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Perspective Article

The Place of Gas Analysis in Forensic Toxicology

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Introduction

Because we need Oxygen (O_2) to live, our organism is in permanent contact with gases explaining why gaseous intoxication is one of the easiest human intoxication. The physical gaseous state makes gaseous substances readily available for a lung administration which facilitates the entrance in the blood circulation. If the deleterious effects of gas intoxication are easily noticeable on the metabolism, gas analysis in biological samples remains a tremendous challenge.

Are Gases Really Toxic?

The discovery of the gaseous state is recent compared to those of liquid and solid states. The word gas is a neologism created by the early XVIIth - century chemist JB van Helmont from the Greek word "Chaos", where the sound "Ch" is pronounced "g" in Dutch. Observations of flammable atmospheres or airs were also reported by the IVth - century BC [1]. The first intoxications diagnosed as gas intoxication were described in the 19th century in studies led on carbon monoxide [2] simultaneously with the development of manufactured gases.

The toxicity of the gases is very variable. The pathways of intoxication can be totally different for a similar molecular structure. Most of gases act as direct or indirect oxygen depletants. However, if most of the gases are oxygen depletants, high amount of oxygen can generate a hyperoxia and producing oxygen toxicity [3]. Therefore, if another gas is present in important quantity in the inhaled air, oxygen is proportionally less present and therefore less available in the organism, leading to anoxia whose magnitude is dependent on the quantity of adulterating gas.

The elemental gases are constituted of two groups: diatomic homonuclear gases and monoatomic noble gases. Diatomic homo nuclear gases correspond to Hydrogen (H_2), Nitrogen (N_2), Oxygen and two halogens, Fluorine (F_2) and Chlorine (Cl_2). Additionally to their oxygen depletion property (except oxygen which toxicity is induced by hyperoxia), these gases have individual dangerous properties. Hydrogen is highly explosive, chlorine and fluorine are strong pulmonary irritant causing internal damages (airways, lungs, liver and kidneys) [4].

Monoatomic noble gases are constituted by Helium (He), Neon (Ne), Argon (Ar), Krypton (Kr), Xenon (Xe) and Radon (Rd) and act as oxygen depletants. Xenon also shows aesthetical properties through an antagonism of glycine-site N-methyl-D-aspartate (NMDA) receptor [5] and was recently added on the list of substances banned by the World Anti-doping Agency (WADA) because of its performance-enhancing properties [6]. Radon is also classified as noble gas but shows radioactivity and carcinogenicity properties [7].

Another category of gases is diatomic heteronuclear gases which are not many. Nitrogen monoxide (NO) and Carbon monoxide (CO) are the most known. Nitrogen oxide is an important cellular signalling molecule involved in many physiological and pathological processes in mammals and a potent vasodilator acting also as neurotransmitter. However, it is rapidly oxidised in nitrogen dioxide which is a strong oxidising agent. Low nitrogen monoxide can be used as therapeutic agent but high levels lead to nitrogen dioxide formation, causing methemoglobinemia. The carbon monoxide toxicity is caused by indirect oxygen depletion in organism. Carbon monoxide affinity for haemoglobin is 250 times higher than this of oxygen. Therefore, carbon monoxide binds the proteins carrying oxygen in the body such as haemoglobin in blood, resulting in formation of carboxyhemoglobin, and myoglobin in muscles causing carboxymyoglobin, leading to an anoxia [8].

Others gases are classified as polyatomic heteronuclear gases. They all act as oxygen depletant such as dioxides (Carbon dioxide (CO_2), Sulphur dioxide (SO_2), Nitrogen dioxide (NO_2), Chlorine dioxide (ClO_2)), Ammonia (NH_3), Hydrogen Sulphide (H_2S), Nitrous Oxide (N_2O), Hydrogen Cyanide (HCN), Phosgene (CCl_2O_2), Arsine (AsH_3), Stibine (SbH_3) and all the polyatomic structures at gaseous state at normal conditions such as gaseous alkanes, gaseous alkenes (e.g., ethylene oxide (C_2H_4O), acetylene (C_2H_2), dimethylether (C_2H_6O) and gaseous halogenated hydrocarbon derivatives such as Chlorofluorocarbons (CFC), Hydrofluorocarbons (HFC) and Hydrochlorofluorocarbons (HCFC).

