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ORIGINAL ARTICLE

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Skin analysis following dermal exposure to kerosene in rats: the effects of postmortem exposure and fire

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Abstract To evaluate the usefulness of skin analysis for the forensic examination of cases involving postmortem dermal exposure to kerosene and/or fire, an experimental study using rats was performed. Rats received dermal exposure to kerosene before or after death, and the effect of fire was determined by burning an area of exposed skin after death. Kerosene concentrations in skin and blood were determined by gas chromatography-mass spectrometry and microscopic observation was performed for skin samples. No differences were observed in skin kerosene levels between antemortem and postmortem exposure. Kerosene concentrations in mildly burned skin where the stratum corneum (SC) was retained were approximately 84% compared to those in non-burned exposed skin, whereas concentrations in severely burned skin where the SC was almost completely burned off were 28% of non-burned skin. Even in non-exposed control skin 14% of the original kerosene concentrations could be detected, which was considered to be caused by contamination during the experimental protocol combined with kerosene's property of a high affinity for the SC. These results suggest that (1) skin analysis is useful in estimating the type of petroleum product involved in crimes or accidents even for postmortem exposure, (2) whether the SC is retained or not primarily determined the kerosene levels in burned skin, and (3) attention must be paid to evaluate the results obtained from skin samples in the light of the circumstances surrounding the case.

Keywords Kerosene · Skin · Dermal exposure · Fire · GC-MS

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Introduction

Kerosene is a petroleum product widely used as fuel all over the world. It consists of 80% saturated hydrocarbons, i.e. aliphatic hydrocarbons (AHCs) and naphthenes, and 20% aromatic hydrocarbons, i.e. trimethylbenzenes (TMBs) and xylenes [1]. The toxicity of kerosene itself is generally low, but it is often involved in accidents or crimes due to its ready availability in Japan and it sometimes results in death in conjunction with other factors, i.e. fire, CO intoxication or aspiration of kerosene into the lungs [2, 3, 4, 5, 6, 7, 8]. Kerosene is analyzed as a petroleum product in forensic investigations to obtain scientific evidence from victims' samples [2, 3, 4, 5, 6, 7].

Dermal exposure is a less toxic route of exposure than inhalation, but it is frequently seen in actual forensic cases, i.e. in fires after pouring kerosene onto victims [2, 3, 4]. Using rats we have previously demonstrated (1) the usefulness of detecting TMBs, which are readily absorbed via the skin, in blood and tissues to estimate postmortem or antemortem dermal exposure [9], (2) the relationship between blood kerosene levels and area or amount of dermal exposure and (3) the usefulness of skin analysis to identify the petroleum product due to high concentrations of AHCs [10]. The purpose of this experimental study using rats was to evaluate whether skin analysis is still useful even after postmortem dermal exposure and/or burning.

Materials and methods

Reagents

Standard kerosene was obtained from Shell Petroleum (Tokyo, Japan). Some of the major kerosene components, eight kinds of saturated AHC (C_9-C_{16}) and three kinds of TMB (1,3,5-TMB, 1,2,4-TMB and 1,2,3-TMB) were purchased in analytical grade. The standard kerosene consisted of 16.8% (w/w) of the eight AHCs and 0.98% (w/w) of the three TMBs.

Dermal exposure to kerosene in rats

All animal experimental protocols were approved by the Shimane University Animal Experiment Committee. The animal experiments were performed in a routine laboratory facility and 12 male Sprague-Dawley rats (body weight 330–387 g, Charles River Breeding Labs, Yokohama, Japan) were randomly divided into 2 groups and treated as indicated in Fig. 1. With the rats under anesthesia (2 ml/kg i.m. of a mixture of 1.25 mg/ml droperidol and 0.025 mg/ml fentanyl), the abdominal and back fur was closely clipped. A piece of cotton (4×4 cm) soaked with 4 ml of standard kerosene and another piece of cotton (4×1 cm) soaked with 1 ml of saline were applied to either abdominal or back skin (Fig. 2a). The cotton was covered with impermeable stretch film and immobilized by adhesive bandage. The dermal exposure was performed for 30 min (antemortem exposure in Fig. 1). The exposed skin was thoroughly washed with soap at the end of the exposure. The rats were sacrificed by decapitation and the trunk blood was collected. Then, an area of exposed skin (portion 3 in Fig 2a) was burned with a portable burner (Minitorch, Coleman, Tokyo, Japan) at a distance of 10 cm from the skin for 30 s for the abdominal (group I) and for 40 s for the back skin (group II). The four portions of skin samples shown in Fig. 2a were collected. After sampling, the rat was turned over and received postmortem exposure for 30 min on the back (group I) and abdomen (group II). Except for decapitation and blood sampling, the procedures for the postmortem exposure, washing, burning and skin sampling were the same as those described for the other area of skin. A small portion of each skin sample was used for microscopic observation and the others for kerosene assay.

