Forensic Science International 353 (2023) 111872

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Correlations between blood volatile hydrocarbon concentrations in different types of fire-related deaths

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ARTICLE INFO

Keywords:
Carboxyhemoglobin
Volatile hydrocarbon
Fire-related death
Gas chromatography-mass spectrometry
Kerosene
Gasoline

ABSTRACT

Analysis of volatile hydrocarbons in blood from fire-related deaths provides useful information such as whether the victim inhaled smoke from the fire before death or whether an accelerant was used in the fire. In this study, we used headspace gas chromatography-mass spectrometry to quantify volatile hydrocarbons in post-mortem heart blood from 121 fire victims. The cases were classified into the following four groups according to the detected volatile hydrocarbons: construction fires without accelerants, kerosene fires, gasoline fires, and a group with no fire-related hydrocarbons detected (other fires). We investigated the relationships between blood concentrations of carboxyhemoglobin (COHb) and volatile hydrocarbons, and between various volatile hydrocarbons. The mean COHb concentrations were higher in the construction fire group than in the kerosene and gasoline fire groups. In the construction fire group, there was a high correlation coefficient between the concentrations of benzene and COHb and relatively high coefficient correlations between the concentrations of benzene and toluene, benzene and xylene, toluene and styrene, and ethylbenzene and styrene. Our results indicate that the relationships between benzene, xylene, and toluene concentrations could be used to distinguish between deaths in construction fires, kerosene fires, and gasoline fires.

1. Introduction

Fire-related deaths are frequently investigated by forensic pathologists to clarify the cause of death. Pathological findings, such as inhalation injuries caused by breathing hot air in a fire, burns with vital reactions, and soot in the respiratory tract, are used along with scientific analyses to determine the cause of death [1]. However, because of the destruction caused by fires, it is often difficult to determine the circumstances around the manner of death.

Carboxyhemoglobin (COHb) and volatile hydrocarbon analysis of a fire victim’s blood are useful scientific analyses to obtain information about the cause of death and/or the circumstances surrounding the death. A high COHb concentration can be fatal and the COHb test reveals whether a fire victim has inhaled carbon monoxide generated by the fire before death. Volatile hydrocarbon analysis in fire-related deaths also provides useful information, such as whether the victim inhaled smoke from the fire before death and whether an accelerant was used in the fire. Morinaga et al. conducted qualitative analysis of blood volatile hydrocarbons in 47 carbon monoxide poisoning cases [2]. They found that some volatile hydrocarbons could be used as indicators to distinguish between construction fires, kerosene fires, and gasoline fires. Specifically, benzene, toluene, and styrene were frequently detected in the blood of victims of construction fires; n-nonane and n-decane were detected in the blood of victims of kerosene fires; and n-hexane, n-heptane, and C_3 alkylbenzenes were detected in the blood of victims of gasoline fires. In other research, the sensitivity of volatile hydrocarbon analysis has been improved and attempts have been made to identify other volatile hydrocarbons that are characteristic of certain circumstances in fire-related deaths [3,4].

In a previous study, we used headspace gas chromatography-mass spectrometry (GC-MS) to quantitatively analyze volatile hydrocarbons in post-mortem blood from 37 victims of fires [5]. Our results showed that the benzene and styrene concentrations in blood were correlated with COHb concentrations and could be evidence that the deceased inhaled hydrocarbons and carbon monoxide simultaneously. Furthermore, the volatile hydrocarbons concentrations in post-mortem blood could be used to classify fires into three types: construction fires, kerosene fires, and gasoline fires. However, in this study, the number of cases was not large enough to examine the relationships between hydrocarbons and COHb concentrations in these three types of fires.

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https://doi.org/10.1016/j.forsciint.2023.111872
Received 18 July 2023; Received in revised form 6 October 2023; Accepted 23 October 2023

The aim of the present study was to examine the relationships between the concentrations of volatile hydrocarbons and COHb and between the concentrations of different volatile hydrocarbons in a large number ($n = 121$) of fire-related autopsy cases. We focused on the concentration ratios of toluene/benzene and xylene/benzene to determine if these ratios could be used as indicators to distinguish cases from the three types of fires. On the basis of our results, we propose criteria for evaluating fire-related deaths.

2. Materials and methods

2.1. Chemicals

Eight aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylene, styrene, propyl benzene, 3-ethyl toluene, and trimethyl benzene) and five aliphatic hydrocarbons (n-octane, n-nonane, n-decane, n-undecane, and n-dodecane) were examined as volatile hydrocarbons in this study. These compounds and toluene-d_8 (all of analytical grade) were purchased from FUJIFILM Wako Chemicals Corporation (Osaka, Japan). Tetraethylene glycol dimethyl ether (analytical grade) was obtained from Merck KGaA (Darmstadt, Germany).

