Forensic Science International 256 (2015) 46–52

Contents lists available at ScienceDirect

Forensic Science International

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

Assessment of carboxyhemoglobin, hydrogen cyanide and methemoglobin in fire victims: a novel approach

Luis A. Ferrari [a,*], Leda Giannuzzi [b]

[a] Cátedra Toxicología y Química Forense, Facultad de Ciencias Exactas y Derecho, U.M, Cabildo 134, 1708 Morón, Buenos Aires, Argentina
[b] Cátedra de Toxicología, Facultad de Ciencias Exactas Universidad Nacional de La Plata, calle 47 y 116, 1900 La Plata, Argentina

ARTICLE INFO

Article history:
Available online 21 August 2015

Keywords:
Carbon monoxide
Methemoglobin
Hydrogen cyanide
Fire deaths
Polyurethane combustion
Blood samples

ABSTRACT

To establish the cause of death, carboxyhemoglobin (COHb), total hemoglobin (tHb), methemoglobin (MetHb), and hydrogen cyanide (HCN) were quantified in the blood of fire victims. We analyzed 32 out of 33 blood samples from forensic autopsy cases in a disastrous polyurethane mattress fire, which caused the deaths of 33 inmates at a prison in Argentina in 2006.

The cadaveric blood samples were collected by femoral vein puncture. These samples were analyzed using the IL80 CO-oximeter system for tHb, MetHb, and COHb levels and by microdiffusion for HCN and COHb levels. Blood alcohol (ethanol) and drugs were examined by headspace gas chromatography–flame ionization detection (HS-GC–FID) and GC–mass spectrometry (MS), respectively. Polyurethane mattress samples were analyzed according to the California 117 protocol.

The saturation of COHb ranged from 10% to 43%, tHb from 2% to 19.7%, MetHb from 0.10% to 35.7%, and HCN from 0.24 to 15 mg/L. These HCN values are higher than the lethal levels reported in the literature. Other toxic components routinely measured (ethanol, methanol, aldehydes, and other volatile compounds) gave negative results in the 32 cases. Neither drugs of abuse nor psychotropic drugs were detected. The results indicate that death in the 32 fire victims was probably caused in part by HCN, generated during the extensive polyurethane decomposition stimulated by a rapid increase in temperature. We also considered the influence of oxygen depletion and the formation of other volatile compounds such as NO_x in this disaster, as well as pathological evidence demonstrating that heat was not the cause of death in all victims. Furthermore, statistical analysis showed that the percentage values of COHb and MetHb in the blood were not independent variables, with $\chi^2 = 11.12$ (theoretical $\chi^2 = 4.09$, degrees of freedom = 12, and $\alpha = 0.05$). However, no correlation was found between HCN and MetHb in the blood of the victims. This is the first report to assess the relationship between COHb and MetHb in forensic blood samples. We further discuss other factors that could lead to a lethal atmosphere generated by the fire and compare the data from this disaster with that of other published fire episodes.

1. Introduction

It is well known that fire generates various gases, some of which are very toxic, such as carbon monoxide (CO) and hydrogen cyanide (HCN), which can be lethal on inhalation [1–6]. However, oxygen depletion and the generation of carbon particles and other volatile compounds at the fire site can directly affect the toxicity of the mixture of gases generated [7].

Although the considerable amount of CO produced could have been the primary cause of death of the exposed individuals, other toxic compounds are sometimes generated, depending on the type of combustion material [8]. Several published papers report the generation of HCN during the combustion of polymers containing nitrogen in their molecular structure [9–13], such as the polyurethane used in the manufacture of mattresses or acoustic soundproofing in nightclubs and music venues.

Variable levels of carboxyhemoglobin (COHb) and HCN are reported in the literature on fire episodes involving polymers [9–16], some mentioning lethal concentrations of HCN and sublethal levels of COHb and others the reverse.

Data on the role of HCN in fires are controversial, with some authors reporting that HCN poisoning may itself be the cause of death. Others, however, consider CO to be the main toxic agent and assert that HCN levels are falsely increased by a number of factors

* Corresponding author.
E-mail address: lferrari@biol.unlp.edu.ar (L.A. Ferrari).

http://dx.doi.org/10.1016/j.forsciint.2015.08.010
0379-0738/

inherent either to the fire episode itself or to the analyzed blood sample. These conclusions are based on COHb and HCN determinations in postmortem blood samples from fire victims [7,12].

The specific type of polymer used in the manufacture of materials affected by the fire and the incorporation of fire retardants in these materials are equally important.

Factors such as oxygen depletion, the generation of carbonized particles that prevent escape from the fire, confined spaces, the high temperatures reached during the episode, and the production of other gases such as hydrochloric acid (HCl) and nitrogen oxides (NO_x) appear to have a direct impact on the intoxication mechanism. Apart from a few case studies, little research has been published to date on this topic and the few publications that do exist rely on data from only one or a few affected individuals [7].

Furthermore, very little research has been carried out on the possible formation of methemoglobin (MetHb)-generating compounds in fires, and only a few publications refer to the likely role of these compounds in the final lethal mechanism [17–19]. It is well known that putrefied blood contains considerable amounts of MetHb [20–23].Several authors have described different methods of quantifying COHb, HCN, and MetHb [24–29].

Several disastrous fire episodes have occurred in Argentina, not only in nightclubs and music venues but also in prisons and/or police cells. In an earlier publication [16], we reported a case involving the mass intoxication of 35 people, of whom 33 died within the first few minutes after polyurethane mattresses and pillows caught fire in a prison cell block forming part of the Province of Buenos Aires Penitentiary Service in Olmos, Argentina, in 1990. Very high levels of HCN and very low levels of COHb were found in the blood of the victims, and the indices of lethal levels of both gases (LI_{CO} and LI_{HCN}) were defined in order to diagnose the cause of death. At the time the forensic study was carried out, at the beginning of the 1990s, knowledge on the subject was very limited and so little inroads were made into researching secondary factors such as those mentioned earlier, which play an important role in determining the cause of death.