If carbon dioxide shows only an oxygen depletant property, sulphur dioxide is a potent irritating compound whereas nitrogen dioxide is a potent oxidising gas. Methemoglobinemia induced by nitrogen dioxide reduces the oxygenation of the organism and long exposure can lead to a fatal anoxia [9]. Sulphur dioxide is also noxious through its metabolites, mainly sulphites, which are potent nucleophilic molecules without acute toxicity but with a long term exposure causing allergy and thiamine (vitamin B1) destruction.

Regularly referred to as "laughing gas", the toxic effects associated with nitrous oxide are caused by the interaction with vitamin B_{12} or the B_{12} coenzyme [10]. Irreversible oxidation of the central cobalt ion inactivates the vitamin and leads to inhibition of the methionine synthase and the N-methyl-D-aspartate (NMDA) glutamate receptor. Methionine synthase is highly important for the

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formation of methyl groups crucial to the synthesis of DNA, RNA, amines and proteins. Furthermore, nitrous oxide effects the production of S-adenosylmethionine leading to a demyelination of nerve tissue. However, lower levels of nitrous oxide are widely used as aesthetic agent for surgical operations.

Ammonia, hydrogen sulphide and hydrogen cyanide are easily noticed because of their odorant properties: ammonia exhibits a characteristic pungent odour, hydrogen sulphide a strong "rotten eggs" odour and hydrogen cyanide a faint "bitter almond-like" odour. Acute inhalation of ammonia may initially cause upper respiratory tract irritation. Substantial exposures can cause burns in the oral cavity, nasopharynx, larynx and trachea, together with airway obstruction, respiratory distress and bronchiolar and alveolar oedema. Ammonia is converted to carbamoyl phosphate by the enzyme carbamoyl phosphate synthase, and then enters the urea cycle to be either incorporated into amino acids or excreted in the urine. Hydrogen sulphide and hydrogen cyanide show a comparable toxicity. They are considered as broad-spectrum poisons, forming a complex bond with the iron atom in the mitochondrial cytochrome c oxidases, thus inhibiting cellular respiration. They act very rapidly that is the reason why they have been used as chemical warfare agents during the World War conflicts. A hydrogen cyanide (Zyklon B) concentration of 300 mg/m^3 in air can kill a human in less than hour. This rapidity of action was used in the German concentration camp mass killing.

Other gases were also employed in war contexts. Phosgene was used during the World War I, the Second Sino-Japanese War and the Second Italian-Ethiopian war. Although phosgene does not contain phosphorus atom, its toxicity is as close to this of phosphane (or phosphine), a phosphorated gas. Phosgene reacts with amines of the proteins in the pulmonary alveoli, disrupting the blood-air barrier and causing suffocation [11] and phosphine denaturates oxyhemoglobin interfering with the synthesis of proteins and enzymes of respiratory tract [12]. Arsine was also proposed as chemical warfare agent but phosgene was preferred because of its non-flammable property. Arsine directly attacks haemoglobin in the erythrocytes, causing them to be destroyed by the organism [13].

Hydrocarbon gaseous derivatives such methane (CH_4), propane (C_2H_8) and butane (C_4H_{10}) constitute the most used gases in the world. Methane is the main component of natural gas used in many heating domestic and industrial applications. Propane and butane are also very frequently employed in cookers and heating devices. These gases also act as oxygen depletants but could also display another toxicity or potential hazard such as gas outbursts [14]. Methane, propane and butane induce a reduction of the available oxygen but are explosive even at low concentrations in ambient air (5% for methane). Moreover, propane but especially butane can induce cardiac arythmia. Butane hypersensitizes cardiac cells to norepinephrine and interfers with the binding or release of oxygen by red blood cells. The resulting hypoxia hypersensitizes the cardiac cells to norepinephrine, causing defibrillation and arythmia [15]. A chronic exposure to this kind of substances can also be responsible for neurotoxic effects.