2.2. Cases

Samples were obtained from fire-related forensic autopsy cases ($n = 121$) processed at the Department of Forensic Medicine, Faculty of Life Sciences, Kumamoto University between 2011 and 2018. Ninety of the deceased were male and 31 were female. The ages of the deceased ranged from 1 to 94 years (mean: 66.4, standard deviation: 19.9). 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.

Heart blood samples, about 10 mL, were collected using a needle from the pericardial cavity after the heart was removed. The COHb concentration was measured using a CO oximeter (AVOXIMETER 4000, Instrumentation Laboratory, USA). When the COHb concentration was above 75%, which is the upper limit of determination of the CO oximeter, a spectrometric method was used for analysis [6]. Blood samples were analyzed immediately after collection or after storage at 4 °C for several days.

2.3. Sample preparation

Samples were prepared according to the method of Morinaga et al. [2]. Briefly, an aliquot (1 mL) of the heart blood sample collected at autopsy and cold ultra-pure (Milli-Q) water (1 mL) were added to a glass vial (Agilent, 5182–0837) with a headspace volume of 18 mL above the blood specimen. The vial was then covered with a silicon-rubber septum and sealed with an aluminum cap. Toluene-d_8 (1 μL, 100 ng/μL) as an internal standard, was added to each vial by micro syringe through the septum.

For quantitative calibration, a volatile hydrocarbons calibration curve (1, 3, 6, 12, 25, 50, 100, 200, and 2000 ng/mL blood) was constructed using standard vials of the volatile hydrocarbons in swine whole blood. First, a stock volatile hydrocarbons solution containing 2 mg/mL of each chemical in tetraethylene glycol dimethyl ether was prepared. The stock volatile hydrocarbons solution was then diluted to the required concentrations using tetraethylene glycol dimethyl ether. An aliquot (1 μL) of each diluted solution was spiked into a vial containing 1 mL of swine whole blood, 1 mL of cold water, and the internal standard. These vials were used for constructing the calibration curve.

2.4. GC-MS analysis

The volatile hydrocarbon concentrations in blood were determined by headspace GC-MS analysis in selected ion monitoring mode using an Agilent GC7890A, 5977MSD, and an Agilent G1888 network headspace sampler. A DB-WAX column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with helium as the carrier gas was used for separation. The oven temperature was initially 50 °C and then increased to 120 °C at a rate of 10 °C/min, increased to 180 °C at a rate of 30 °C/min, and maintained at 180 °C for 3 min. The injector temperature was 150 °C, the oven temperature was 150 °C, and the ion source was 200 °C. The injection was performed in split mode (11:1). The mass spectrometer was used in electron ionization mode with an electron energy of 70 eV. The analytical information for each volatile hydrocarbon is listed in Table 1.

2.5. Method validation

A 10-point calibration curve was constructed by plotting the peak area ratio of each chemical to the internal standard against its concentration, and fitting was performed via least-squares linear regression without a weighting factor. The precision (% coefficients of validation) and accuracy (%) were determined by analyzing four control samples containing known amounts of the chemical. The precision is expressed as the relative standard deviation of the concentrations. The accuracy is expressed as a percentage for the difference between the observed concentration and the expected concentration.

2.6. Classification of the cases

The volatile hydrocarbons detected in the autopsy samples by GC-MS analysis were used as indicators, and the 121 cases were classified into four groups according to the criteria reported by Morinaga et al. (Table 2) [2]. C_5–C_7 aliphatic compounds were not analyzed in this study. When C_8–C_{12} aliphatic hydrocarbons (n-octane, n-nonane, n-decane, n-undecane, and n-dodecane) were detected, the case was classified into the kerosene fire group. When propyl benzene, 3-ethyl toluene, and trimethyl benzene were detected without the aliphatic hydrocarbons mentioned above, the case was classified into the gasoline fire group. In cases where benzene, toluene, styrene, and other several aromatic compounds were detected, the case was classified into the construction fire group. For cases where fewer than two hydrocarbons were detected in the blood and at low concentrations, the case was classified into the other fire group. The relationships between blood concentrations of COHb and volatile hydrocarbons and between various volatile hydrocarbons were investigated in each group.