Our study focuses on the main factors involved in interpreting the cause of death of the 33 victims of the fire episode based on data of COHb, HCN, and MetHb levels in the blood. We also studied the gases generated once the fire had started and the factors that lead to a lethal atmosphere.

2. Case description

The fire began during a riot at a state penitentiary in Argentina in 2006, resulting in 33 deaths. The fire was started by the combustion of mattresses and pillows made of polyurethane. Within a few minutes, the fire had spread to all the beds in the cellblock, producing vast quantities of smoke, or particles suspended in the air, and high temperatures. Some of the inmates were evacuated at the outset of the fire. However, 33 bodies were found after a brief interval, one of them a survivor who died a few hours later in hospital.

The autopsies after the episode were timed as follows:

1–2 h post mortem: one case
3–8 h post mortem: seven cases
9–12 h post mortem: 10 cases
12–24 h post mortem: 15 cases*

*One of the victims was hospitalized but died a few hours later.

In the case of all victims, the autopsy report described the presence of carbon particles (black smoke) in the larynx and in some cases in the trachea, first- and second-degree burns in most of the victims, and congestion of all the organs. The reports also mentioned vasocongestion in the liver, lungs, brain, heart, kidney, and spleen, especially severe in the meninges; extensive areas of intra-alveolar edema; and the presence of black smoke in the lingual epithelium and larynx. In all cases, death was attributed to an asphyxia syndrome, ruling out burns as the primary cause of death.

3. Materials and methods

3.1. In biological samples

COHb, total hemoglobin (tHb), MetHb, HCN, and other toxic compounds were quantified in the blood of fire victims in order to establish the cause of death.

Blood samples were collected from the bodies of the victims with sterile syringes and needles from the femoral and/or intracardiac vein, depositing 10 mL in heparinized flasks.

COHb (%COHb), MetHb (%MetHb) and tHb were analyzed using an IL80 CO-oximeter following the technique described by Lee et al. [23] and the manufacturer's instructions: the sample was diluted in a ratio of 1:2 with ultrapure Milli-Q Water, homogenized, and centrifuged. The microdiffusion technique described by Klendshoj et al. [29] for COHb was also performed. Briefly, 1 mL of whole blood was placed in the outer Conway chamber, and 2 mL of 0.01 N palladium chloride solution was added in the central compartment. Then, 10% sulfuric acid was added to the blood sample and pulled ensuring tightness for 2 h at room temperature or in an oven at 37 °C.

The internal compartment solution (metallic palladium and palladium salt excess) was centrifuged, and then 0.1 mL of the supernatant solution was transferred to a 10-mL volumetric flask. Finally, 0.1 mL of 0.1% Arabic gum and 1 mL of 15% potassium iodide were added and mixed well. This mixture was read in a spectrophotometer at 500 nm. The calibration curve was established with six points of COHb concentration at 10%, 20%, 40%, 60%, 80%, and 90% ($r^2 = 0.970$).

HCN was quantitatively determined by the microdiffusion method described by Guatelli [30] and reviewed in detail by Ballantyne [31]. Briefly, the technique involves separating the cyanide from blood by microdiffusion and converting it into cyanogen chloride with chloroamine T. This is then made to react with a mixture of 1-phenyl-3 methyl-2-pyrazoline-5-one and 4,4′-bis-(1-phenyl-3 methyl-2-pyrazolin-5-one) in pyridine, which results in the formation of a blue dye that can be estimated spectrophotometrically at 630 nm. The calibration curve was established with six points of cyanide concentration (μg/100 mL), at 5, 50, 200, 500, and 1000 ($r^2 = 0.995$).

Further relevant toxic components such as ethanol, methanol, aldehydes, and other volatile compounds were studied using the headspace gas chromatography–flame ionization detection (HS-GC–FID) technique [32]. After isolation by the solid-phase extraction (SPE) method using Clean Screen DAU 303 cartridges (World Wide Monitoring Corp.), the blood, liver, and kidney were screened for drugs of abuse (cocaine, tetrahydrocannabinol, and psychotropic drugs) and other acidic, neutral, and basic drugs. The extracts were analyzed by the capillary column GC–mass spectrometry (MS) method [33], as described by Drummer [34].

Pearson's Chi-squared test was performed to determine the statistical relationship between HCN, COHb, and MetHb in the blood of the fire victims.

3.2. In mattresses

3.2.1. Study of the velocity of fire propagation, flame duration, possible existence of fire retardants in the combustion materials, and presence of HCN in the cell block

A fire simulation was performed using the unburned remains of the 16 mattresses ignited in the episode. An experimental

combustion chamber in conformity with the California 117 protocol [35] was used to calculate the velocity of ignition; total burning time; and concentration of oxygen (O_2), CO, and NO_x. The calculated volume of the experimental chamber was 0.768 m^3 (the volume of the precinct where the fire occurred was 1015 m^3).

Six pieces of the polymer extracted from the mattress samples collected from the fire site and submitted to the laboratory by the coroners were used for the experiments, each piece weighing 9.2 g. The number and weight of the sample pieces were proportional to the number of burned mattresses and the volume of the cellblock. The pieces were cut with a special device in accordance with the cited norms and placed in test tubes measuring 300 mm × 72 mm × 13 mm. The test tubes were in turn placed inside the combustion chamber stipulated in the California 117 protocol, and combustion was initiated with a pilot light.