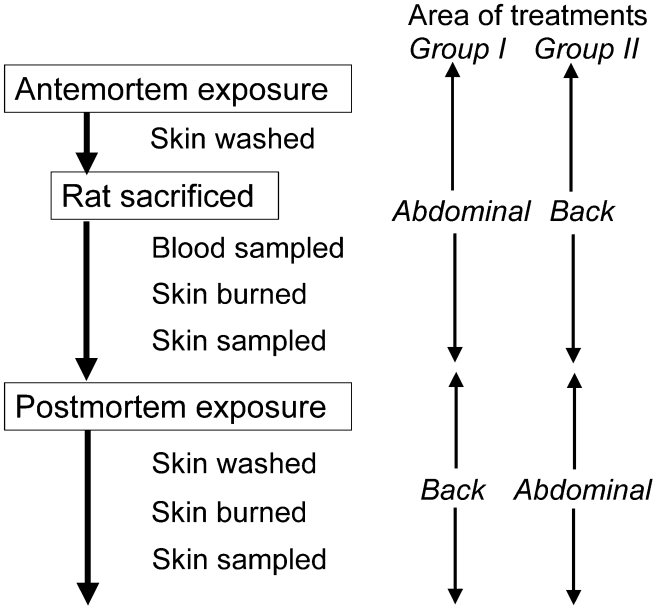

Fig. 1 The flow diagram of experimental procedures for each animal group

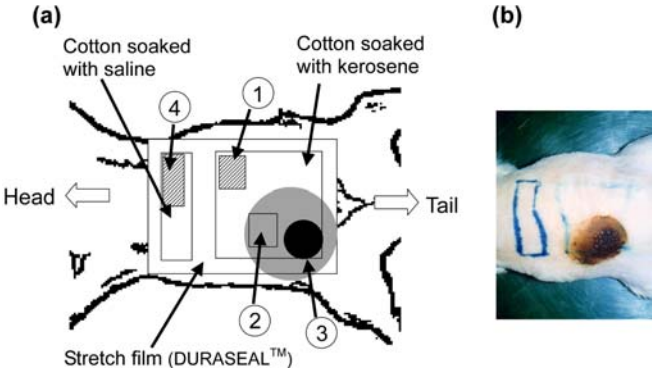

Fig. 2 **a** Schematic of dermal exposure to kerosene and the sampling portions. Dermal exposure was performed by applying a piece of cotton soaked with kerosene and another piece of cotton soaked with saline for 30 min. The rat was sacrificed after the exposure, and then an area of kerosene-exposed skin (*portion 3*) was burned with a portable burner for 30 or 40 s for abdomen or back, respectively. The skin samples were excised from four portions; (*portion 1*) the exposed skin without burning, (*portion 2*) the mildly burned exposed skin, (*portion 3*) the severely burned exposed skin and (*portion 4*) the control skin. **b** The actual abdominal skin after burning for 30 s in group I

Kerosene assay

Kerosene concentrations in whole blood (0.5 g) and skin (0.02–0.04 g) were determined by gas chromatography-mass spectrometry utilizing our previously described method [11]. The skin used for this assay consisted mainly of epidermis and some dermis, which was confirmed by microscopic observation.