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

	Retention time (min)	Fragment ions (*m/z*)
Toluene-d_8 (internal standard)	2.96	98
Aromatic hydrocarbons		
Benzene	2.30	78
Toluene	2.95	92, 91
Ethylbenzene	3.65	106, 91
Xylene (*o*-, *m*-, and *p*-)	4.29, 3.79, and 3.72	106, 91
Styrene	5.16	104, 78
Propylbenzene	4.55	120, 91
3-Ethyltoluene	5.21	120, 105
Trimethylbenzene (1,3,5-, 1,2,4-, and 1,2,3-)	4.98, 5.45, and 6.07	120, 105
Aliphatic hydrocarbons		
n-Octane (C_8)	1.74	85, 57
n-Nonane (C_9)	2.03	85, 57
n-Decane (C_{10})	2.55	85, 57
n-Undecane (C_{11})	3.26	71, 57
n-Dodecane (C_{12})	4.21	71, 57

Table 2

Classification criteria for the present cases using the detected hydrocarbons [2].

	Aromatic compounds		Aliphatic compounds
Group	BZ, TL, EBZ, XL, SR	PBZ, ETL, TMB	C_8–C_{12}
Construction fire	+	−	−
Kerosene fire	+	+	+
Gasoline fire	+	+	−

BZ, benzene; TL, toluene; EBZ, ethylbenzene; XL, xylene; SR, styrene; PBZ, propylbenzene; ETL, 3-ethyltoluene; TMB, trimethylbenzene (1,3,5-, 1,2,4-, and 1,2,3-); + , detected; − , not detected.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 for Windows. One-way analysis of variance followed by Tukey's multiple comparisons test was used to determine whether the differences between the groups (construction fire, kerosene fire, and gasoline fire) were statistically significant. Pearson's correlation analysis was used to determine any correlations between the blood concentrations of COHb and volatile hydrocarbons (benzene, toluene, ethylbenzene, xylene, and styrene) and between the different volatile hydrocarbons.

2.8. Ethics

This study was approved by the Ethics Committee for Epidemiology Research and General Research at the Faculty of Life Sciences, Kumamoto University, Japan (approval No. 1152).

3. Results

Validation data for the volatile hydrocarbon quantitative analysis are given in Table S1. The calibration curve of each volatile hydrocarbon (0–2000 ng/mL) was linear with a coefficient of determination (R^2) of 0.901–0.996. The precision and accuracy for the volatile hydrocarbons, except for n-decane and n-undecane, were within ± 20% and thus considered acceptable. Some of the n-decane and n-undecane results were greater than ± 20% and these data were only used qualitatively to establish that these compounds were present.

According to the detected volatile hydrocarbons, the 121 cases in this study were classified as follows: 82 cases in the construction fire group, 17 cases in the kerosene fire group, 10 cases in the gasoline fire group, and 12 cases in the other fire group. There were no discrepancies between the results of the volatile hydrocarbon analysis and the scene information from the police investigation in each case. The other fire group (12 cases) consisted of 5 cases of outdoor fires (e.g., field burning) and 7 cases of indoor fires.

The blood COHb concentrations in the four groups were compared (Fig. 1). The mean COHb concentration was significantly higher in the construction fire group than in the kerosene fire group or the other fire group ($p < 0.0001$). No other significant differences were found between the four groups. It should be noted that the cases in the other fire group had lower COHb concentrations than the cases in the other groups.

Focusing on the construction fire group, we investigated the relationship between the concentrations of blood COHb and those of three volatile hydrocarbons (benzene, toluene, and styrene), which were detected at high concentrations in most cases (Fig. 2). The concentrations of the three volatile hydrocarbons were positively correlated with the COHb concentrations, particularly for benzene ($p < 0.001$), which was consistent with our previous report [5].

Table 3 shows the correlation coefficients between the concentrations of five volatile hydrocarbons (benzene, toluene, ethylbenzene, xylene, and styrene) that were frequently detected in the construction fire group. There were positive correlations between their concentrations (Fig. 3). High correlations were found between toluene and benzene ($r = 0.78$), xylene and benzene ($r = 0.72$), toluene and styrene ($r = 0.75$), and ethylbenzene and styrene ($r = 0.83$) (Table 3 and Fig. 3).

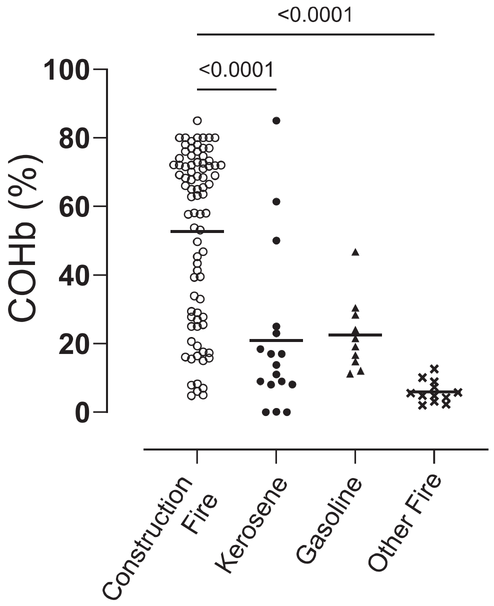

Fig. 1. Blood carboxyhemoglobin (COHb) concentrations in cases grouped according to the type of fire. The horizonal line for each group shows the mean COHb concentration. The mean COHb concentration in the construction fire group was significantly higher than in the kerosene fire group or the other fire group ($p < 0.0001$).