Ignition was standardized to a brief 12 s, after which the pilot flame was extinguished. A chronometer was used to calculate the time that elapsed between ignition at one end of the chamber and the flame reaching the other end, 30 cm away.

The results were compared with values of regulation-compliant commercial mattresses containing fire retardants.

Based on the data provided by the sensor placed in the chamber, the gases generated after the fire ignited were analyzed with XILIX-EMA2000; calibrated; and validated to measure O_2, CO, CO_2, NO_x, hydrocarbons (HCs), and SO_x.

4. Results

4.1. In biological material

Table 1 shows the percentage of tHb, COHb, MetHb, and hydrogen cyanide in the blood of each of the fire victims, including the victim who died later in the hospital.

The %COHb varied between 10% and 43% (average 30.13%), %tHb between 2% and 19.7% (average 5.46%), %MetHb between 0.10% and 35.7% (mean value 10.29%), and HCN between 0.24 and 15 mg/L (mean value 5.46). The values are higher than the lethal levels reported in the literature.

Fig. 1 shows the data in a frequency histogram for %MetHb, %COHb, and HCN level (mg/L) in the blood of the 32 fire victims. The frequency distribution for COHb and HCN concentration in the blood of the 32 fire victims is shown in Table 2. In most cases, the level is higher than 2 mg/L of HCN and 30–40% of COHb. Table 3 shows the frequency distribution for %COHb and %MetHb, the values in most cases ranging from 20% to 40% for %COHb and from 10% to 40% for %MetHb. The results indicate that the blood of the fire victims contained lethal levels of cyanide together with low levels of %COHb. The test results were negative for other toxic compounds (ethanol, methanol, aldehydes, and other volatile compounds) in all 32 cases (i.e., not including the hospitalized victim who died later). No drugs of abuse or psychotropic substances were detected.

4.2. In polyurethane mattresses and pillows

The average speed of propagation of the fire was 109 mm/min, the material being completely consumed within 3 min. The flames remained incandescent until the material was consumed entirely.

The gases in the chamber were measured 2 min after ignition: the oxygen levels (initial level 21%) were found to have fallen by 11%; the CO values increased to slightly above 4000 ppm; the HC level increased to >10,000 ppm; the NO_x level reached 95 ppm; and the total combustion time, with only slight variations, was around 3 min. The quantitative results correspond to mean values from three successive determinations.

Table 1
Levels of total hemoglobin (tHb), methemoglobin (MetHb), carboxyhemoglobin (COHb), and hydrogen cyanide (HCN) in the blood of fire victims.

Cases	tHb (g/dL)	MetHb (%)	(% COHb)	HCN (mg/L)
1	19.7	0.1	10	2.0
2	5.1	4.5	40	1.3
3	5.5	2.9	18	14.0
4	2.3	1.8	42	10.0
5	8.0	2.0	26	0.24
6	4.9	31.5	35	0.24
7	6.7	11.4	30	4.0
8	0	0	0	0[a]
9	5.2	6.8	42	5.2
10	2.0	13.6	31	0.36
11	6.2	5.5	40	15
12	3.7	11.9	30	0.5
13	4.1	11.4	30	6.0
14	5.7	7.8	24	0.6
15	6.1	3.8	12	4.0
16	5.5	11.2	25	3.2
17	9.5	1.2	31	5.0
18	7.0	2.4	32	7.0
19	2.8	35.7	36	6.5
20	6.8	3.3	43	7.0
21	5.7	23.2	37	3.8
22	4.3	15.4	40	4.5
23	5.1	4.5	24	4.5
24	3.5	33.0	33	9.0
25	6.3	4.4	37	6.0
26	3.0	8.3	23	4.0
27	4.6	18	15	5.0
28	9.2	11	34	0.5
29	5.5	4.4	13	3.8
30	3.1	18.5	28	3.8
31	2.3	4.3	40	5.0
32	2.3	10.2	37	0.5
33	3.0	5.5	26	1.4

[a] Victim died in hospital a few hours.

5. Discussion

5.1. Evaluation of the role of CO and HCN in the present episode and comparison with data published by other authors

In a similar episode published by Ferrari et al. [16], the lethality index (LI) was defined as follows:

$$LI_{CO} = \frac{\%COHb - 50\%}{50\%}$$

where %COHb is the percentage of COHb in the blood of victims. When $LI_{CO} = 0$, then the COHb in the sample is 50%. LI_{CO} is >0 when COHb is >50%, and a <0 LI_{CO} indicates a COHb <50%. The level of 50% COHb was selected for our LI_{CO} definition, because levels above 50–60% saturation were often lethal [18].

A second LI was defined as follows:

$$LI_{CNH} = \frac{HCN - 1\,(mg/L)}{1\,(mg/L)}$$

where HCN is the level of cyanide in the blood. Similarly, when LI_{HCN} equals 0, HCN in the sample is 1 mg/L. LI_{HCN} >0 corresponds to HCN >1 mg/L, whereas $LI_{(HCN)}$ <0 indicates HCN <1 mg/L. A blood cyanide level >1 mg/L is associated with lethal cases [4,25].

