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Journal: International journal of legal medicine

Year: 2019 Feb 7

DOI: 10.1007/s00414-019-02014-3

Helium poisoning: new procedure for sampling and analysis

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Abstract

An increasing number of suicidal asphyxiation with a plastic bag with inert gases, and in particular helium (He), have been reported from numerous countries over the last decade. These cases are differently managed and lead to different and variable interpretations. Based on the twelve last cases analysed in the laboratory and on the review of the most recent literature about this topic, updated autopsy guidelines for sampling have been proposed regarding to the samples choice and analytical challenges required by the gaseous state of this substance. Biological samples from airways (lungs lobe) followed by brain and cardiac blood are the best matrices to take during the autopsy to diagnose He exposure. Gaseous samples from trachea, pulmonary bronchi, gastric and cardiac areas are also recommended as alternative samples. The anatomical site of sampling must be carefully detailed, and to this end, forensic imaging constitutes a beneficial tool. Even if He detection is sufficient to conclude to He exposure, He concentrations in samples may be related to He exposure conditions (duration, breathing rate, etc.). A quantification in biological samples could be helpful to document more precisely the case. He concentrations in gaseous samples are reported up to 6.0 µmol/mL (tracheal gas), 2.4 µmol/mL (pulmonary gas), 0.64 µmol/mL (cardiac gas) and 12 µmol/mL (gastric gas). He concentrations in solid/liquid samples are reported up to 28 µmol/g (lungs) and 0.03 µmol/g (cardiac blood). The other matrices usually sampled during autopsy such as urine, peripheral blood, liver, fat matter and kidney appear as not relevant.

Keywords

Helium; Suicide; Suffocation; Asphyxia; GC-MS

Introduction

Helium is the lightest gas among the noble gases group, historically also named the inert gases, gathering odourless, colorless and monoatomic gases with very low chemical reactivity [1]. The six natural noble gases located in the last raw of the periodic table are Helium (He), Neon (Ne), Argon (Ar), Krypton (Kr), Xenon (Xe) and Radon (Rn). Among this group, He appears as a non-toxic and not flammable inert gas and is extracted from natural gas using low-temperature gas liquefaction and fractional distillation. At standard conditions, He is much lighter than air (0.178 kg/m^3) hence its use in numerous applications such as cryogenic cooler (Magnetic Resonance Imaging (MRI) magnets), gas for inflatable (from balloon to airship/dirigible balloon) and laparoscopy [2] or other medical applications [3]. He is also used for scuba diving in Trimix (He, Oxygen - O_2 and Nitrogen - N_2), heliox (He, O_2) or heliair (He, Air) gases mixtures for deep dives in order to reduce nitrogen narcosis, the oxygen toxicity and the respiratory effort due to its low density [4].

In forensic medicine, concerns about He araised in the last years due to its use as fatal anoxiant in recreative sniffing (to change the timbre of speech when inhaled) [5], but especially in self-administered and assisted suicides [6-12]. This method was first recorded in 1990s and described in books and article of right-to-die advocates as “suicide bag method” [13-16], consisting of a large plastic bag with a drawcord [17] usually used in conjunction with a flow of an inert gas like helium or nitrogen [18, 19].

From a physiopathological point of view, He in excess can lead to anoxia by oxygen depletion. This causes O_2 concentration imbalance in lungs, transiently compensated by a transfer from O_2 of blood and alveolar capillaries, but finally causing a quick depletion in O_2. The deoxygenated blood then passes through the systemic circulation to the vital organs, including the brain, and rapidly lowers oxygen concentration below the level required to sustain consciousness, and cardiac arrest occurs [20, 21]. As recommended, a hypoxic or oxygen free (such as He or N_2) but also carbon dioxide (CO_2) free metabolically inert gas should be used to reduce the panic, sense of suffocation and struggling before unconsciousness, known as the hypercapnic alarm response caused by the presence of important CO_2 concentrations in the blood [22]. With breathing gas free of CO_2, the blood CO_2 levels will remain low while breathing occurs, and there will be no distress or urge to increase breathing rate. To this end, He and N_2 [23] are the most used suicide bags gases but argon [24] and volatile alkanes such as methane [25], propane [26], butane [27] and their mixtures [28] have also been described.

Other suicides by gassing have been also described such as charcoal gas or car exhausts where carbon monoxide exposure is clearly identified as the anoxic cause of death [29].

