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# Quantitative evaluation of volatile hydrocarbons in post-mortem blood in forensic autopsy cases of fire-related deaths

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ABSTRACT

Volatile hydrocarbons in post-mortem blood from victims of fires were analyzed quantitatively by headspace gas chromatography mass spectrometry. The benzene and styrene concentrations in the blood were positively correlated with the carboxyhemoglobin (CO-Hb) concentration, which is evidence that the deceased inhaled the hydrocarbons and carbon monoxide simultaneously. By contrast, the concentrations of toluene and CO-Hb in the blood were not significantly correlated. This lack of correlation could be explained by two different sources of toluene, with low blood concentrations of toluene arising when the deceased inhaled smoke and high blood concentrations of toluene arising when the deceased inhaled petroleum vapor or other unknown vapors. The quantity of soot deposited in the respiratory tract was classified into four grades (–, 1+, 2+, 3+). The mean CO-Hb concentration in the 1+ soot group was significantly lower than those in the 2+ ($p < 0.05$) and 3+ ($p < 0.01$) soot groups. The blood CO-Hb concentrations in the 1+ soot group were all below 30%. Those indicated that the deceased aspirated smoke that contained both soot and carbon monoxide. The wide variation in CO-Hb concentrations for each soot classification could be caused by the different types of smoke produced by different materials. For example, petroleum combustion with a limited supply of oxygen, like in a compartment fire, may produce a large volume of dense black smoke that contains a large quantity of soot. Soot deposits in the airways and the blood CO-Hb concentration are basic and essential autopsy findings that are used to investigate fire-related deaths. The quantitative GC–MS analysis of blood volatile hydrocarbons can provide additional useful information on the cause of the fire and the circumstances surrounding the death. In combination, these three findings are useful for the reconstruction of cases.



## 1. Introduction

Chromatographic analysis of volatile hydrocarbons in fire debris [1–4] and post-mortem blood from fire related deaths [5–7] can provide useful information on the cause of the fire and the circumstances surrounding the death of individuals in the fire. The analysis of volatile hydrocarbons in the blood can indicate if the deceased inhaled fire accelerant and/or smoke. Kimura et al. proposed a method for hydrocarbon analysis in post-mortem blood to determine the conditions before death, and suggested that it could be used to discriminate between gasoline and kerosene components in the blood [5]. Morinaga et al. performed qualitatively headspace gas chromatography mass spectrometry (GC–MS) analysis of combustion- and petroleum-related hydrocarbons in 47 blood samples from carbon monoxide (CO) poisoning cases [7]. These blood samples showed four typical hydrocarbon profiles, which could be used to classify the fire as construction, kerosene, gasoline, or exhaust gas related. Construction fire cases were characterized by the presence of styrene and relatively high levels of benzene and toluene in the blood. Similarly, n-nonane and n-decane were detected in kerosene-related fires, and n-hexane, n-heptane, and C3 alkyl-benzenes in gasoline-related fires.

However, volatile hydrocarbons in post-mortem blood from fire related deaths have not been evaluated quantitatively yet. In the present study, quantitative analysis of hydrocarbon was conducted on post-mortem blood from forensic autopsy cases of deceased found at the scene of a fire. The concentrations of the hydrocarbons were evaluated in relation to the blood CO-Hb concentration, quantity of soot in the airways, and the situation of the fire.

## 2. Materials and methods

### 2.1. Sample preparation

Sample preparation was performed according to the method of Morinaga et al. [7]. An aliquot (1 mL) of the heart blood sample collected at autopsy was added with cold water (1 mL) to a 15 mL glass vial with a silicon-rubber septum, which was then covered with the septum and sealed with an aluminum cap. The glass vials

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were cooled on ice before use and then kept on ice during sample preparation. An internal standard solution (1 μL) was injected through the septum into the vial. The vial was warmed to 60 °C on an aluminum block heater (Shibata Scientific Technology Ltd., Japan) and shaken manually at frequent interval for 20 min. Aliquots (200 μL) of the headspace gas were collected in a 1 mL gas-tight glass syringe, and injected into the GC–MS instrument.

### 2.2. Quantitative analysis

Eight aromatic hydrocarbons (benzene, toluene, ethylbenzene, *p*-xylene, styrene, propylbenzene, 1,3,5-trimethylbenzene, 3-ethyltoluene) and six aliphatic hydrocarbons (n-heptane, n-octane, n-nonane, n-decane, n-undecane and n-dodecane) were determined by headspace GC–MS analysis in selected ion monitoring (SIM) mode [8].

For the calibration standard, an aliquot (1 mL) of blank blood sample from individuals that did not die in fires and cold water (1 mL) were placed in a 15 mL glass vial. After sealing the vial, 0–10 μL of a standard mixture solution was injected, and the subsequent preparation was the same as for the blood samples. For the standard stock solution for analysis, 1 μL of each of the 14 compounds stated above and the internal standard (toluene–d8) were separately dissolved in 10 mL of tetraethylene glycol dimethyl ether. The internal standard solution was prepared by diluting the stock solution with tetraethylene glycol dimethyl ether to give a concentration of 0.1 μL/mL for each of the compounds. The standard solution for calibration was prepared by mixing an equal volume of each of the 14 standard stock solutions. The volume (μL) of each compound was converted to a mass (μg) using the corresponding specific gravities. Toluene-d8 was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Standard compounds and other chemicals were of GC analytical grade.