The data for %COHb and HCN for the 32 victims expressed in terms of the LIs (LI_{CNH} and LI_{CO}) are presented in Fig. 2. Data from other published episodes are included for the purpose of comparison. Four zones (I–IV) were defined in which the abscissas correspond to LI_{CO} and the ordinates to LI_{CN}. As can be observed in the graph, the interception of the lines corresponding to $LI_{CO} = 0$ and $LI_{CN} = 0$ determines the following attributes for the four zones:

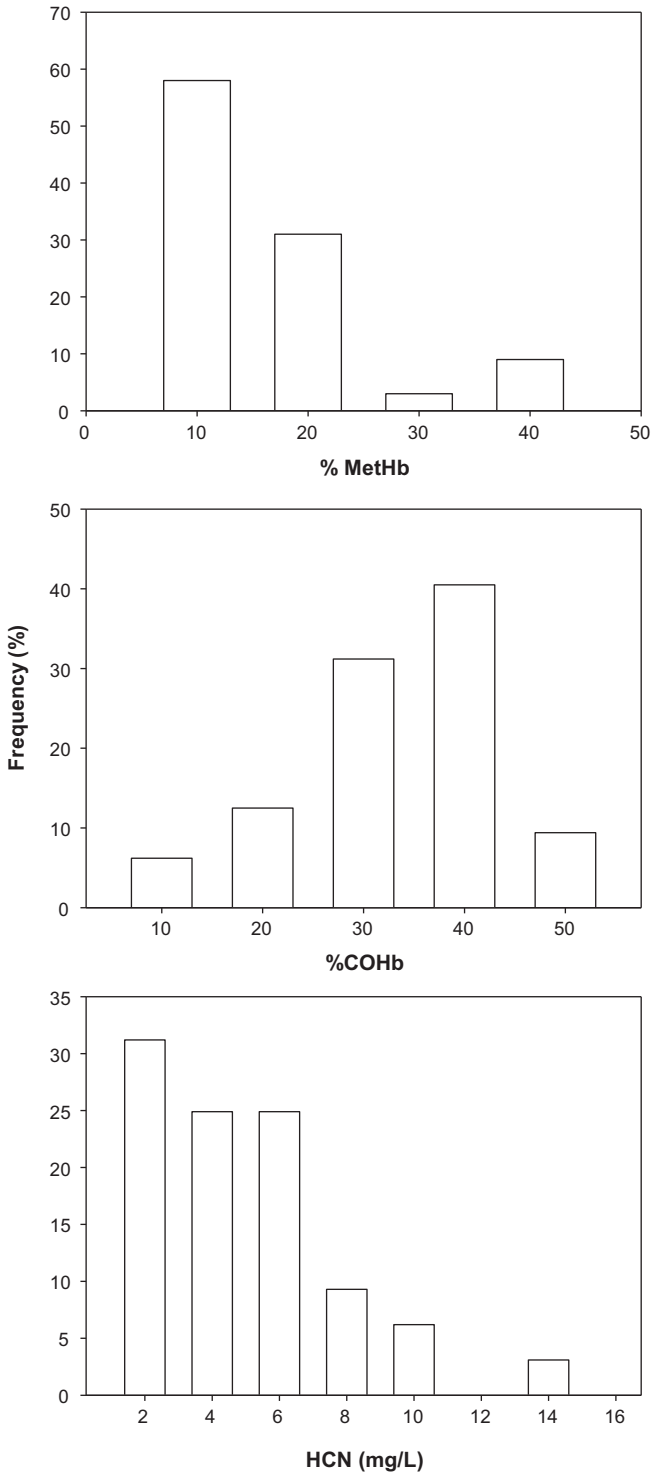

Fig. 1. Frequency histogram of %MetHb, %COHb, and HCN (mg/L) in 32 fire victims studied.

Zone I: This zone contains sublethal levels of %COHb and HCN in the blood.
Zone II: The highest levels of gas in the blood correspond to COHb.
Zone III: The highest levels of gas in the blood correspond to HCN.
Zone IV: Both gases are present in the blood in lethal concentrations and are toxic.

Having established the toxic gas content in the blood of victims of fires involving nitrogenated plastics known to generate HCN and CO, it can indirectly be assumed that either of the gases was present in the atmosphere. In 30 of the 32 cases in the present episode and in those reported by Ferrari et al. [16] and Zameckinc and Tam [13], the concentration of HCN in the blood of victims was higher than the lethal level (Zone III).

All the victims of a fire in a nightclub reported by Repetto and Martínez [10] fall within Zone II; that is, they had high levels of %COHb and sublethal levels of HCN.

The cases reported by Ishii et al. [14] fall under Zones I and II, with sublethal levels of both gases and a higher concentration of %COHb than of HCN. Two of the 32 cases from our episode also fall under Zone I. Only a few cases reported by Lundquist et al. [2] and one case published by Ishii et al. [14] describe quantitative values higher than the toxic range for both COHb and HCN.

From the cases in the literature, it can be concluded that the characteristics of each fire episode are different, translating into higher or lower levels of %COHb or HCN found in the blood of victims of mass intoxication. It is worth noting that few cases have shown high levels of toxicity for both gases simultaneously (Zone IV).

5.2. Factors that complicate the interpretation of data on COHb and HCN in the blood of fire victims

The processes involved in CO and HCN generation and their toxic effects are still not fully understood [3–7,9–16,19,22,36]. Repetto and Martínez [10] found high levels of carbon monoxide and sublethal doses of hydrogen cyanide in four of six fatal victims of a fire in a nightclub. Coleman [37] reports a higher level of CO than of HCN in the decomposition of nitrogenated plastics, and Montgomery [38] states that HCN is released in excess in environments with little oxygen.

The production of HCN has been shown to occur through a variety of processes, depending on the materials involved [2,4–8,12,15,38]. However, there is a tendency to attribute a greater toxicological role to the CO generated than to HCN [7].

Purser et al. [3] reported that the association of HCN with products of the pyrolysis of polyacrylonitriles and carbon monoxide was less toxic than HCN, leading to an actual diminution of the clinical symptoms. The authors surmised that a competitive mechanism between the nitriles and the hydrogen cyanide limited the effects of the latter. Therefore, these studies suggest that, in cases of fire, HCN can result in a rapid initial incapacity or inhibition of movement, after which death is caused by carbon monoxide poisoning. Our findings in the present case contradict this hypothesis, as we observed that COHb concentrations were <40% in almost all cases (29 of 32).