Fig. 4a shows the correlation between toluene and benzene concentrations in the three groups (construction fires, kerosene fires, and gasoline fires). In the gasoline fire group, the concentration of toluene was extremely high compared with that of benzene, and the concentration ratio of toluene/benzene (4.89 ± 3.37) was significantly higher than in the construction fire (0.34 ± 0.88) and kerosene fire (0.84 ± 0.84) groups (Fig. 4b). The difference between the construction fire and kerosene fire groups was not significant.

Fig. 5a shows the correlation between the xylene and benzene concentrations in the three groups. In the kerosene fire and gasoline fire groups, the xylene concentrations were higher than those of benzene and the concentration ratios of xylene/benzene (kerosene fire: 0.60 ± 0.58, gasoline fire: 0.73 ± 0.41) were significantly higher than that in the construction fire group (0.02 ± 0.03) (Fig. 5b). The difference between the kerosene fire and gasoline fire groups was not significant.

4. Discussion

The concentrations of volatile hydrocarbons in 121 fire-related cases were quantified and the cases were classified into four groups according to the criteria from Morinaga et al. We then examined the relationship between the concentrations of COHb and volatile hydrocarbons, and between those of different volatile hydrocarbons.

Fig. 1 shows that the blood COHb concentrations in the two groups associated with fire accelerants (kerosene and gasoline) were significantly lower than those in the construction fire group. This difference has been mentioned in a previous report [7]; however, to the best of our knowledge, the present study is the first to report clear data for this. On the basis of these data, we propose that, in cases of fire involving the use of accelerants, flame propagation is more extreme and death occurs rapidly before a significant quantity of carbon monoxide can be inhaled. The blood COHb concentrations in the other fire group were very low (mean ± standard deviation: 6.0% ± 3.1%). Five of these cases were outdoor fires and the other 7 cases were indoor fires. In outdoor fire cases, people are less likely to suffer from carbon monoxide poisoning because carbon monoxide that is produced by the fire will readily diffuse. Additionally, compared with construction fires, there are fewer