CFC, HFC and HCFC derivatives show low toxicity; low reactivity and act as oxygen depletants but most of them are highy flammable [16]. They are mainly used as refrigerants, solvents and propellants [17]. This group of compounds constitutes the limit between volatile compounds and pure gases because some of them are found at gaseous state under normal conditions such as hexafluoroethane (C_2F_6, boiling point at -78.2°C) or chloroethane (C_2H_5Cl, boiling point at 12.5°C), whereas others become gaseous with a slight temperature increase. Many mixtures of these compounds are available and marketed with R-xxx denomination as industrial refrigerants or propellants. Several of these HFC and HCFC can exhibit anesthetic properties such as halothane (C_2F_3HBrCl), sevoflurane ($C_4H_3F_7O$), desflurane ($C_2H_2F_6O$) and isoflurane ($C_3H_2ClF_5O$) but at temperature close to 50°C. Others appear more toxic such as enflurane ($C_3H_2ClF_5O$) which was abandoned in 1980's due to its hepatotoxicity. At high levels, some cases of cardiac arythmia have been described but the concern is relatively low in a forensic context except for perioperative anesthesia. However, from a physic-chemical point of view, these last molecules are volatile compounds and not pure gases.

Finally, other very toxic gases exist but are expensive, generated in small quantities, rarely used and for specific applications, and sometimes unstable so they react rapidly into other compounds (pyrophosphate derivatives). However, even if less employed and rarely described in intoxications, some of them are highly toxic (Table 1). This is the case of many chemical and industrial intermediaries such as perchloryl fluoride (ClO_3F), perfluoroisobutylene (C_4F_8), diborane (H_6B_2), dichlorosilane (SiH_2Cl_2) and boron trifluoride (BF_3), such as germane (H_4Ge) and hexafluorides of tungsten (WF_6), tellurium (TeF_6), selenium (SeF_6) employed in semiconductor chemistry, such as hydrogen selenide (H_2Se) employed for metals preparations or bromine monochloride (BrCl), bromomethane ($BrCH_3$), diazomethane (CH_2N_2) and trifluoroacetyl chloride (C_2ClF_3O) as chemical reagents and biocides. Other gases are just observed reactions products such as hydrogen telluride (H_2Te), oxygen difluoride (OF_2), phosphorus pentafluoride (PF_5). Their toxicities refers generally to this of the substance they originate as for example cyanogen ($(CN)_2$) deriving from hydrogen cyanide.

What is the Importance of Gases in Forensic Toxicology?

Gases constitute a subcategory among volatile compounds. Indeed, they are gaseous under normal conditions (normal temperature: 20°C, and pressure: 1 atm) whereas volatile compounds can be transformed at gaseous phase as soon as the temperature overcomes their boiling point. Therefore, due to their gaseous physical state and their variable and sometimes very high toxicity, gases must be considered as important poisonous and lethal agent.

Two ways of exposure should be differentiated: domestic and professional exposure. Moreover, among domestic exposure cases, two categories must be defined - accidental exposure and deliberate exposure - whereas professional exposure is mainly accidental.

Most of gases are widely used in industry. Therefore, workers can be subjected to gas outburst or gas leaks leading to fatal anoxia. Gaseous residues should be sampled from the outburst site or the death scene to investigate the nature of the intoxication. Coolant and can fillers (N_2, CO_2), sewage (H_2S, CH_4), oil and gases (hydrocarbon gaseous derivatives) industries, volcano/geological and scuba diving activities are examples where numerous fatalities implying gas intoxications were reported. Numerous highly toxic gases are employed in the industry (Table 1) but their uses are very specific and controlled.