In his comprehensive review, Alarie [7] asserts that the main actors in fire episodes are CO, a large number of chemical irritants, a low oxygen level in the environment, and high temperatures. His opinion can be summarized as follows:

1. CO remains the main toxic substance in fires presently.
2. Appreciable amounts of HCN can be found in the blood of fire victims.
3. Oxygen depletion and increased temperatures are difficult to establish, although they may play a larger role at the spot where the fire started.
4. Studies using experimental animals indicate that the lower the level of oxygen, the faster the onset of death.

In the experiments cited by Alarie, the levels of COHb at the time of death are lower than would be expected had the victims been exposed to CO alone. However, HCN was lower by a factor of 10 than the level of hydrogen cyanide alone [7], thus complicating the assessment of the contribution of HCN to the deaths caused by

Table 2
Percentage of victims with different levels of HCN (mg/L) and %COHb in blood.

		%COHb					
		0–10.0	10.1–15.0	15.1–20.0	20.1–30.0	30.1–40.0	>40.0
HCN (mg/L)	0–2.0	3.1	0	0	12.5	15.6	0
	2.1–4.0	0	6.2	3.1	12.5	3.1	0
	4.1–6.0	0	3.1	0	6.2	12.5	3.1
	6.1–8.0	0	0	0	0	6.2	3.1
	8.1–10.0	0	0	0	0	3.1	3.1
	>10.0	0	0	3.1	0	0	0

the fire, as interpreted from the blood analyses. Several researchers have suggested that, in the case of both animals and humans, concentrations of 1–3 mg/L are lethal when HCN is inhaled during the fire [39,40]. These concentrations were surpassed in the present and other reported episodes. It appears that the magnitude of the generated toxicity is time–space dependent, in accordance with the scenario of each fire.

It should further be considered that lethality also depends on whether the person is awake, asleep, or capable of escaping the fire. Our combustion experiment in the experimental chamber using pieces of polyurethane mattresses showed that NO_x and HC were generated and oxygen was depleted within 1–2 min, accompanied by the formation of highly dense carbon particles. Furthermore, data from our episode confirm the generation of NO_x, a known MetHb-generating agent, both in the combustion of the polymer and in the blood of many of the victims.

Therefore, we can hypothesize that the %MetHb produced in the episode affected the action of the incorporated HCN, as it has greater affinity for HCN. However, HCN could not be uniquely incorporated into blood at such high levels. In addition, even after death, HCN may diffuse through the skin or other parts of the corpse, which may partly explain the HCN levels found in the blood of fire victims, several times higher than the lethal dose.

Based on reports, Uncontrollable fires involving polyurethane often involve rapid spread of the fire and smoke, which could contribute significantly to the lethality of the episode. As mentioned earlier, we tested these conditions via the California 117 protocol.

The thermal decomposition of complex products whose structure varies considerably under different temperature and oxygen conditions can have a marked impact on the composition of the toxic atmosphere generated by the fire. It is clear that this further complicates determining the incapacitating effect of the atmosphere. The low level of oxygen and high temperatures generated in this episode are relevant to the ability of the victims to escape the fire, and the incapacitating effect could even be a significant factor leading to death. The dense smoke with black particles observed in our experiments also has an incapacitating effect, impeding escape due to vision impairment and irritation in the upper respiratory tract. A further incapacitating factor is CO, which at levels above 30% also hampers escape [7].

We were able to corroborate that several bodies in our study case were found close to the ignition spots within the cellblock. In our view, the experiments conducted with the mattress samples, the blood analyses of the victims, the position of the bodies of the victims, and the published data from other episodes indicate that the victims were severely incapacitated soon after the fire ignited, preventing their escape due to the high temperatures generated by the fire, the swift spread of the flames to the polyurethane materials, and the excessive liberation of HCN and CO.

5.3. Incidence of %MetHb and correlation with %COHb and HCN

%MetHb was detected in the blood of victims of only few episodes, although MetHb appears to provoke functional anemia, especially at levels above 20% [26]. In our episode, five of the 32 cases showed MetHb values between 18% and 35.7%, with HCN values in the blood of 3.8–9.0 mg/L; one case showed 31.5 MetHb and 0.24 mg/L; and the rest showed between 0.1% and 11.4% and 0.24–15 mg/L, respectively. Although these values could have reduced during storage, it has been reported that blood samples stored at 3–4 °C remain fairly stable for up to weeks. In our cases, the analysis commenced 12.5 days after death.

As can be observed in Table 1, some victims showed high concentrations of HCN and low concentrations of %MetHb whereas others showed the reverse. In our opinion, the position of the victims within the cell block – whether or not they were close to external windows and whether MetHb-generating agents such as NO_x were present – likely affected the level of %MetHb registered in their blood.

In the context of the present episode, a possible correlation between %MetHb and %COHb was also analyzed, with a resulting $\chi^2 = 11.12$ (theoretical case $\chi^2 = 4.09$, degree of freedom = 12, and $\alpha = 0.05$). Based on this finding, different ratios between the two

Table 3
Percentage of victims with different levels of %COHb and %MetHb in blood.