### 2.3. GC–MS conditions

The GC–MS analysis was performed on a GC-MS QP-5000 (Shimadzu, Kyoto, Japan). Chromatographic separation was performed with a DB-1 capillary column (30 m × 0.25 mm i.d., 2.5 μm film thickness, J&W Scientific, Folsom, CA). The carrier gas was helium in constant pressure mode. The temperature of the interface was set at 250 °C. The oven temperature was held at 35 °C for 2 min and then increased to 250 °C at 20 °C/min. The injector temperature was set at 200 °C. The mass spectrometer was operated in electron ionization (EI) mode with an electron energy of 70 eV. The separator temperature was set at 250 °C and the ion source at 250 °C. Analysis was performed in SIM mode. The analytical information for each volatile hydrocarbon is listed in Table 1.

### 2.4. Cases examined

Thirty-seven forensic autopsy cases from fire related fatalities performed by the Department of Forensic Medicine, Graduate School of Medical Sciences, Kumamoto University from 2005 to 2007 were examined. Twenty-one of the deceased were male and 16 were female. The ages of the deceased ranged from 22 to 92 years (mean ± S.D., 65.1 ± 20.0). All the deceased were found at fire scenes, and the cause of death was burning in most of the cases. The physical state of the body differed from case to case. Cases with missing respiratory organs or no blood in the heart were not included in this study. At the autopsy, heart blood samples were collected and the CO-Hb concentration was determined by a spectrometric method [9]. The blood sample was analyzed immediately by GC–MS. The quantity of soot deposited in the respiratory tract was classified into four grades (–, 1+, 2+, 3+) (Fig. 1). Information on the cause of the fire and the circumstances surrounding the death of the individual was limited, and results for analysis of scene residues for volatiles could not be obtained from the police.

**Table 1**
Analytical information for volatile hydrocarbons in post-mortem blood.

| | Retention time (min) | Fragment ions (m/z) |
|---|---|---|
| Toluene–d8 (IS) | 4.24 | 98 |
| Aromatic hydrocarbon | | |
| Benzene (BZ) | 2.81 | 78 |
| Toluene (TL) | 4.30 | 92 |
| Ethylbenzene (EBZ) | 6.02 | 106, 91 |
| *p*-Xylene (XL) | 6.21 | 106, 91 |
| Styrene (SR) | 6.52 | 104, 78 |
| Propylbenzene (PBZ) | 7.79 | 120, 105, 91 |
| Trimethylbenzene (TMB) | 8.08 | 120, 105, 91 |
| 3-Ethyltoluene (ETL) | 8.25 | 120, 105, 91 |
| Aliphatic hydrocarbon | | |
| n-Heptane (C7) | 3.41 | 71, 57 |
| n-Octane (C8) | 5.17 | 85, 57 |
| n-Nonane (C9) | 7.05 | 85, 57 |
| n-Decane (C10) | 8.90 | 71, 57 |
| n-Undecane (C11) | 10.65 | 71, 57 |
| n-Dodecane (C12) | 12.06 | 71, 57 |

### 2.5. Statistical analysis

Statistical analysis was performed using SPSS 15.0J for Windows. Pearson's correlation analysis was used to determine any correlations between the blood CO-Hb and three hydrocarbon (benzene, toluene and styrene) concentrations. Results were considered statistically significant if $p < 0.05$.

## 3. Results

Fig. 2 shows the GC–MS (SIM) chromatogram for the results from a kerosene detected case. The calibration curve of each

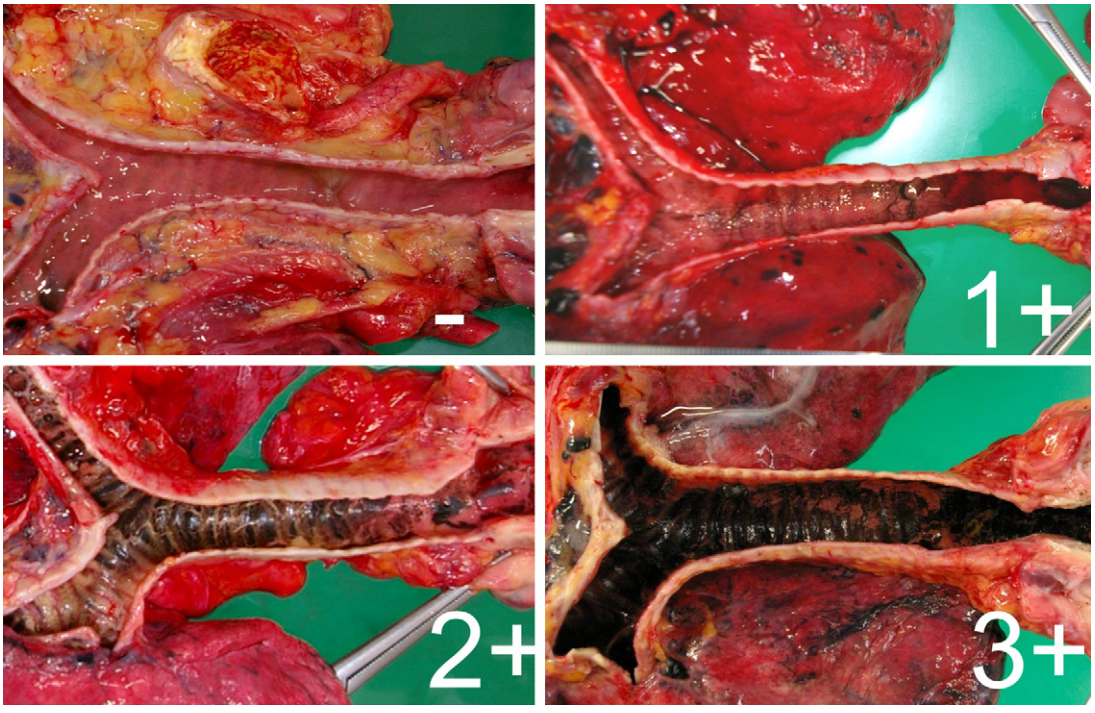


**Fig. 1.** Grading of the quantity of soot in the respiratory tract.