From a forensic point of view, this method of suicide is very challenging. Indeed, the direct cause of death becomes difficult to trace if the bag and/or gas canister are removed before the death is reported [22]. At autopsy, unspecific signs of anoxia can be noticed but the identity of anoxic agent cannot be determined. He exposure can be put in evidence only by forensic toxicology investigation at concentrations widely higher than those found in air. To this end, analytical instruments are really helpful and gas chromatography coupled to mass spectrometry (GC-MS) or thermal conductivity detection (GC-TCD) constitute methods of choice. However, as He is widely used as GC carrier gas, He analysis in forensic samples requires the use of another carrier gas [30]. Nitrogen can be used [31] but hydrogen (H_2) is the best alternative [24, 32, 33] and MS detection should be therefore preferred to TCD detection due to the influence of this gas mixture on the TCD response [34] or to the better sensitivity of MS [35]. Hydrogen was proposed as the optimal carrier gas for GC-MS analysis to obtain best sensitivity and selectivity. Indeed, the analysis of a gas which is lighter than the carrier chromatographic gas can lead to saturation of the mass spectrometer (pumps are not efficient enough to pump out these gases of the MS) causing a decrease in sensitivity.

However, if the analytical challenge can be addressed easily using H_2, the presence of He in forensic samples can be observed only if the sampling during autopsy is rigorously done in order to avoid leaks and loss of representativeness. Indeed, due to manual handlings of the body during the forensic chain of custody, leaks of He can occur by passive diffusion as He has no affinity for biological tissues. Considering this fact, the selection of biological samples is of high importance in order to maximize the chances to put He in evidence. Similarly, until now, only screenings of biological samples such as lungs biopsies have been taken and analysed because they were found to be sufficient to prove He exposure. Indeed, due to the speed of the asphyxiation, the He analysis of blood and other organs such as liver or kidneys appear less relevant. However, with the progress of analytical chemistry, it is become possible to quantify He in samples of interest (biopsies of lungs and gases from gastric, tracheal and pulmonary cavities). To our knowledge, no study has been done to evaluate the relevance of He quantification in those matrices.

Consequently, the goals of this study are to define the standard biological samples for He determination through new autopsy procedures and to investigate the relevance of He concentration according to the biological sample.

Material and methods

Collection of gas samples

Biological samples came from various forensic institutes, which had to deal with possible helium death cases (France: Montpellier, Bordeaux, Angers and Lyon, Germany: Bremen, Cologne, Hamburg and Frankfurt, Switzerland: Geneva). The results of 14 different cases were compiled in this study and concerned various biological samples such as brain, cardiac and peripheral blood, lungs (different lobes) and gases from trachea, pulmonary, intracardiac (right ventricle) and gastric cavities. Tissue samples were taken according to the particular standard procedure (rapid sampling during autopsy in HS vials of 10 or 20 mL, sealed with aluminium cap and cold storage). Gaseous samples were taken following the protocol of noble gases poisoning or intracadaveric gases [36-38].

Gas analysis

Pure helium (He), methane (CH_4) and nitrous oxide (N_2O) were purchased from Carbagas (Lausanne, Switzerland). Further dilutions when required were perfomed in 20 mL HS vials (BGB Analytik, Boeckten, Switzerland) with airtight gas syringe equipped with a push-button valve (Vici, Baton Rouge, USA).

For quantification, a calibration curve was done using pure He dilutions with an airtight gas syringe of 100 µL and at the following concentrations: 41.6, 83.2, 208, 416 and 830 nmol/mL of Headspace (HS) (respectively 1, 2, 5, 10 and 20 µL of pure He). Injections were performed manually and 20 µL of internal standard (CH_4 or N_2O) at 980 nmol/mL HS, sampled in the same syringe, was co-injected with the points of the calibration range, as well as with the biological samples (50 µL, for a total injected volume of 70 µL).

GC-MS procedure

An Agilent 6890N GC (Agilent Technologies, Palo Alto, CA) equipped with an Agilent Select Permanent Gases column was used [37]. This column consisted of two capillary columns set in parallel: a molecular sieve 5 Å PLOT capillary column (50 m x 0.53 mm) and a Porabond Q (10 m x 0.32 mm). Thanks to this design, He elutes on both columns during the same run,

leading to a double peak detection, before O_2 peak on molecular sieve 5 Å and before air peak on Porabond Q. The temperature program was as follows: 30°C, held for 3 min; the injector (splitless) set to 180°C and the interface MS temperature to 250°C. H_2 was used as the carrier gas at a flow rate of 11 mL/min. The detection was performed with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA), operating in the electron ionization mode (EI) at 70 eV. The selected ion monitoring (SIM) mode was used to acquire the He signal (m/z = 4) and the internal standard signal (CH_4 m/z = 16 or N_2O m/z = 44, according to laboratory availability and sample state). The analytical strategy is only based on He screening and does not require a full method validation.