		% COHb				
		0–10.0	11–20	21–30	31–40	>40
MetHb (%)	0–10.0	6.2	9.4	15.6	15.6	9.4
	10.1–20.0	0	3.1	15.6	12.5	0
	20.1–30.0	0	0	0	3.1	0
	30.0–40.0	0	0	0	9.3	0

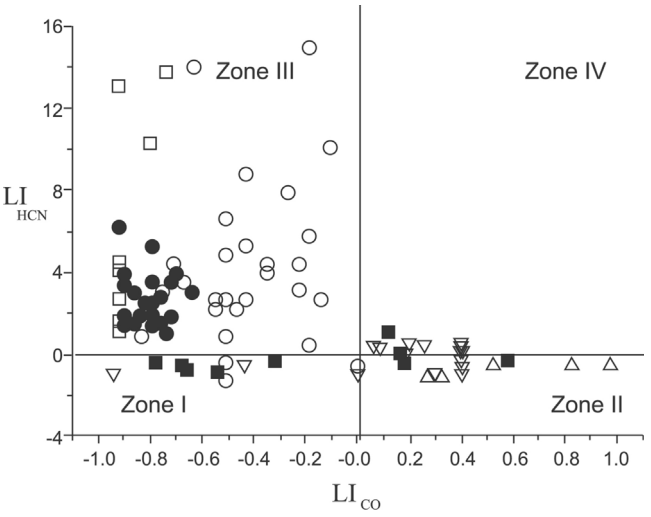

Fig. 2. Plot of the lethality index (LI) for this work and data of other authors, described under the zones defined previously. (○) Present work, (●) Ferrari et al. [16], (□) Zamecnik and Tam [13], (△) Repetto and Martinez [10], (■) Ishii et al. [14]; (▽) Lundquist et al. [2].

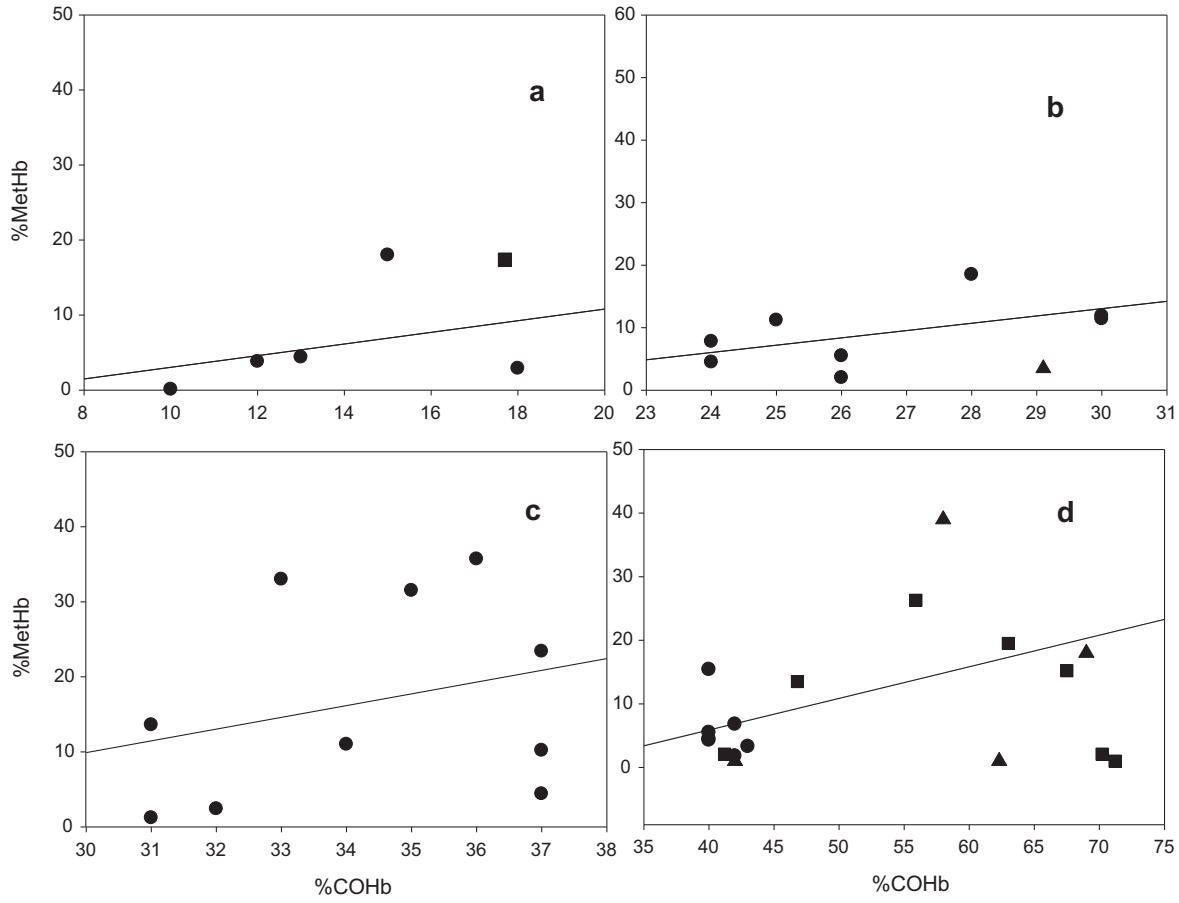

Fig. 3. Relationship between the values of %COHb and %MetHb in the 32 cases studied. Data have been separated at intervals of COHb levels as (a) 0–20% COHb, (b) 20–30% COHb, (c) 30–39% COHb, (d) >40% COHb. (●) Present work, (▲) data from Seto [41], (■) data from Katsumata [42].

variables were studied and the best fit was found to be the linear correlation between %COHb and %MetHb. Fig. 3 shows the ratios between the values for %COHb and %MetHb in each of the 32 cases studied. Intervals of levels of COHB were separated as follows: (a) 0–20% COHb, (b) 20–30% COHb, (c) 30–39% COHb, and (d) >40% COHb. In graphs a–d, MetHb is found to increase with increase in COHb. The coefficient of determination between the two variables ranged from 0.43 to 0.65.

In order to verify the validity of the proposed ratio, the %COHb and %MetHb data reported by Seto et al. [41] and Katsumata [42] taken from fatal fire casualties were also included in the same figure.

