Chapter 9

Simplified Analysis of Toxic Gaseous Substance in Forensic Practice: Experiences from Japan

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Abstract

Toxicological examination in forensic practice is important for the proper diagnosis of acute poisoning. We have discussed the properties and features of poisoning incidents due to gaseous substances and elaborated on the simplified analytical techniques and apparatus used for their identification and quantitation for forensic purposes. Briefly, we have explained the simplified analysis of toxic gaseous substances such as carbon monoxide, hydrogen cyanide, hydrogen sulfide, and helium in blood. The techniques used include color testing, gas chromatography, detector tube, oximeter, and spectrophotometric method. In doing so, we have shared our experiences and highlighted the fact that the analysis of gaseous substances can be performed using readily available laboratory tools and equipment. We have emphasized the need and usefulness of the reference data tables for guiding forensic diagnosis. We hope that the above overview will assist other colleagues to implement such simplified techniques for the advancement of forensic medicine practice.

Keywords: toxicological examination, toxic gaseous substances, simplified analysis

1. Introduction

Toxicological examination in forensic practice is important for the proper diagnosis of acute poisoning [1]. The forensic pathologist requests toxicological analysis to forensic toxicologist

in case of poisoning or poisoning suspected cases. It will usually consist of two-stage testing at autopsy [2]. The first step is usually performed as a screening test. The second step is required for the identification and quantification of its causative agent following the confirmatory test. Toxic gaseous substances are one of the targets for toxicological examination in a daily forensic practice. Simplified analysis of gaseous substances involves both first and second step of toxicological examination in forensic practice.

In the present chapter, we share our experiences about the analysis of gaseous substances such as carbon monoxide, hydrogen sulfide, cyanide, and helium.

2. Autopsy and subsequent toxicological examination in gas-related poisoning incidents

Gaseous substances can cause acute poisoning. They get absorbed into the body by inhalation. Most of them do not produce specific symptoms but they can induce dizziness, lethargy, headaches, and suffocation. There is no specific finding at autopsy in most poisoning cases [1, 2]. Most of gaseous substances cause little or no tissue damages. However, there will be observed unique findings in some poisoning cases, such as cherry red appearance of postmortem lividity in carbon monoxide poisoning, bright pink lividity with bitter almond odor in cyanide poisoning, and dark green coloration and rotten egg smell in hydrogen sulfide poisoning [2]. Nonspecific findings such as generalized organ congestion and pulmonary edema may be observed in most cases [1]. The presence of gaseous poisons is usually indicated by the circumstances of the incident, and involvement of gaseous substances is sometimes indicated by circumstantial evidence [3].

Although detailed management is out of scope of this chapter, it is sufficient to mention that the management of poisoning by gaseous substances involves the use of antidotes when available; decontamination; artificial respiration with demand-valve resuscitators, bag-valve-mask devices; administration of hyperbaric oxygen; performance of cardiopulmonary resuscitation (CPR); and close observation and monitoring of vital signs.

In our laboratories, toxicological examination is routinely performed on a daily basis—from the screening test using immunoassay kits to subsequent identification and quantification using gas chromatography mass spectrometry or liquid chromatography mass spectrometry techniques. Since the physiological effects of most gaseous substances correlate with the concentration in blood, it becomes the best indicator of toxicity [4]. As numerous reference tables for fatal levels of chemicals have been reported [5–9], forensic diagnosis is made in reference to the values reported in the data tables. In addition, several other factors have to be considered for toxicological evaluation; these include the properties of the sample, diffusion and redistribution, degradation, and metabolism [1, 2, 10, 11]. In the light of the above, we have instituted simplified analysis techniques for gaseous substances; these techniques provide a lot of information promptly that aid forensic diagnosis. In the following paragraphs, we describe these techniques for each of the main gaseous substances.

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3. Specific substances and its simplified analysis methods