Results

A picture of a rat in group I after burning is shown in Fig. 2b. The skin surface was partially and completely charred in portions 2 and 3, respectively. The microscopic observations of each portion (Fig. 3) revealed that extensive necrosis was observed in burned skin (portion 2 and 3) and the primary difference was whether the stratum corneum (SC) was retained or not. The SC in portion 2 was retained even though the structure was denatured, while the SC in portion 3 was almost completely burned off and some epidermal layer was also flayed off. No change was observed in the exposed skin (portion 1 vs. 4) and the much thicker SC and epidermis were observed in the back skin. The concentrations of kerosene in each portion of skin are shown in Fig. 4. The kerosene concentrations in the mildly burned skin (portion 2) and the severely burned skin (portion 3) were 84% and 28% of those in the non-burned exposed skin (portion 1), respectively (Table 1). The kerosene components were detected even in the non-exposed control skin (portion 4) and the concentrations were 14% of those in the exposed skin (portion 1). The back skin had consistently lower concentrations (43–69%) than the corresponding portions of the abdominal skin (Table 2). The TMB and AHC levels in blood were 100-fold and 300-fold less than those in the exposed skin (portion 1), respec-

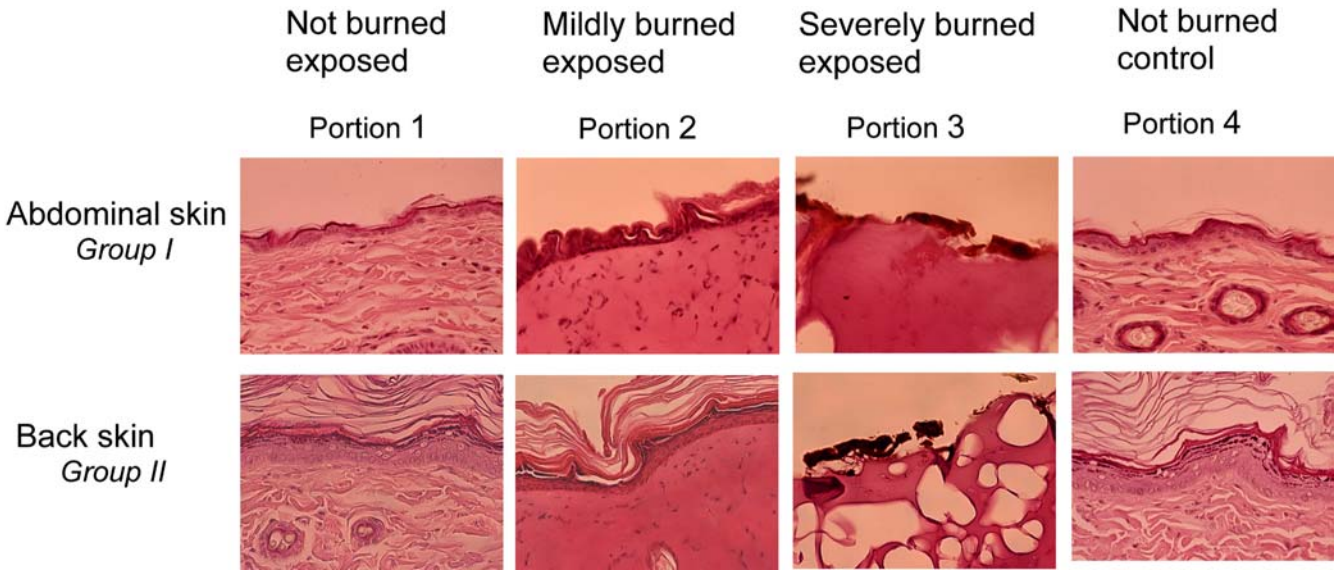

Fig. 3 The microscopic observations in four portions of abdominal (group I) and back skin (group II) obtained following antemortem exposure and burning (HE ×400). The exposed skin (*portion 1*) appeared to be normal compared with the control skin (*portion 4*). Extensive necrosis was observed in both burned skins (*portions 2 and 3*). The stratum corneum was retained in mildly burned skin (*portion 2*) and completely burned off in the severely burned skin (*portion 3*). The much thicker stratum corneum and the epidermal layer were observed in back skin

tively. No difference was observed between the kerosene levels in skin after antemortem and postmortem exposure (Fig. 5).