We propose that in fire episodes it is possible to find elevated levels of MetHb that are linearly correlated with COHb values. MetHb may have been formed by the inhalation of nitrogen oxides produced by the burning plastic.

In an in vitro experiment, Seto [41] reported considerable changes in Hb forms and CO levels when blood samples were heated. MetHb was formed from Hb-O_2 under mild heating, leading to the mistaken diagnosis of poisoning by oxidizable gases. COHb was stable when heated.

However, possible COHb losses due to the two different spectrophotometric methods used was minimized by refrigerating the blood and adding 2% NaF preservative. Blackmore [22] stated that, under the latter conditions, COHb is stable for many months and losses due to bacterial denaturation of blood can be prevented.

According to Katsumata [42], heat denaturation was considered to be the main cause of MetHb production in fire episodes, and inhalation of nitrogen oxides in cases of carbon monoxide poisoning. The appearance of MetHb was dependent on COHb content and heating time, with more drastic changes between 75 and 80 °C [43].

In our case, no blood coagulation was observed; the victims were removed from the fire site a few minutes after the fatal episode.

Other authors have found no correlation between COHB and soot in victims, although this is now under investigation [44].

The present paper is the first to find a linear relationship between COHb and MetHb levels in the blood of fire victims in forensic cases.

6. Conclusions

1. The level of COHb found in the blood of the majority of fire victims was below the level considered to be lethal.
2. The level of hydrogen cyanide in the blood of the majority of fire victims was >1 mg/L.
3. Other substances such as NO_x were detected in the environment of the fire.
4. The disparate results between various fire episodes can be attributed, for the most part, to the different temperatures reached, the specific type of polymer that was burned, and whether or not the polymers contained fire-retarding agents.
5. The heat generated during the present episode, beyond 400 °C, and the substantial depletion of oxygen in the cell block are important factors to be considered when interpreting the laboratory data, as they affect the capacity or incapacity to escape the fire.

6. The spot where the victims were found – most of them on the side opposite the windows – could have affected the levels of MetHb, CO, and HCN found in the blood.
7. The LI proposed can provide insight into the type of atmosphere generated and the incidence of HCN in the different published episodes, providing diagnostic data, which should be included among all the other factors mentioned.
8. Heat denaturation is considered to be the main cause of MetHb production in fire episodes and inhalation of nitrogen oxides in cases of carbon monoxide poisoning.