3.1. Carbon monoxide (CO)

CO is an odorless, nonirritable, and colorless gas and is slightly lighter than air (specific gravity for the air; 0.97). It is mainly produced by incomplete combustion of fuels or carbon compounds [8, 12]. Its common sources are vehicle exhaust, smoke from fire, and improperly maintained heating systems. CO is the leading cause of poisoning death in Japan [13–15], and also a common cause of poisoning in the United States [16, 17]. The annual number of victims by CO poisoning is about 2000–4000 in Japan, including accidental or suicidal cases [13–15]. CO is absorbed from the lung into the bloodstream. As the affinity of CO for hemoglobin is 230–270 times greater than that of oxygen, it binds to hemoglobin in erythrocyte, and forms carboxyhemoglobin (CO-Hb) [12, 18, 19]. The formation of CO-Hb (represented as a percentage of the total hemoglobin) in blood depends on various factors such as the concentration of inspired CO, duration of CO exposure, pulmonary ventilation, exercise, and health status [12, 18]. The toxicity of CO is thought to be tissue hypoxia due to the formation of CO-Hb. Its binding is a reversible process; however, as the binding between CO and hemoglobin is strong, the CO elimination half-life is long, about 4–5 hours under room air ventilation for a resting adult at sea level. The formation of CO-Hb decreases the capacity of oxygen transport, and it causes insufficient oxygen supply in tissues [12, 18, 19].

The hypoxia due to CO-Hb formation causes signs and symptoms. Clinical symptoms roughly correlate with CO-Hb levels (**Table 1**). The CO-Hb concentration of nonsmoking healthy subjects is 1–3%, and around 5–8% in smokers. No symptom is observed below 10% of CO-Hb levels. Neurological symptoms such as headache, dizziness, nausea, and weakness are observed in CO-Hb level from 10 to 30%. Increase of respiration and heart rate, syncope, and confusion are observed in 30–50% of CO-Hb level. When the level of CO-Hb exceeds 50%, it becomes life-threatening. It is noteworthy that the value of CO-Hb is important for the diagnosis of CO-poisoning or fire-related death [12, 18, 19]. In addition to hemoglobin, CO

CO-Hb (%)	Clinical symptom
0–10	No symptom
10–20	Headache, ear ringing, fatigue
20–30	Headache, weakness, nausea, vomiting
30–40	Severe headache, dizziness, nausea, vomiting
40–50	Syncope, confusion, increased respiration and heart rate, muscle weakness
50–60	Coma, convulsions, depressed respiration
60–70	Coma, convulsions, cardiorespiratory depression, often fatal
70+	Respiratory failure, death

Table 1. Correlation of carboxyhemoglobin (CO-Hb) levels to clinical symptoms.

combines with heme-proteins such as myoglobin and cytochrome oxidase, and it may cause the impairment of cardiac and neurological functions [12, 18].

The most characteristic appearance of the body in poisoning case is a cherry red color of the skin. It is usually observed in cases where CO-Hb exceeds 30% [1]. At autopsy, the common findings include discoloration of blood, organs, and muscle that become cherry red color, as a result of CO-Hb and carboxymyoglobin. Other autopsy findings such as pulmonary edema and generalized organ congestion are also observed [1].

With regard to the identification and quantification of CO, several methods and techniques have been reported [20]. Spectrophotometric methods and gas chromatography techniques are widely used. The CO-Hb is relatively stable under storage in cool and dark conditions [18, 21–23]. It is important to note that postmortem production of CO has been reported in some conditions, and therefore, it is recommended not to use body cavity fluids such as pleural effusion for the measurement of CO in severe putrefied case [24–28].

For identification purposes, the qualitative test for CO includes color test and microdiffusion tests. Color test is a simple procedure where a blood sample mixed with 0.01 M ammonia solution (1:20) [29] or a few drops of blood are added to some 10% sodium hydroxide solution [1]. This test is based on the fact that CO-Hb is relatively tolerant to alkaline condition. However, as other simple methods have been established, color testing for CO poisoning is now rarely required and not recommended [1, 29]. The microdiffusion test using Conway cell [30] or on the filter paper [31] have been reported. It is based on reaction with palladium chloride. This is still the most widely used method since it was invented by Conway in 1944.

With regard to quantification, spectrophotometric method, gas chromatography, detection tube, and oximeter are used. The spectrophotometric method is the most popular, and various assay procedures have been reported. CO-Hb could be determined by the changes of absorption spectrum in either Soret (410–425 nm) [32–34] or visible region (500–600 nm) [30, 35–38]. In our laboratories, we perform the measurement of the spectrum of blood sample by adding sodium hydrosulfite. The addition of sodium dithionite reduces oxyhemoglobin without affecting the CO-Hb. This procedure is simple, and it does not need an extraction from the sample. **Figure 1** shows the spectra of blood samples from a normal nonsmoker (CO-Hb: 0%) and CO poisoning victim (CO-Hb: 68 and 95%, respectively). Twin-peaked spectrum was observed in CO poisoning sample.