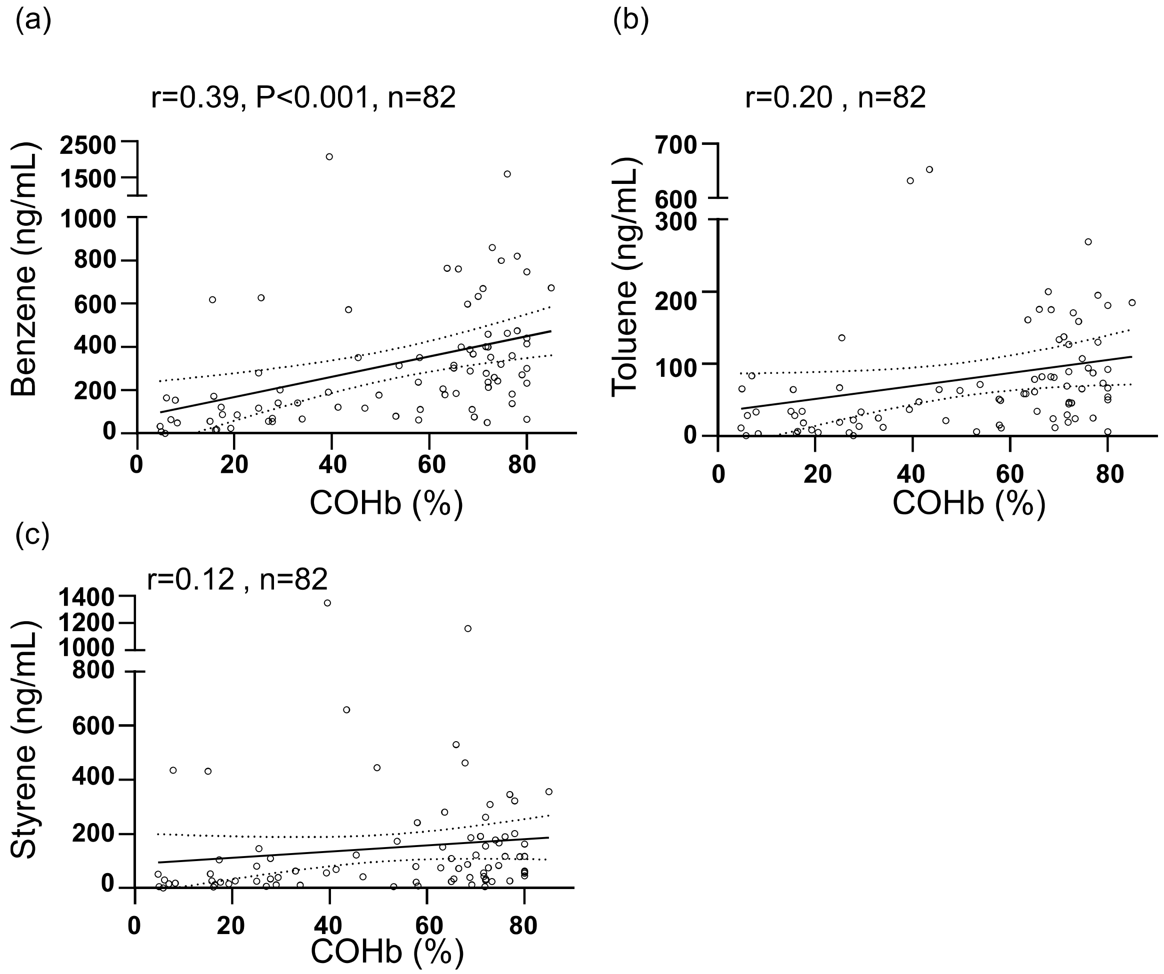

Fig. 2. Correlations between the carboxyhemoglobin (COHb) concentrations and blood volatile hydrocarbon concentrations for benzene (a), toluene (b), and styrene (c) in the construction fire group. The dotted lines show 95% confidence intervals for the linear regression equation.

Table 3
Correlation coefficients between the concentrations of five volatile hydrocarbons (benzene, toluene, ethylbenzene, xylene, and styrene) in the construction fire group.

Correlation coefficient	TL	EBZ	XL	SR
BZ	0.78	0.63	0.72	0.60
TL	–	0.59	0.61	0.75
EBZ	0.59	–	0.71	0.83
XL	0.61	0.71	–	0.58

BZ, benzene; TL, toluene; EBZ, ethylbenzene; XL, xylene; SR, styrene; –, not determined.

combustible materials to generate volatile hydrocarbons in outdoor fires. Therefore, very low concentrations of volatile hydrocarbons will be detected in the blood in these cases. The reason for the lack of volatile hydrocarbons in the indoor fire cases was not clear. Because the individuals in these 7 cases were elderly (mean ± standard deviation: 84.3 ± 5.6 years), we speculated that they did not survive at the scene for a long time, which reduced their inhalation of volatile hydrocarbons.

The concentrations of styrene, toluene, and especially benzene were correlated with the COHb concentrations, which is consistent with our previous report (Fig. 2) [5,8]. In the cases with high COHb concentrations ($>$ 60%), the COHb concentrations deviated from the line produced by linear regression. This might be because the blood COHb concentration would become saturated, unlike those of the volatile hydrocarbons. Incidentally, Austin et al. investigated the characteristics of volatile hydrocarbons in smoke at municipal structure fires [9]. They found that the combustion products were similar even though there were differences in the combustible materials, and benzene, toluene, and naphthalene were commonly detected in these types of fires. Moreover, benzene, styrene, and toluene are known toxic chemicals generated in fires and their metabolites are sometimes used for biomonitoring to assess firefighters' smoke exposure [10–12]. These results all show that construction fires generally produce benzene, styrene, and toluene in addition to carbon monoxide. Therefore, a high correlation between the concentrations of these chemicals and COHb in blood could be taken as evidence that a victim was exposed to smoke before death. In consideration of the detection sensitivity, we recommend using heart blood rather than peripheral blood for volatile hydrocarbon analysis. Volatile hydrocarbons are thoroughly distributed to tissues during blood circulation and their concentrations in a fire victim's peripheral blood are usually lower than those in heart blood (data not shown) [13].

