concentrations were much greater than blood concentrations, especially in aliphatics (Fig. 2). These results indicate that skin sample, compared to blood sample, is advantageous in discriminating petroleum products, especially in the amount needed for analysis. Greater retention of aliphatics (essential components to discriminate petroleum products in skin) results in less systemic absorption, which in turn results in easier detection of petroleum products in skin. The results of the human cases support the results of the animal experiment and suggest that skin analyses can be applicable in both clinical and forensic fields (Fig. 3).

In case 1, we confirmed that the patient fueled the stove several hours before the first awareness of redness on her thigh from the previous afternoon. Therefore, she was probably exposed to unnoticeable kerosene from her clothes. The duration of dermal exposure was about 8 h and the initial visit was approximately 12 h post-exposure. Systemic absorption of the kerosene components was considered mainly from dermal exposure. The concentrations in the blood sample were too low to obtain individual mass spectra, which is reasonable considering the elimination half-life of TMBs was about 3 h in a human case and Sprague-Dawley rats [3]. And the aromatic components almost disappeared at 24 h postexposure, which results in higher %AHCs than soon after the dermal exposure [12]. On the other hand, it was easy to identify kerosene from the detached roof of bulla. This was also reasonable considering the patient did not wash the lesion surfaces allowing the majority of components to remain except for some evaporation, absorption and metabolism within the skin. Further study is needed to evaluate how long the causative agents can be identified from skin samples.

In case 2, the essential components of aliphatics of kerosene with mass spectra were obtained from the skin sample, which strongly suggests that the victim's skin was in direct contact with kerosene. In the blood, aliphatics of C_9–C_{13} were detected, which was different from the results of case 1 and the animal experiment. The aliphatics would be derived mainly from inhalation of vaporized kerosene or combusted kerosene based on the result reported by Kimura et al. [13], in which lower molecules of aliphatics and aromatics were easily inhaled compared to larger molecules. Skin concentrations are mainly affected by direct contact with chemicals, but blood concentrations can be affected by many factors, i.e. inhalation and dermal exposure, suggesting that the skin is a feasible specimen to determine the dermal exposure. In addition, case 2 involved various factors besides dermal exposure, i.e. fire and post-mortem changes, which suggests that analyses using all available specimens rather than just one sample are always recommended to get a comprehensive interpretation for individual cases.

The petroleum products used as individual standard products in this study were commercially available products. Attention should be paid via practical examination to identify the petroleum product involved, since petroleum product hydrocarbons are highly varied depending on company, season or refinery or the composition and blend of constituents. Identifying the constituent chemicals, especially in aliphatic hydrocarbons, would be the fundamental procedure to be determined, and the approximate proportion of major components may be of secondary help. Aromatic components, i.e. TMBs, are contained in almost all kinds of petroleum products, but aliphatic hydrocarbons are highly correlated to the boiling points of products, which can help to discriminate gasoline, kerosene or light oil, even if the proportion of aliphatic (%AHCs) is greatly distorted. For example, alkylate gasoline (not commercially available by itself) contains almost no aromatic components, but through identifying AHC molecules size, it can be determined as gasoline [14]. The stratum corneum of epidermis is considered to be the main reservoir of AHCs among skin layers from our previous study [2]. Detached roof of bulla, which mainly consists of epidermis, could be the most advantageous skin sample because high concentrations of components remain in it and it is easy and painless sampling.

Skin is a convenient and useful specimen, but thorough attention should be paid to sample handling, since skin is easily contaminated with any nearby chemicals. The skin surface has to be washed with excessive amounts of water or an appropriate solvent prior to sampling to remove contaminants or raw materials at high concentration on the surface, except when the purpose is to analyze residue on the surface. Only chemicals permeated in skin have to be analyzed to confirm dermal exposure.

In conclusion, skin analysis is useful to identify the causative chemical in cases of dermal exposure. The causative chemicals would not only be limited to the three kinds of petroleum products examined in this study, but would include other hydrophobic chemicals that can be trapped in skin.

References

[1] Y. Tsujino, Y. Hieda, K. Kimura, S. Dekio, Forensic Sci. Int. 133 (2003) 141.
[2] Y. Hieda, Y. Tsujino, Y. Xue, K. Takayama, J. Fujihara, K. Kimura, S. Dekio, Int. J. Legal Med. 118 (2004) 41.
[3] Y. Tsujino, Y. Hieda, K. Kimura, H. Eto, T. Yakabe, K. Takayama, S. Dekio, Int. J. Legal Med. 116 (2002) 207.
[4] K. Matsubara, A. Akane, S. Takahashi, H. Shiono, Y. Fukui, J. Chromatogr. 424 (1988) 49.
[5] M. Morinaga, S. Kashimura, K. Hara, Y. Hieda, M. Kageura, Int. J. Legal Med. 109 (1996) 75.
[6] G.S. Douglas, W.A. Burns, A.E. Bence, D.S. Page, P. Boehm, Environ. Sci. Technol. 38 (2004) 3958.
[7] D.L. Wetzel, E.S. VanVleet, Mar. Pollut. Bull. 48 (2004) 927.
[8] K. Kimura, S. Fukushima, K. Matsubara, T. Idzu, S. Takahashi, Adv. Legal Med. 3 (1997) 535.

[9] J.V. Rogers, P.G. Gunasekar, C.M. Garrett, M.B. Kabbur, J.N. McDougal, J. Appl. Toxicol. 21 (2001) 521.
[10] J.N. McDougal, D.L. Pollard, W. Weisman, C.M. Garett, T.E. Miller, Toxicol. Sci. 55 (2000) 247.
[11] R.E. Baynes, J.D. Brooks, K. Budsaba, C.E. Smith, J.E. Riviere, Toxicol. Appl. Pharmacol. 175 (2001) 269.
[12] J. Fujihara, Y. Hieda, Y. Tsujino, Y. Xue, K. Takayama, K. Kimura, S. Dekio, Legal Med. 6 (2004) 109.
[13] K. Kimura, T. Nagata, T. Imamura, K. Hara, Biomed. Mass Spectrom. 20 (1991) 493.
[14] T.M. Mata, R.L. Smith, D.M. Young, C.A. Costa, Environ. Sci. Technol. 15 (2003) 3724.