The CO is extracted and introduced in gas chromatography. Various methods and apparatus have been reported for its extraction [39–44]. And the released CO is detected by the thermal conductivity detector (TCD) [39–42, 44], or the flame ionization detector with the catalytic reduction of the CO to methane [43]. As this method is a direct measurement of CO contents in the sample as well as a measurement of the hemoglobin, two measures represent the percentage of CO-Hb. Application of gas chromatography equipped with semiconductor detector has been reported for forensic practice [45]. This gas chromatography system (sensor gas chromatography, sGC) is highly sensitive for CO and has some advantages such as portability and easy handling. This apparatus does not need a gas cylinder as it uses the room air as the career gas. Although it is not commonly in use, further application in the field of forensic medicine would be expected.

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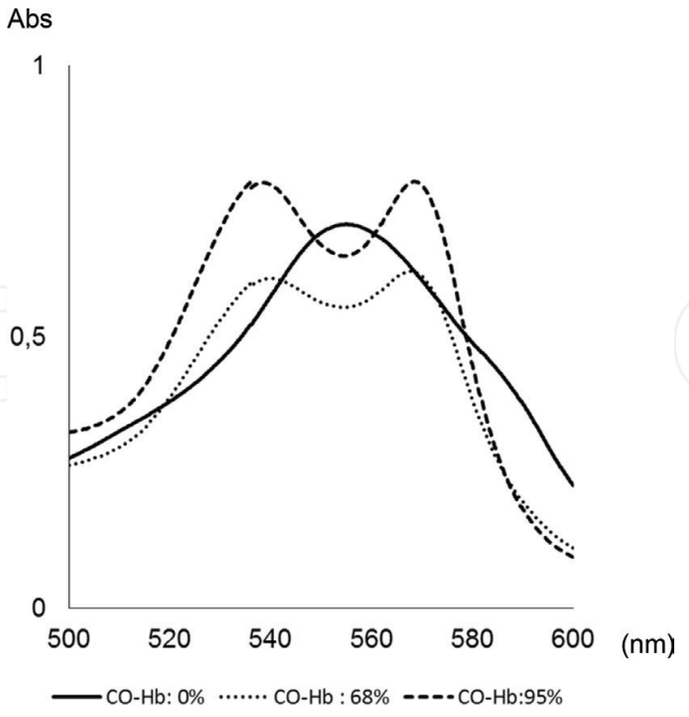

Figure 1. Spectra of blood sample from CO-Hb: 0, 68, and 95%, respectively.

The detector tube method is widely used for the determination of various gaseous substances [46, 47]. It is also applied for the quantitation of CO in blood [48]. This apparatus consists of a CO-separator tube, CO-detector tube, and aspirating pump. The CO-separator tube is packed with silica gel particles coated with ferricyanide [48]. The CO-detector tube is packed with silica gel particles coated with sulfite palladium potassium [46, 49]. These tubes and pump are connected in series. The CO in blood is released following the injection of blood sample (200 μL) in CO-separator tube, and the released CO gas is detected by the CO-detector tube, followed by the aspirating of the pump. As the detector tube is easy to carry at the scene where an incident has taken place or to a point-of-care testing, it is applied to not only screening test, but also for quantitation.

Oximeter is routinely used for laboratory test [50, 51], and it is also applied in forensic medicines [52–64]. This instrument uses seven wavelengths in the visible region for the determination of various hemoglobin species, such as oxyhemoglobin, CO-Hb, reduced hemoglobin, and methemoglobin. It automatically analyzes the proportion of each species of hemoglobin and oxygen contents. This oximeter system (**Figure 2**) requires 50 μL of blood for a single measurement, and it may be a valid option in case of difficult blood sampling due to severe blood loss. As there are many advantages such as no necessity of sample preparation, easy handling, and portability, it is suitable for forensic practice. In a recent study, it has been reported that squeezed splenic blood can be used as an alternative specimen for CO-Hb measurement using oximeter [65].

3.2. Cyanide

Hydrogen cyanide (molecular weight, 27; boiling point, 25.7°C) is a colorless gas or liquid, with a bitter almond like odor [8, 12]. Cyanide is used for various purposes, such as fumigate,

Figure 2. Portable oximeter (AVOX 4000) and its operation. Sample cartridge is shown in lower left-hand corner.

fungicide, insecticide, metal polishes, and electroplating. Hydrogen cyanide is present in fire smoke from burning nitrogen-containing plastics. Poisoning occurs by hydrogen cyanide gas inhalation or ingestion of cyanide salts. It is a highly toxic substance with a rapid onset of toxic effects as it is absorbed quickly from lung or the stomach [12]. Cyanide binds to heme iron in cytochrome complex, and it inhibits cellular respiration. The symptoms of acute poisoning include headaches, tachypnea, dizziness, coma, seizure, and death within 10–20 minutes in severe cases [12].