Discussion

In our previous study skin was proven to be useful for forensic investigations to identify the type of petroleum product, because (1) sampling of skin is easy, (2) a small skin sample is sufficient (<0.03 g), and (3) aliphatics which are essential components for discrimination of petroleum products are found at much higher levels in skin compared to blood samples [10]. Our present study was designed to perform further evaluation of skin analysis in situations of postmortem dermal exposure and/or fire. Regarding the exposure period in this study, our preliminary experiments confirmed approximately 39% and 55% of skin kerosene

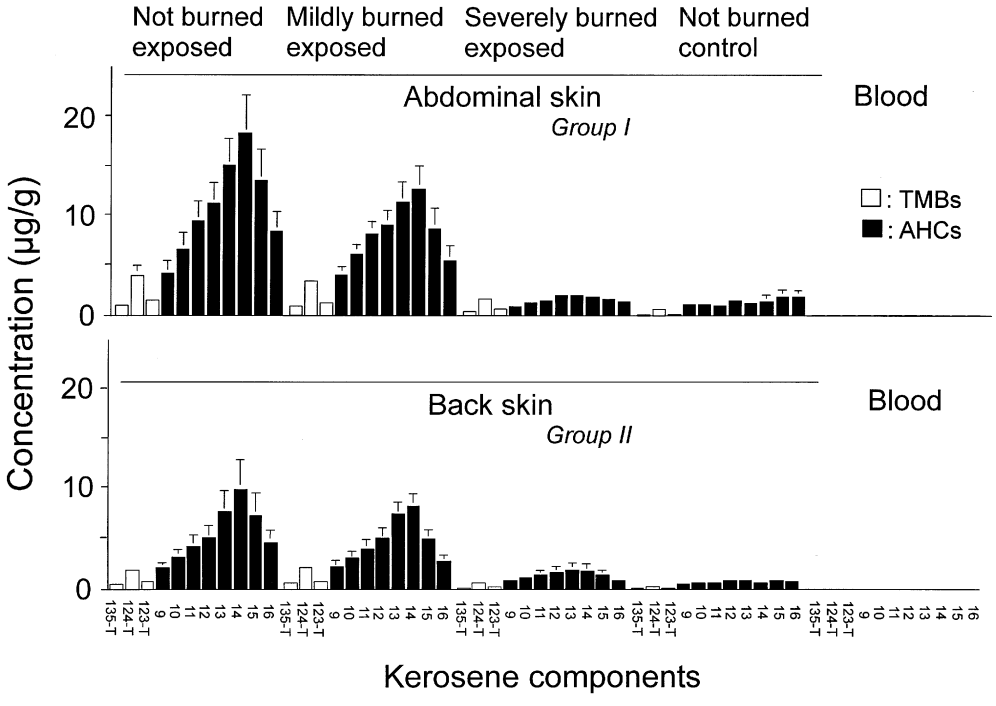

Fig. 4 The concentrations of kerosene components in four portions of skin and blood samples obtained following antemortem exposure. The back skin (group II, *bottom*) detected 42% lower kerosene concentrations compared to those in abdominal skin (group I, *top*). Kerosene levels in the mildly burned skin (*portion 2*) were detected 25% lower than those in the non-burned exposed skin (*portion 1*), while those in the severely burned skin (*portion 3*) were 73% lower and close to those in the control skin (*portion 4*). The blood levels were less than 100-fold lower than those in the exposed skin (*portion 1*). The data express mean ±S.E. (*N*=6). *135-T* 1,3,5-trimethylbenzene, *124-T* 1,2,4-trimethylbenzene, *123-T* 1,2,3-trimethylbenzene, *9* nonane (C_9), *10* decane (C_{10}), *11* undecane (C_{11}), *12* dodecane (C_{12}), *13* tridecane (C_{13}), *14* tetradecane (C_{14}), *15* pentadecane (C_{15}), *16* hexadecane (C_{16})

Table 1 The percentages of the concentrations in the mildly burned skin (portion 2) compared to those in the non-burned exposed skin (portion 1)

Exposure/Skin	Abdominal	Back	Average
Antemortem	70±5 (group I)	84±6 (group II)	75±4
Postmortem	94±5* (group II)	92±4** (group I)	93±3***
Average	79±4	89±3	84±3

*p<0.05 vs. antemortem abdominal.
**p<0.01 vs. antemortem abdominal.
***p<0.001 vs. antemortem (Scheffe's test).
The percentage was calculated as follows, (1) the concentration of each component in portion 2 was divided by the concentration of the corresponding component in portion 1 in each individual rat, and then (2) the percentages obtained from all components in all rats were averaged and the standard errors were calculated.
The percentages for severely burned skin (portion 3) and non-exposed control skin (portion 4) were calculated as same as this method (data not shown).
Data express mean ± S.E.

levels following 10 and 20 min of exposure, respectively, compared to those following 30 min exposure and concentrations varied greatly between samples following shorter periods of exposure (data not shown). Although 30 min of exposure may be longer than the period which would possibly occur in practical human cases, the experimentally reproducible shortest period (30 min) was chosen in this study.