The domestic gaseous intoxications are also complex to diagnose because except with carbon monoxide (where blood can rapidly turns into a reddish-cherry colour giving a characteristic colour to the body), hydrogen cyanide (potent almond odour) and hydrogen sulphide (potent rotten eggs odour), gases are generally invisible and

Chemical name	Chemical formula	Boiling point (°C)
Arsenic pentafluoride	AsF_5	-52.8°C
Arsine	AsH_3	−62.5 °C
Boron trichloride	BCl_3	12.6°C
Boron trifluoride	BF_3	-100.3°C
Bromine monochloride	BrCl	5°C
Bromomethane	CH_3Br	4°C
Carbon monoxide	CO	-191.5°C
Chlorine	Cl_2	-34°C
Chlorine pentafluoride	ClF_5	-13.1°C
Chlorine trifluoride	ClF_3	11.8°C
Cyanogen	C_2N_2	-21°C
Cyanogen chloride	CNCl	13°C
Diazomethane	CH_2N_2	-23°C
Diborane	B_2H_6	-92.4°C
Dichlorosilane	H_2Cl_2Si	8.3°C
Fluorine	F_2	-188.1°C
Formaldehyde	CH_2O	-19°C
Germane	GeH_4	-88.5°C
Hydrogen cyanide	HCN	25.6°C
Hydrogen selenide	H_2Se	-41.2°C
Hydrogen sulfide	H_2S	-60°C
Hydrogen telluride	H_2Te	-2.2°C
Nitrogen dioxide	NO_2	21°C
Oxygen difluoride	OF_2	-145°C
Perchloryl fluoride	$ClFO_3$	-46.7°C
Perfluoroisobutylene	C_4F_8	7°C
Phosgene	CCl_2O	8.3°C
Phosphine	PH_3	-87.7°C
Phosphorus pentafluoride	PF_5	-84.5°C
Selenium hexafluoride	SeF_6	-46.6°C
Silicon tetrafluoride	SiF_4	-65°C
Stibine	H_3Sb	-17°C
Sulfur tetrafluoride	SF_4	-38°C
Tellurium hexafluoride	TeF_6	-37.6°C
Trifluoroacetyl chloride	C_2ClF_3O	-27°C
Tungsten hexafluoride	WF_6	17.1°C

Table 1: Chemical formula and boiling points of the most toxic gases.

odourless. The clues on the death scene are of great importance. Individual protections should be worn from the investigators arrival until the end of the autopsy. Accidental domestic fatalities are often related to natural gas outburst, H_2S knock-outs or individual works (soldering, cleaning). Deliberate domestic exposure is increasingly popular as cheap substance abuse in order to get high. Huffing practice implies spray aerosols such as hydrocarbon gaseous derivatives (butane/isobutane or lighters refills mixtures), N_2O and CO_2. Finally, fatal deliberate gas exposure is also increasingly used to commit suicide. Asphyxias with helium, argon or hydrogen sulphide are more and more numerous.

Thus, the monitoring and quantification of gases should be mandatory in forensic laboratories.

What is the Analytical Strategy?

First of all, even if there are obvious clues on the death scene, the sampling should be carried out without targeting a specific gas to avoid the possibility of scene make-up. Indeed, until now, the analyses were directly planned taking into consideration only the macroscopic evidences. However, a gas screening must be obligatory as a preliminary step preferentially on blood.

According to the context and the magnitude of the intoxication, the gas can be at gaseous state or dissolved in the body tissues. Therefore, it is of importance to sample a lot of different samples to reconstitute the magnitude of the intoxication and a potential metabolism. Samples of lungs, blood, urine, brain, heart, kidney, liver, skeletal muscle and fat tissue are required. A high gas concentration in lungs and weak concentrations in other samples such as brain and kidney would favorize the hypothesis of an acute exposure and rapid death. A lower concentration in lungs but high concentrations in liver, kidney, brain, urine would favorize the hypothesis of a longer exposure and a certain metabolisation of the gas. Similarly, gas samples should be of interest in case of vital gas embolisms, scuba diving accidents (Decompression Compression Sickness) or suicides with noble gases [18]. Gas sampling under laser guidance is an efficient and precise tool to recover gas samples located into the body [19]. For suicide with noble gas, the samples of interest are gastric gas, tracheal gas and lungs samples [20]. These matrices can easily be sampled during conventional autopsy (with airtight gas syringe for gaseous samples) without the aid of forensic imaging.