Table 3 and Fig. 3 showed high correlations between toluene and benzene, xylene and benzene, toluene and styrene, and ethylbenzene and styrene in the construction fire group. There are no other reports for comparison with our data; however, in experimental structure fires, the concentrations of benzene in smoke are positively linearly correlated with six substances, including xylenes (*o*, *m*, and *p* isomers) and toluene [9,14], which is consistent with our results. Our results indicate that the concentrations of volatile hydrocarbons in the blood of fire victims reflect the victim's circumstances at the fire scene. The concentration ratios between toluene/benzene, xylene/benzene, toluene/styrene, and ethylbenzene/styrene could be used in volatile hydrocarbon analysis to

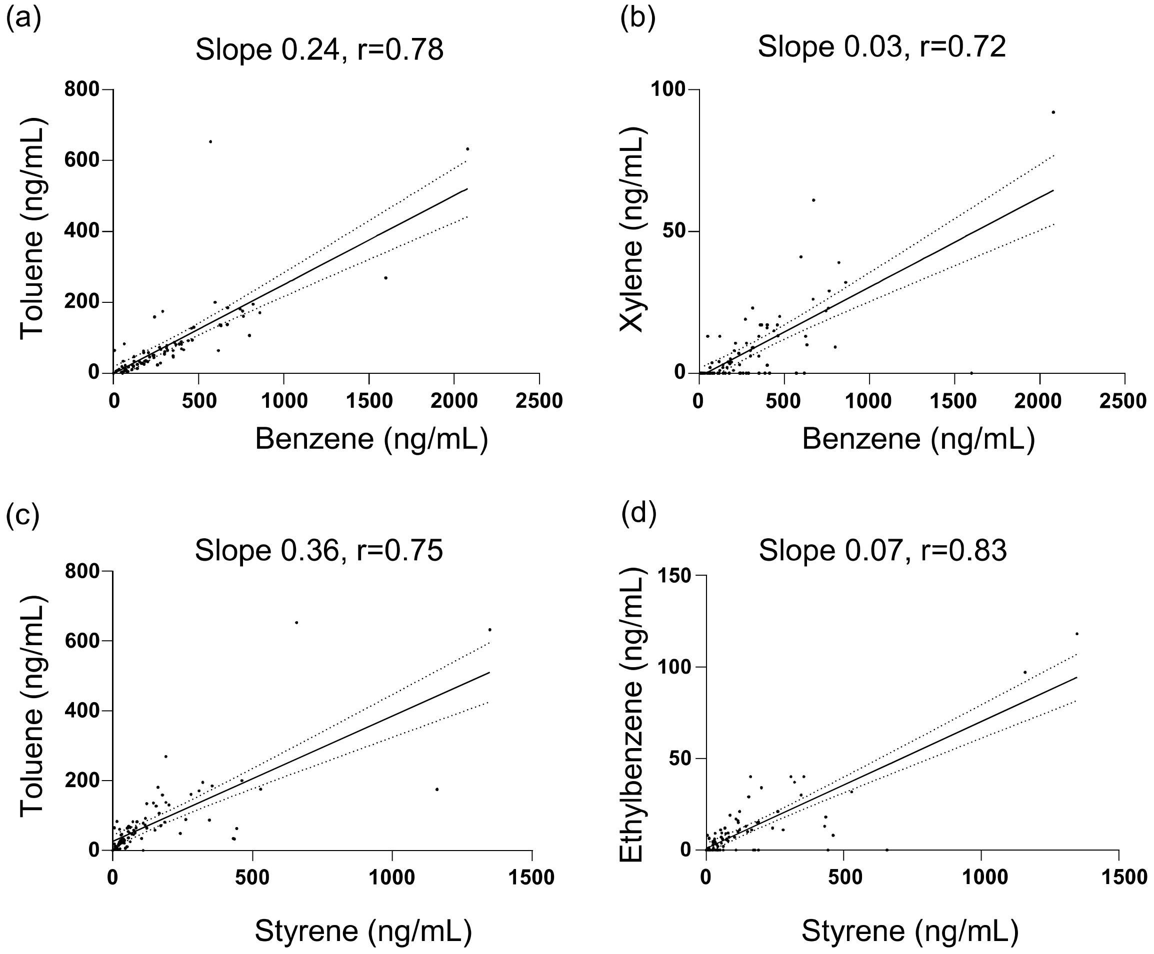

Fig. 3. Correlations between blood toluene and benzene (a), xylene and benzene (b), toluene and styrene (c), and ethylbenzene and styrene (d) concentrations in the construction fire group. The dotted lines show 95% confidence intervals for the linear regression equation.