Kerosene concentrations in skin following postmortem exposure were the same as for antemortem exposure (Fig. 5), which is reasonable considering that a functional lipid SC consists of dead cells as a primary barrier in living subjects. Blood circulation is not necessary at this site for absorption. Since kerosene levels in blood and other tissues following postmortem exposure have been demonstrated to be significantly lower than those following antemortem exposure in our previous study [9] and the concentrations in blood compared to those in skin samples were more than 100-fold lower even following antemortem exposure in this study and our previous study [10], the results of skin analysis suggest the usefulness of skin as a forensic sample especially when postmortem exposure has occurred.

Although this study was not designed to clarify the mechanism of dermal absorption or to determine the kerosene in each skin layer, the SC is estimated to be an important component to determine the kerosene levels in skin. Most of the kerosene would remain in the SC, but small amounts of aromatics and a few aliphatics would diffuse to the viable epidermis and dermis, and would then be systemically absorbed in living subjects. High levels of kerosene were detected when the SC layer was retained in the mildly

Table 2 The percentages of the concentrations in back skin compared to those in abdominal skin

Treatment	Not burned exposed	Mildly burned exposed	Severely burned exposed	Not burned control	All types
Portion	1	2	3	4	1–4
Antemortem (Group II / I)	52±3**	60±2*	69±7** ^{aa}	46±3	57±2
Postmortem (Group I / II)	67±4^{pp}	69±3^{ppp}	43±3	48±3	56±2
Average	59±3†	65±2††	56±5	47±2	57±2

*p<0.05 or **p<0.01 vs. corresponding postmortem.
$^{\dagger}p$<0.05 or $^{\dagger\dagger}p$<0.01 vs. control.
^{aa}p<0.01 vs. antemortem control.
^{pp}p<0.01 or ^{ppp}p<0.001 vs. postmortem severely burned or postmortem control (Scheffe's test).
The percentage was calculated as follows, (1) the mean concentration of each component in the back skin in one group was divided by the mean concentration of the corresponding component in the abdominal skin in the other group, and then (2) the percentages obtained from all components were averaged and the standard errors were calculated.
Data express mean ± S.E.

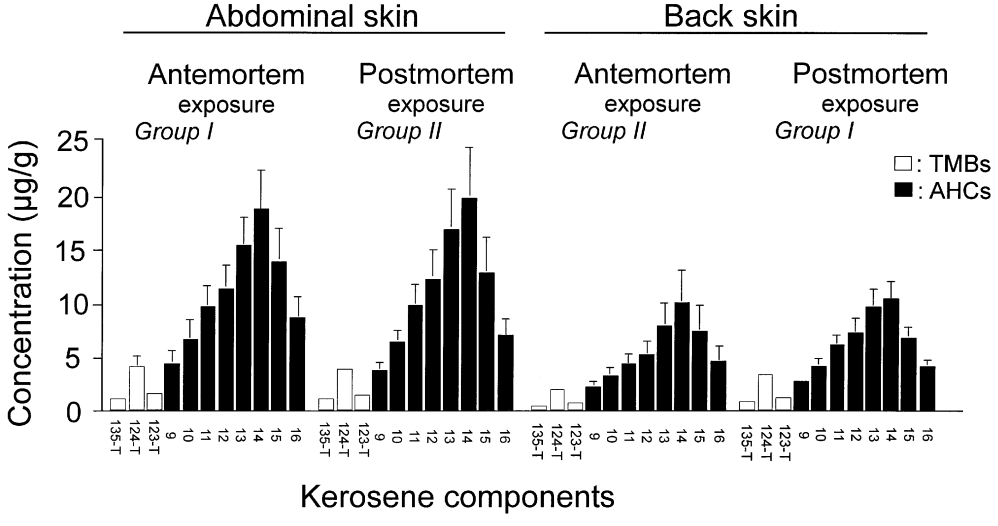

Fig. 5 The kerosene levels in the exposed skin (*portion 1*) were compared between antemortem and postmortem exposure in the abdominal and back skin. The concentrations were almost identical between antemortem and postmortem exposure. The data express mean ±S.E. (N=6). The abbreviations of kerosene components were the same as those in Fig. 4

burned skin (portion 2), while only low concentrations of kerosene were detected when partial epidermis and dermis remained with no SC in the severely burned skin (portion 3). Kerosene levels in skin samples in this study would be largely derived from kerosene in the SC.