At autopsy, the appearance of the body is slightly bright pink. This is thought to be due to the presence of excess oxyhemoglobin [1]. Moreover, because cyanide inhibits cellular respiration, tissue oxygen consumption would be decreased. The stomach wall is damaged by the alkaline nature of stomach contents in case of cyanide salt ingestion [1]. It is well known that almond-like odor is one of the characteristics of cyanide poisoning [1, 8, 12, 19]. However, it can be detectable only approximately in one-third to half of the victims [19, 66], as this characteristic depends on the genetic trait [1]. Other autopsy findings are nonspecific in cyanide poisoning [1].

Cyanide level in blood is useful to confirm its toxicity [12]. The blood sample should be taken from the peripheral sites in case of cyanide salt ingestion, to exclude the effect of postmortem diffusion [66]. The normal blood cyanide level is 0.016 μg/mL for nonsmoker and 0.041 μg/mL for smoker. Cyanide concentration of less than 0.2 μg/mL in blood does not usually elicit any symptom [12]. Fatal concentration of cyanide in blood has been reported to be not lower than 3–5 μg/mL [67]. Lethal dose of potassium cyanide ingestion for adults is 200–300 mg.

For identification purposes, various methods have been devised. Color and microdiffusion tests are the most common [67, 68]. The Schöenbein-Pagenstecher method, using guaiac-cupper paper, is employed as a preliminary test [68]. This coated paper turns blue in the presence of

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cyanide. This method is user-friendly and highly sensitive for cyanide. However, since it is difficult to store the guaiac-cupper paper for a long period, we prepare it immediately before examination. Other color test or commercially available test tube methods and test papers have also been used to test cyanide in blood samples [29, 66]. The microdiffusion test using Conway cell (pyridine-pyrazolone method) have been reported [67]. Although this method is also highly sensitive for cyanide, and also used as a quantitative examination, its main drawback is that it is relatively time-consuming.

For quantitative testing, gas chromatography and detection tube are employed in our laboratories. To do so, cyanide is extracted from the sample by adding the concentrated phosphoric acid or sulfuric acid, and detected by nitrogen phosphorus detector (NPD) or flame thermoionic detector (FTD), equipped with a gas chromatography device [69, 70]. Application of sGC has also been reported for cyanide measurement [71]. The sGC system is also highly sensitive for cyanide and has some advantages such as it is easy to operate and portable. Further application would be expected in the field of forensic medicine (**Figure 3**).

The detector tube is also used for the quantitation of cyanide in blood [46, 47, 72]. This apparatus consists of a cyanide-separator tube, cyanide-detector tube, and aspirating pump. These tubes and the pump are connected in series. The cyanide-separator tube is packed with silica gel particles coated with sulfuric acid, and the released cyanide gas is detected by the cyanide-detector tube, followed by the aspirating of the pump (**Figure 4**). The cyanide-detector tube is packed with silica gel particles coated with mercuric chloride and pH indicator [46], and the hydrochloric acid formation by the reaction between cyanide and mercuric chloride was observed. Because the detector tube is easy to handle and portable, it can be carried to the scene of accident or poisoning and at the point of care, and it is well applied in forensic medicine practice.