References

[1] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, 9th ed., Biomedical Publications, Foster City, CA, 2011, pp. 411–414.
[2] P. Lundquist, L. Rammer, B. Sorbo, The role of hydrogen cyanide and carbon monoxide in fire causalities: a prospective study, Forensic Sci. Int. 43 (1989) 9–14.
[3] D.A. Purser, P. Grimshaw, K.R. Berril, Intoxication by cyanide in fires: a study in monkey using polyacrylonitrile, Arch. Environ. Health 39 (1984) 394–400.
[4] I.S. Symington, R.A. Anderson, I. Thomson, J.S. Oliver, W.A. Harland, J.W. Kerr, Cyanide exposure in fires, Lancet 2 (1978) 91–92.
[5] B.J. Terril, R.R. Montgomery, C.F. Reinhardt, Toxic gases from fires, Science 200 (1978) 1343–1347.
[6] M.J. Yeoh, G. Braitberg, Carbon monoxide and cyanide poisoning in fire related death in Victoria, Australia, J. Toxicol. Clin. Toxicol. 42 (6) (2004) 855–863.
[7] Y. Alarie, Toxicity of fire smoke, Crit. Rev. Toxicol. 32 (4) (2002) 259–289.
[8] Y. Alarie, R.C. Anderson, Toxicologic classification of thermal decomposition products of synthetics and natural polymers, Toxicol. Appl. Pharmacol. 57 (1981) 181–188.
[9] T. Grabowska, R. Skowronek, J. Nowicka, H. Sybirska, Prevalence of hydrogen cyanide and carboxyhaemoglobin in victims of smoke inhalation during enclosed-space fires: a combined toxicological risk, Clin. Toxicol. 50 (8) (2012) 759–763.
[10] M. Repetto, M. Martínez, Presencia de cianuro en sangre de las víctimas de fuego de materiales plásticos, Medicamenta 63 (1974) 193–195.
[11] A.K. Charturvedi, D.R. Smith, D.V. Canfield, Blood carbon monoxide and hydrogen cyanide concentrations in the fatalities of fire and non-fire associated civil aviation accidents, 1991–1998, Forensic Sci. Int. 121 (2001) 183–188.
[12] D.W. Walsh, D.J. O'Brien, J.L. Arnold, Cyanide poisoning: not just a HAZMAT issue, Fire EMS J. (2004), www.Jems.com/free-resource.htlm/.
[13] J. Zamecnik, J. Tam, Cyanide in blood by gas chromatography with NP detector and acetonitrile as internal standard: application on air accident fire victims, J. Anal. Toxicol. 11 (1987) 47–48.
[14] A. Ishii, H. Seno, K. Watanabe-Suzuki, O. Suzuki, T. Kumazawa, Determination of cyanide in whole blood by capillary gas chromatography with cryogenic oven trapping, Anal. Chem. 70 (1998) 4873–4876.
[15] M.M. Birky, F.B. Clarke, Inhalation of toxic products from fires, Bull. N. Y. Acad. Med. 57 (1981) 97–1013.
[16] L.A. Ferrari, M.G. Arado, L. Giannuzzi, G. Mastrantonio, M.A. Guatelli, Hydrogen cyanide and carbon monoxide in blood of convicted dead in polyurethane combustion: a proposition for the data analysis, Forensic Sci. Int. 121 (2001) 140–143.
[17] Y. Katsumata, M. Aoky, M. Oya, O. Suzuki, S. Yada, Simultaneous determination of carboxyhemoglobin and methemoglobin in victims of carbon monoxide poisoning, J. Forensic Sci. 25 (1980) 546–549.
[18] W. Schwerd, E. Schulz, Carboxyhemoglobin and methemoglobin findings in burnt bodies, Forensic Sci. Int. 12 (1978) 233–235.
[19] F. Moriya, Y. Hashimoto, Potential for error when assessing blood cyanide concentration in fire victims, J. Forensic Sci. 46 (2001) 1421–1425.
[20] K. Sato, A method for determination of methemoglobin in blood containing carboxyhemoglobin, Jpn. J. Legal Med. 37 (1983) 133–144.
[21] D.T. Reay, J.S. Insalaco, J.W. Eisele, Postmortem methemoglobin concentration and their significance, J. Forensic Sci. 29 (1984) 1160–1163.
[22] D.J. Blackmore, Interpretation of carbon monoxide levels found at postmortem, in: B. Ballantyne (Ed.), Forensic Toxicology, John Wright and Sons, Bristol, 1974, pp. 114–120.
[23] C.W. Lee, J.C.N. Tam, L.K. Kung, L.K. Yim, Validity of CO-oximetric determination of carboxyhaemoglobin in putrefying blood and body cavity fluid, Forensic Sci. Int. 132 (2003) 153–156.
[24] C. Brehmer, P.X. Iten, Rapid determination of carbon monoxide in blood by oximeter, Forensic Sci. Int. 133 (2003) 179–181.
[25] N. Yukawa, T. Suzuoka, T. Saito, A.R.W. Forrest, M. Osawa, S. Takeichi, Current CO-oximeter, Forensic Sci. Int. 94 (1998) 211–215.
[26] M. Vevelstad, I. Morlid, Lethal methemoglobin and automobile exhaust inhalation, Forensic Sci. Int. 187 (2009) e1–e5.
[27] J. Van Dam, P. Daenens, Microanalysis of carbon monoxide in blood by head space capillary gas chromatography using thermal conductivity detector, J. Forensic Sci. 39 (1994) 473–478.
[28] B.C. Levine, P. Rechani, J.L. Gurman, F. Landron, H. Clark, M.F. Yoklavich, J.R. Rodriguez, F. Mattos de Cabrera, S. Kaye, Analysis of carboxyhemoglobin and cyanide in blood from victims of the DuPont Plaza Hotel fire in Puerto Rico, J. Forensic Sci. 35 (1990) 151–168.
[29] N.C. Klendshoj, M. Feldstein, A.L. Sprague, Spectrophotometric determination of carbon monoxide, J. Biol. Chem. 183 (1950) 297.
[30] M.A. Guatelli, in: S.A. Curry (Ed.), Toxicity of Cyanides in Methods of Forensic Sciences, vol. III, Academic Press, New York, 1964, pp. 233–265.
[31] B. Ballantyne, The forensic diagnosis of acute cyanide poisoning, in: Proceedings of a Symposium Held at the Chemical Defense Establishment, Wright, Bristol, (1974), pp. 99–113.
[32] J.D. Ramsey, R.J. Flanagan, Detection and identification of volatile organic compounds in blood headspace gas chromatography as an aid to the diagnosis of solvent abuse, J. Chromatogr. 50 (1982) 423–442.
[33] H.H. Maurer, Systematic toxicological analysis procedures for acidic drugs and/or metabolites relevant to clinical and forensic toxicology and/or doping control, J. Chromatogr. B 733 (1999) 3–25.
[34] O.H. Drummer, Chromatographic screening techniques in systematic toxicological analysis, J. Chromatogr. B: Biomed. Sci. Appl. 733 (1999) 27–45.
[35] Requirements, Test Procedure and Apparatus for Testing the Flame Retardance of Resilient Filling Materials Used in Upholstered Furniture. Technical Bulletin 117, 2000 www.bhfti.ca.gov/industry/117.pdf.
[36] R.A. Anderson, I. Thomson, W.A. Harland, The importance of cyanide and organic nitriles in fire fatalities, Fire Mater. 3 (1979) 91–99.
[37] E.H. Coleman, Gaseous combustion products from plastics, Plastics 24 (1959) 416.
[38] R.R. Montgomery, in: G.D Clayton, F.E. Clayton (Eds.), Patty's Industrial Hygiene and Toxicology, 3rd revised, John Wiley and Sons, New York, 1982, pp. 4209–4526.
[39] W.T. Lowry, L. Juarez, C.S. Petty, B. Roberts, Studies of toxics gas production during actual structural fires in the Dallas area, J. Forensic Sci. 30 (1985) 59–72.
[40] A.H. Hall, B.H. Rumack, Clinical toxicology of cyanide, Ann. Emerg. Med. 15 (1986) 1067–1074.
[41] Y. Seto, M. Kataoka, K. Tsuge, Stability of blood carbon monoxide and hemoglobins during heating, Forensic Sci. Int. 121 (1–2) (2001) 144–150.
[42] Y. Katsumata, M. Aoki, K. Sato, M. Oya, S. Yada, A simple spectrophotometric method for the determination of carboxyhemoglobin in blood, Forensic Sci. Int. 18 (1981) 175–179.
[43] S. Oritani, K. Nagai, B. Zhu, H. Maeda, Estimation of carboxyhemoglobin concentrations in thermo-coagulated blood on a CO-oximeter system: an experimental study, Forensic Sci. Int. 83 (1996) 211–218.
[44] D. Gerostamoulos, J. Beyer, K. Wong, C. Wort, O.H. Drummer, Carbon monoxide concentrations in the 2009 Victorian Bushfire disaster victims, Forensic Sci. Int. 205 (2011) 69–72.