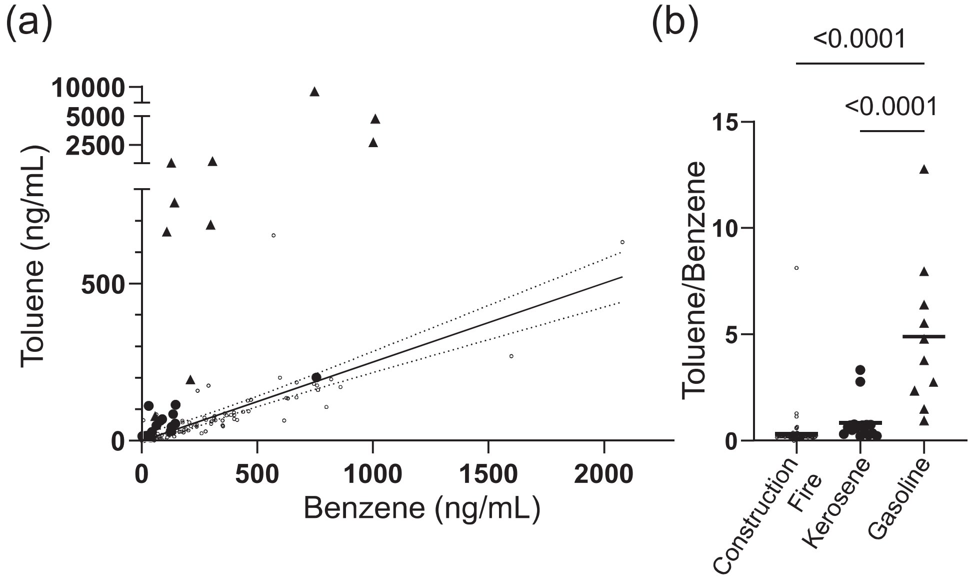

Fig. 4. (a) Correlation between blood toluene and benzene concentrations in three of the groups. The dotted lines show 95% confidence intervals for the linear regression equation of the construction fire group. (b) The toluene/benzene concentration ratio in blood from cases in the three groups. The horizonal line for each group shows the mean toluene/benzene concentration ratio. Symbols: construction fires (open circles), kerosene fires (closed circles), and gasoline fires (closed triangles).

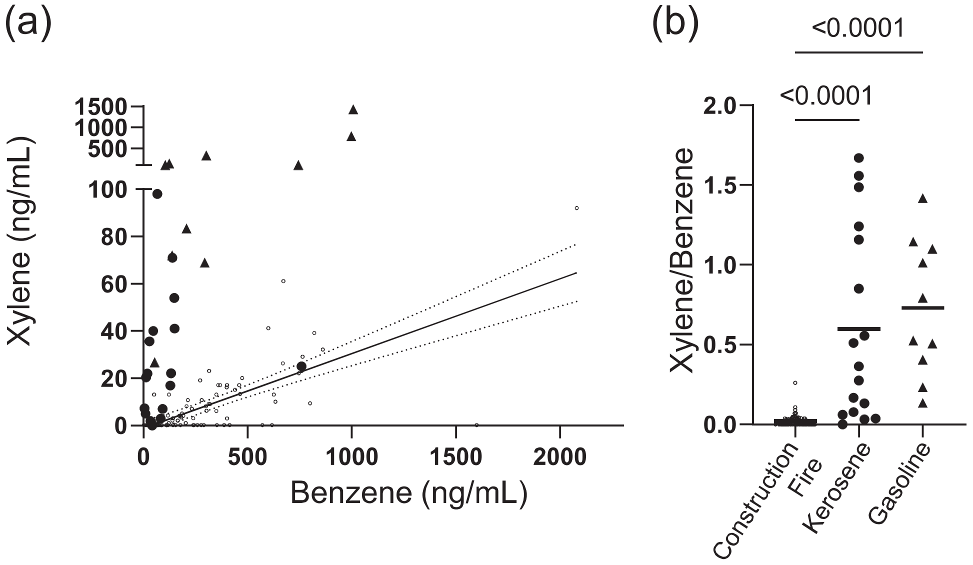

Fig. 5. (a) Correlation between the blood xylene and benzene concentrations in three of the groups. The dotted lines show 95% confidence intervals for the linear regression equation of the construction fire group. (b) The xylene/benzene concentration ratio in blood from cases in the three groups. The horizontal line for each group shows the mean xylene/benzene concentration ratio. Symbols: construction fires (open circles), kerosene fires (closed circles), and gasoline fires (closed triangles).

identify construction fires started without accelerants.