The procedures for gas sampling and analysis must be strictly followed and fully documented to avoid gas leaks in order to guarantee the operator safety and precision of the quantification. The best approach to quantify gases in forensic samples is Gas Chromatography (GC) for the separation and common detectors such as Mass Spectrometry (MS), Thermal Conductimetry (TCD) or Flame Ionisation Detector (FID) for the analysis. Taking into account the wide variety of gases, today no universal gas chromatography column is available. Molecular sieve and Porous Layer Open Tubular (PLOT) columns offer alternatives but they must be placed in series (or in parallel to be less time-consuming) to offer the most exhaustive gas screening as possible.

Thus, the analytical strategy is based on two steps: a first untargeted screening, for identification, and a second analysis for quantification. The best approach for untargeted screening is MS. The identification is performed thanks to retention time and mass spectrum to provide a reliable identification of the gas analysed thanks to comparison with mass spectra libraries. Two screening analyses should be done with two different carrier gases since it is possible that the carrier gas of the first screening analysis could be involved in the intoxication (helium suicide for example).

Once detected and identified, the gases of forensic interest must be measured. According to their identity and their estimated concentration, the choice of the detector is of importance. Once again, MS is the best compromise for accuracy of quantification. Moreover, thanks to the resolution improvement of the analyzer and its ability to separate isotopes of a gas, it becomes possible to use stable labelled isotopes as internal standards. Indeed, the possibility to use specific internal standard is of great help to take into account the possible analytical problems (leaks) that can occur during analysis. Deuterated alkanes have been specifically used for methane [21], propane [14] and butane [22]. Similarly, ^{13}C compounds have been used for CO and

CO_2 quantification [18,23]. However, if tandem Mass Spectrometry (MS/MS) can improve the sensitivity of most of analytical methods and substances quantification, in the particular case of gases, MS/MS appears as not relevant because of the weak molecular mass of the gases and the impossibility to monitor specific transitions. GC carrier gas should be selected to achieve the best quantification. Operating with hydrogen as carrier gas, GC-MS provides good chromatograms in short-time analysis.

Nevertheless, some particular cases are better solved with other detectors. For example, hydrogen cannot directly be analysed by GC-MS. With most of mass spectrometers, gases lighter than the carrier gas cannot be detected. Using MS, hydrogen can only be put in evidence indirectly by carrier gas protonation but it can be directly and easily measured with TCD [24]. However, TCD quantification requires external calibration which can introduce some bias (in case of leaks from an analysis to another) and is not as sensitive as MS. CO quantification is also another case where MS performance is questionable. Indeed, FID equipped with a methanizer reaches the lowest limit of detection for carbon monoxide, but the identification is only based on retention time.

Conclusion

Gas analysis is an exciting analytical challenge. The technology for gas detection appears as sufficiently mature (tandem mass spectrometry is not relevant due to the weakness of the molecular weight of these compounds). Improvements can be done for the separation to get only one column for this analysis. Until now, forensic analytical chemistry was not very focused on gases analysis because of uncertainties of sampling and difficulties of analysis. Today, reliable strategies for sampling have arisen and new quantification approaches are developed. There is not anymore reason not to perform this really informative analysis.

