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

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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

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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