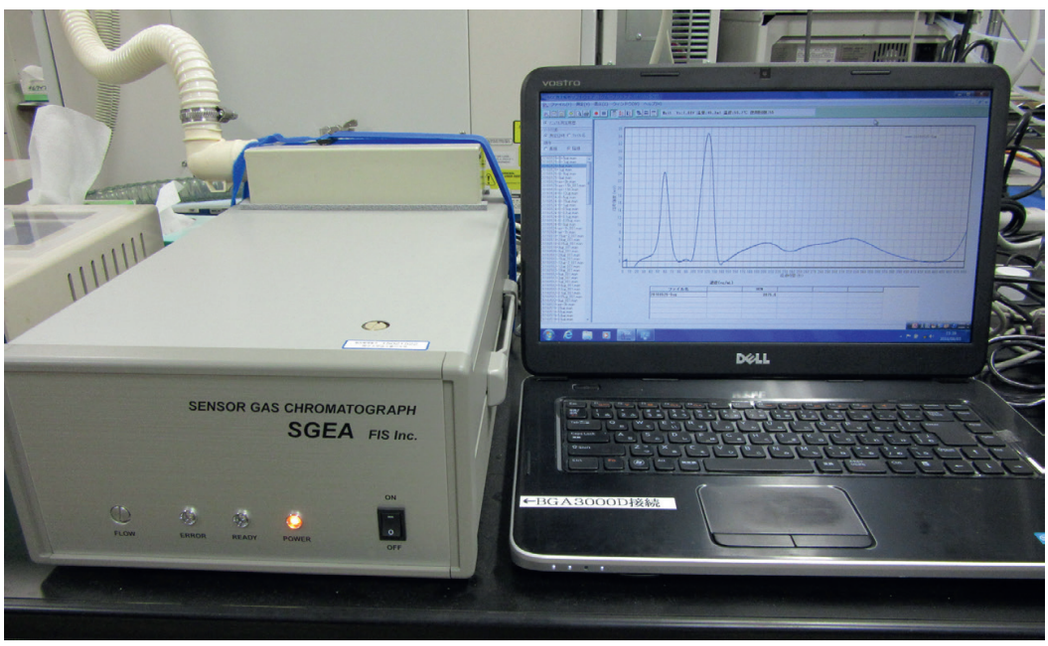

Figure 3. Equipment of the sensor gas chromatography for hydrogen cyanide quantification.

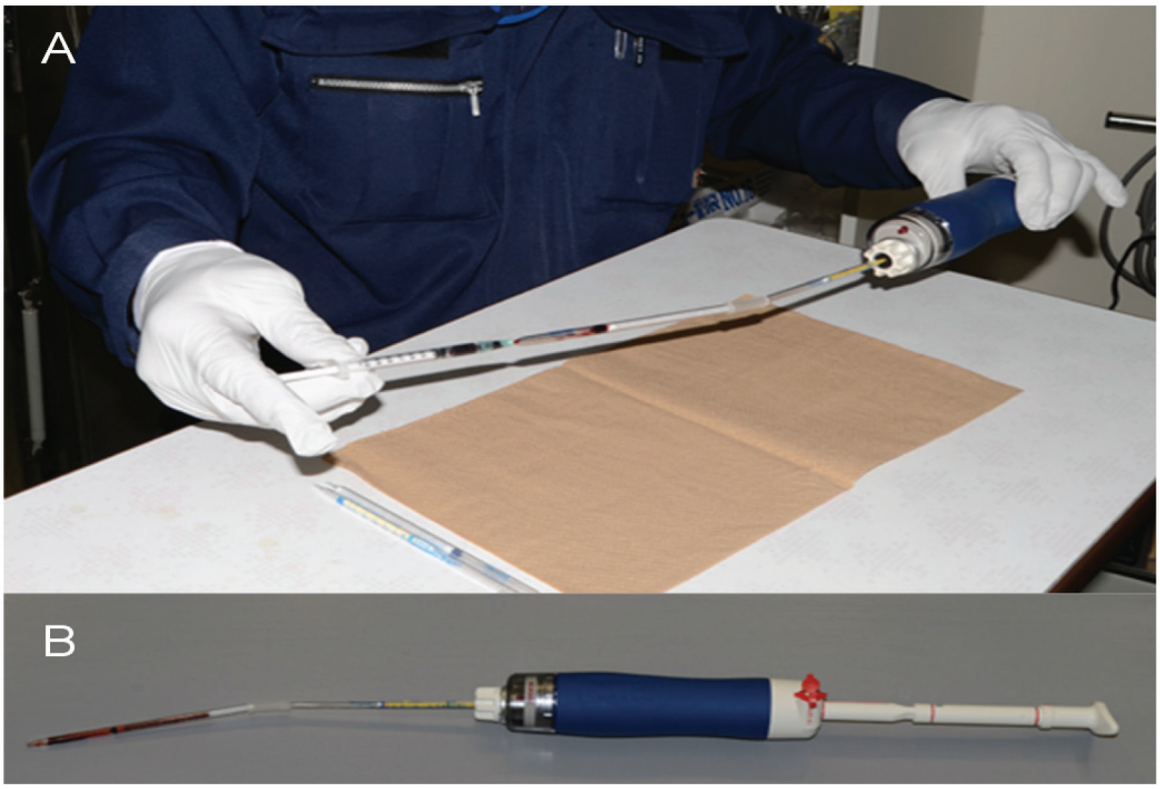

Figure 4. Procedure of the measurement of hydrogen cyanide in blood using detector tube (A). The detector tube is connected to the aspirating pump and separator tube (B).