Our results suggested that the three groups could be distinguished using the correlations between benzene, toluene, and xylene (Figs. 4 and 5). Toluene is a main component of gasoline, and xylene is a component of kerosene and gasoline. High concentrations of toluene were detected in the blood of the cases in the gasoline fire group and the concentration ratio of toluene/benzene in the gasoline fire group was significantly higher than those in the construction fire and kerosene fire groups. Moreover, high concentrations of xylene were detected in the blood of the cases in the kerosene fire and gasoline fire groups, and the concentration ratios of xylene/benzene in the kerosene and gasoline fire groups were significantly higher than that in the construction fire group. Therefore, the concentration ratios of toluene/benzene and xylene/benzene could be used as indicators to distinguish the three groups. Incidentally, Suzuki et al. also examined volatile substances in the blood of fire victims using NeedlEx® headspace GC-MS and compared the approximate levels of volatile compounds in cases from building fires, self-immolation using kerosene, and self-immolation using gasoline [3]. They found that the highest toluene concentrations occurred in the self-immolation cases using gasoline, which is consistent with our results. According to our results, and adding to the criteria proposed by Morinaga et al., we developed criteria for classification of cases into the different groups (Table 4). The modified criteria could help to identify fire cases involving accelerants.

Table 4
Proposed criteria for classification of fire-related death cases into different groups using the detected volatile hydrocarbons in the blood.

	Aromatic compounds		Aliphatic compounds
Group	BZ, TL, XL	PBZ, ETL, TMB	C_8–C_{12}
Construction fire	$BZ^+ > TL^+$ $BZ^+ > XL^+$	−	−
Kerosene fire	$BZ^+ > TL^+$ XL^+	+	+
Gasoline fire	$TL^+ > BZ^+$ XL^+	+	−

BZ, benzene; TL, toluene; XL, xylene; PBZ, propylbenzene; ETL, 3-ethyltoluene; TMB, trimethylbenzene (1,3,5-, 1,2,4-, and 1,2,3-); + , detected; − , not detected; A>B, the concentration A is higher than the concentration B

5. Limitations

While the data presented here are useful for the estimation of the circumstances surrounding the fire-related death, there were some limitations with this study. The bodies of the deceased were stored at 4 °C before autopsy. Their postmortem time were all within 3 days. Any potential changes in blood hydrocarbon concentrations resulting from delays in the postmortem were not examined in this work. Deceased smokers would likely have a COHb concentration between 5% and 6% [15]. However, in this work, we did not collect data to ascertain whether or not the deceased had been smokers. It should also be noted that information concerning the cause of each fire and the circumstances surrounding the death of the individual were limited, even though such details would have assisted in explaining outlying results. The quantities of cases that were evaluated that involved kerosene fires ($n = 17$) and gasoline fires ($n = 10$) were relatively small. The accuracy of the results could be improved in future by increasing the number of cases assessed, in cooperation with other institutions.

The majority of the blood samples were analyzed immediately after collection but some samples were analyzed after storage at 4 °C for several days. The effect of this storage on the concentrations of COHb and hydrocarbons in blood was not examined. This work assessed only 13 hydrocarbons, based on a previous report by Morinaga et al. [2]. A few prior studies examined a greater number of volatile hydrocarbons in association with fire-related deaths [3,4] but none of these presented quantitative results. By contrast, the data we present are quantitative. The 13 hydrocarbons we selected represent the major hydrocarbons found in kerosene and gasoline and the compounds primarily generated by combustion. Future quantitative studies involving other compounds could provide additional insight.

6. Conclusion

We evaluated the relationships between the concentrations of blood COHb and volatile hydrocarbons, and between the concentrations of different blood volatile hydrocarbons in the 121 fire-related cases. The mean COHb concentrations were higher in the construction fire group than in the kerosene fire group or the gasoline fire group. A high coefficient correlation was observed between the benzene and COHb concentrations, which was consistent with our previous report. In the construction fire group, the coefficient correlations between toluene and benzene, xylene and benzene, toluene and styrene, and ethylbenzene and styrene concentrations were relatively high. The correlations between the benzene, xylene, and toluene concentrations may be a novel indicator to distinguish between deaths in construction fires, kerosene fires, and gasoline fires. Quantitation of volatile hydrocarbons in fire-related deaths provides information to support the determination of the circumstances surrounding the death. We expect that this method will be helpful in forensic laboratories when investigating fire-related deaths. In the future, this analytical technique could also provide valuable information if additional data are collected for volatile hydrocarbons to establish correlations with the circumstances at fire scenes.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Ako Sasao: Conceptualization, Data curation, Visualization, Writing – original draft. **Kosei Yonemitsu:** Conceptualization, Supervision, Writing – review & editing. **Yuki Ohtsu:** Data curation, Writing – review & editing. **Hiroshi Tsutsumi:** Resources, Writing – review & editing. **Shota Furukawa:** Resources, Writing – review & editing. Yoko Nishitani: Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank our reviewers for helping us to improve this manuscript. We thank Gabrielle David, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2023.111872.

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