References

1. Magnier MD (1849) New Complete Manual of gas lighting or elementary treatise and practical for use by engineers, managers and masters of against-gas plants. Roret encyclopedic library, Paris, France.
2. Haldane JS (1895) The action of carbonic oxide on man. J Physiol 18: 430-462.
3. Jamieson D (1989) Oxygen toxicity and reactive oxygen metabolites in mammals. Free Radic Biol Med 7: 87-108.
4. Ammoura AM, Aqqad SS, Hamdan AT (2004) Chlorine gas poisoning. J Royal Medical Services 11: 34-37.
5. Sun P, Gu J, Maze M, Ma D (2009) Is Xenon a future neuroprotectant ? Future Neurology 14: 483-492.
6. Thevis M, Piper T, Geyer H, Thomas A, Schaefer MS, et al. (2014) Measuring xenon in human plasma and blood by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 28: 1501-1506.
7. Field RW (2003) Exposure to residential radon causes lung cancer. For the proposition. Med Phys 30: 485-486.
8. Vreman HJ, Wong RJ, Kadotani T, Stevenson DK (2005) Determination of Carbon monoxide (CO) in rodent tissue: effect of heme administration and environmental CO exposure. Anal Biochem 341: 280-289.
9. Vevelstad M, Morild I (2009) Lethal methemoglobinemia and automobile exhaust inhalation. Forensic Sci Int 187: 1-5.
10. Weimann J (2003) Toxicity of nitrous oxide. Best Pract Res Clin Anaesthesiol 17: 47-61.
11. Wyatt JP, Allister CA (1995) Occupational phosgene poisoning: a case report and review. J Accid Emerg Med 12: 212-213.
12. Nath NS, Bhattacharya I, Tuck AG, Schlipalius DI, Ebert PR (2011) Mechanisms of phosphine toxicity. J Toxicol 2011: 494168.
13. Saltzman BE (1961) Preparation and analysis of calibrated low concentrations of sixteen toxic gases. Ammonia, arsine, bromine, carbon dioxide, carbon monoxide, chlorine, chlorine dioxide, ethylene oxide, hydrogen chloride, hydrogen cyanide, hydrogen fluoride, monoethanolamine, nitric oxide, nitrogen dioxide, phosgene and stibine. Anal Chem 33: 1100-1112.
14. Smith F, Augsburger M, Varlet V (2014) Accuracy profile validation of a new analytical method for propane measurement using headspace-gas chromatography-mass spectrometry. J Anal Toxicol 38: 73-79.
15. Williams DR, Cole SJ (1998) Ventricular fibrillation following butane gas inhalation. Resuscitation 37: 43-45.
16. Crawford WV (1976) Death due to fluorocarbon inhalation. South Med J 69: 506-507.
17. Groppi A, Polettini A, Lunetta P, Achille G, Montagna M (1994) A fatal case of trichlorofluoromethane (Freon 11) poisoning. Tissue distribution study by gas chromatography-mass spectrometry. J Forensic Sci 39: 871-876.
18. Varlet V, Smith F, De Froidmont S, Dominguez A, Rinaldi A, et al. (2013) Innovative method for carbon dioxide determination in human postmortem cardiac gas samples using headspace-gas chromatography-mass spectrometry and stable labelled isotope as internal standard. Anal Chim Acta 784: 42-46.
19. Varlet V, Bruguier C, Grabherr S, Augsburger M, Mangin P, et al. (2014) Gas analysis of exhumed cadavers buried for 30 years: a case report about long time alteration. Int J Legal Med 128: 719-724.
20. Varlet V, Bouvet A, Augsburger M (2012) L'argon: utilisations, toxicité et stratégie analytique en toxicologie médicolégale. Ann Toxicol Anal 24: 185-192.
21. Varlet V, Smith F, Augsburger M (2013) Validation of methane measurement using headspace-GC-MS and quantification by stable isotope-labeled internal standard generated in-situ. J Sep Sci 36: 1967-1972.
22. Varlet V, Smith F, Augsburger M (2013) Accuracy profile validation of a new analytical method for butane measurement using headspace-gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 913-914: 155-60.
23. Varlet V, De Croutte EL, Augsburger M, Mangin P (2012) Accuracy profile validation of a new method for carbon monoxide measurement in the human blood using Headspace-gas Chromatography-mass Spectrometry (HS-GC-MS). J Chromatogr B Analyt Technol Biomed Life Sci 880: 125-131.
24. Varlet V, Smith F, Augsburger M (2013) Indirect hydrogen analysis by Gas Chromatography coupled to Mass Spectrometry (GC-MS). J Mass Spectrom 48: 914-918.