3.3. Hydrogen sulfide (H_2S)

The H_2S is a gas with a rotten egg smell. It is colorless, flammable, and heavier than air (specific gravity for the air, 1.19) [8, 12]. It is formed as a by-product of the chemical industry. It occurs in volcanic gases and hot springs, and it is also formed at the process during putrefaction of organic substances. It is highly toxic and causes cellular asphyxia by the inhibition of cytochrome oxidase, like cyanide. Its toxicity depends on various factors such as its concentration in air and duration of exposure [12]. An odor is detectable at a concentration of 0.2 ppm in air, but olfactory paralysis is observed at 100–150 ppm. Inflammatory conditions such as rhinitis, pharyngitis, bronchitis, and pulmonary edema are also observed as a result of its irritant properties [12]. The systemic toxicity is shown by headaches, nausea, vomiting, dizziness, loss of consciousness, and respiratory failure that are observed following high levels of H_2S exposure (above 500 ppm); unfortunately, most fatalities occur at the scene [12].

Nonspecific findings such as generalized organ congestion and pulmonary edema can be observed. The dark-greenish discoloration of cerebral gray matter, organ, and skin with rotten egg smell has been reported [1, 12].

The blood sulfide concentration is less than 0.05 μg/mL in normal healthy subjects [8]. Fatal concentration of sulfide in blood has been reported to be not lower than 0.13–0.45 μg/mL [73]. Since the formation of sulfide by postmortem degradation of protein has been reported, the interpretation of the results requires caution and expertise [12].

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As a qualitative test for H_2S, color testing is commonly used. The lead acetate paper is used as a preliminary test [66, 68]. The sample is mixed with sulfuric acid and heated, then the lead acetate paper is suspended; if the paper turns black, it indicates the presence of H_2S. This procedure is easy to perform [66].

As a quantitative test for H_2S, gas chromatography and detection tube are used. The gas chromatography method measures extracted H_2S using flame photometric detector (FPD) [74, 75]. The detector tube is also used for the quantitation of H_2S in blood [46, 47, 76]. This apparatus consist of a H_2S-separator tube, H_2S-detector tube, and aspirating pump. As explained previously, these tubes and pump are connected in series. The H_2S-separator tube is packed with silica gel particles coated with phosphoric acid, and the released H_2S gas is detected by the H_2S-detector tube, followed by the aspirating of the pump. The H_2S-detector tube is packed with silica gel particles coated with lead acetate [46, 49], and the indicator range is then observed. As this method is easy to operate and portable, it can be carried to the scene or point of care, and it is well suited for forensic practice.

3.4. Helium

Helium (He), a colorless and odorless inert gas, acts as a simple asphyxiant agent. It causes oxygen depletion by the replacement of the inspired air [77]. It has highly diffusive properties and low solubility in water. It is used as a career gas for party balloons or cryogenic liquids. Medically, mixture gas of He and oxygen improve the oxygen flow in patient with upper airway obstruction [78].

The identification and quantification of helium in forensic samples is usually performed using a headspace gas chromatography with TCD detector or gas chromatography mass spectrometry [79–84]. It has been reported that lung tissue, intratracheal, and stomach gas are suitable matrices for the analysis of the inert gases [79–84]. The gas sampling at the time of autopsy is relatively easy, it is a good practice to consider gas sampling in case helium exposure is suspected.

3.5. Other toxic gases

There are a lot of toxic gases that cause tissue damages, such as ammonia gas or chlorine gas [19]. These gases are widely used as industrial chemicals and cause irritation and inflammation at the points of contact. They may cause tissue necrosis in severe cases. Although the incidence of poisoning cases by these gases is relatively low, the detector tube method is often used as one of the simplified analytical methods.

4. Conclusion

We have discussed the properties and features of poisoning incidents due to gaseous substances and elaborated on the simplified analytical techniques and apparatus used for their identification and quantitation for forensic purposes.

In doing so, we have shared our experiences and highlighted the fact that the analysis of gaseous substances can be performed using readily available laboratory tools and equipment. We have emphasized the need and usefulness of the reference data tables for guiding forensic diagnosis.

We hope that the above overview will assist other colleagues to implement such simplified techniques for the advancement of forensic practice.

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