In recent studies in vitro reported by Riviere et al., they demonstrated that the concentrations of aliphatics and aromatics in the SC were 8.2-fold and 4.6-fold higher, respectively than those in other parts of skin following contact with the jet fuel JP-A [12]. Baynes et al. [13] discussed absorption and penetration of aromatics and aliphatics in vitro based on different properties between the SC and viable epidermis. These results in vitro are in accordance with the results obtained in the present study in vivo. Further investigation, however, is needed to fully discuss the distribution of kerosene in each skin layer in vivo.

In addition, the evaporation and combustion of kerosene during fires are significant factors effecting kerosene levels in burned skin. Higher temperature and a longer duration of fire stimulate the evaporation or combustion of kerosene, and they also simultaneously burn the skin starting from the SC. These synergistic effects would be the reasons for the difference in kerosene levels between portions 2 and 3.

The results obtained from back and abdominal skin showed the same patterns of kerosene levels and microscopic observation (Figs. 3 and 4). Approximately 57% of kerosene concentrations in back skin compared to those in abdominal skin (Table 2) is probably due to the difference in the thickness of skin layers between back and abdomen, but this also needs further study.

This study suggests that skin is a valuable sample to detect petroleum components even following postmortem exposure or fire, but careful evaluation is indispensable in practical cases because skin can absorb anything that has an affinity to the SC, i.e. similar volatile hydrocarbons derived from household goods or combustion of inflammable furniture during a fire [14], and these components could be detected in skin. The control skin in this study is an example in which low levels of kerosene were detected even though saline was applied. Possible reasons for this would be (1) the overwhelming kerosene level on the surface of the exposed skin which was spread over the skin under the running water, (2) the soap could accelerate the spread of kerosene by dissolving kerosene into the soap, and (3) a burner in which petroleum products are contained, could have sprayed hydrocarbon gas onto the exposed skin surface. Since kerosene components and especially aliphatics have a high affinity for the SC due to their high octanol/water partition coefficients (Log K_{ow}= 6.25–7.24 for C_9–C_{13}) [15], small amounts of these components could be easily absorbed in a short time period, while water-soluble components (low Log K_{ow}) would be washed away under running water without absorption.

Although this washing process may create low levels of unwanted contamination, this process is necessary to obtain the accurate skin kerosene levels. Without the washing process, the amount of kerosene that was on the surface of the skin and not absorbed into the skin would affect the skin kerosene levels since the concentrations of kerosene itself and absorbed kerosene in skin are not comparable. Although the source of kerosene in the control skin cannot clearly be explained except by unavoidable contamination during the necessary experimental procedures, this kind of contamination would often occur in practical human cases such as fire fighting. In our experience, it is not easy to obtain a completely negative chromatogram for hydrocarbons, especially aliphatics in skin samples even in untreated rats although the detected levels are low, while there is usually no problem with blood samples. This suggests that the skin samples need special attention to evaluate the data, since small amounts of aliphatics could be detected in skin samples regardless of dermal exposure to kerosene. Collection of data in various practical human cases is clearly needed to verify the cut-off levels and to obtain reference data. Postmortem redistribution or passive absorption is also taken into consideration in forensic practice, i.e. organic vapors such as dichloromethane can easily be absorbed via the skin and be distributed to various tissues postmortem [16] and 3,4-methylenedioxymethamphetamine (MDMA "ecstasy") can also show substantial post-mortem redistribution [17, 18].

In conclusion, this study experimentally evaluated the usefulness of skin analysis using rats in the situation of postmortem dermal exposure to kerosene and/or fire. Skin analysis is useful in estimating the type of petroleum product involved in crimes or accidents, but simultaneous attention should be paid to evaluate the results obtained from skin samples in the light of the circumstances surrounding the victim. In addition, analyzing more than one site of skin samples is always recommended combined with blood and tissue samples to obtain the total picture.

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