Results and discussion

Concerning solid tissues, 7 right lobes lung biopsies, 5 left lobes lung biopsies, 10 lung biopsies without anatomical precision, 10 cardiac and 8 peripheral blood samples, 3 brain biopsies and 1 biopsy of liver, kidney, urine and fat matter, from a total of 13 cases were analysed. Concerning gaseous samples, 6 gastric gas samples, 6 tracheal gas samples, 2 right and 2 left pulmonary gas samples, 6 pulmonary gas samples without anatomical precision and 1 cardiac gas sample from 8 cases were analysed. The results are displayed in Table 1. The results compiled show an important variability because the different teams have worked without any common guidelines. For a same anatomical site, negative to highly positive results can be registered, and even on the same sample, repetitions lead to very variable results. This variability proves the need for sampling and analysis harmonization.

Choice of samples: lung biopsies

Taking into account the physiopathology of He, the first obvious biological samples are those related to airways. Therefore, lungs biopsies have been considered primarily as the gold standard in available literature and textbooks. To our knowledge, no anatomical information concerning these biopsies were given (e.g. right inferior lobe, left superior lobe, etc.). However, this approach has a major drawback consisting in a lack of representativeness. Indeed, even if the biopsy is done rapidly and stored in freezer right away, He may not be identified because of the heterogeneity of the organ. Indeed, He concentration in right lung could not be the same as in left lung, and similarly between each lung lobes. This is confirmed by the results from the

Swiss cases in Table 1, where the He concentrations in lung lobular biopsies are very different between the cases - which can be explained by the exposure conditions (different inter-case concentrations) and sampling -, but even for each case (different intra-case concentrations). As result, lung multilobular biopsies of several grams should be done to put in evidence He. To this end, considering the Swiss results in Table 1, the inferior lobes (right and left) seem to lead to the less variable concentrations (9.4 - 14 µmol/g). However, additional analyses on supplementary cases should be done to confirm this trend. Various He concentrations are obtained in the Table 1 concerning these samples. Right lung samples ranges from not detected to 41 µmol/g (n=7), left lung samples from 1.2 to 14 µmol/g (n=5), and taking into consideration the cases where the anatomical position of lung biopsy was not reported, the He lung biopsy concentration ranges ranges from not detected to 28 µmol/g (n=10). These results show an important variability directly related to the exposure protocol (exposure duration, breathing volume and rate, body mass index of the victim, bag volume, bag closing system/leaks, etc.) and sampling conditions. However, this range is consistent with the He concentrations found in lung biopsies reported in the literature: 0.17 – 2.1 µmol/g [31], 6.5 µmol/g [39].

Therefore, as a single lung biopsy cannot be representative of the whole organ, protocols using lung lobectomy (mono, bi, trilobular biopsies) have been developed to avoid false negative results due to sampling. These protocols have been well described [3, 24, 36, 37, 40, 41] but some authors found them too sophisticated compared to rapid lung biopsy in HS [32]. However, this comment was formulated because the HS lungs biopsies were detected as He positive. Consequently, the whole lung protocol should not be totally discarded and the sampling should be done just in case of He absence in HS lung biopsies. Nevertheless, the protocol of He release from a whole lung or lung lobe requires a standardization because several approaches were described with different containers types (plastic boxes, plastic bags, etc.). Briefly, the technique requires the immersion of the whole biopsy in water and a container [36, 37]. The aim is to agitate/press the biopsy to mechanically release trapped residual He, thanks to direct manual pressure [3] or with sticks [40]. The released gas is easily noticeable as a gaseous bubble in the container which can be a bag or a plastic container [24].

Choice of samples: tracheal and pulmonary gases

An alternative to the lung lobectomy is represented by gaseous tracheal and pulmonary gases. Indeed, gaseous biopsies have the major advantage to constitute a homogenous matrix from the concentration point of view. Indeed, in a gaseous sample, the concentration is the same

wherever the sampling site. Consequently, gaseous samplings from airways have been described. (Intra)tracheal and pulmonary gases were found as very informative samples [37, 39]. (Intra)tracheal gas is described as the first sample to be taken. The gas is sampled by gas syringe directly in trachea, after clamping. Various He concentrations are obtained in the Table 1 concerning this anatomical sampling site (not detected – 6.0 µmol/mL HS). As result, when no He concentration is detected in lung biopsies, tracheal gas could constitute an useful alternative. Secondly, pulmonary gases (right or left) can be sampled directly in the primary bronchus after lung massage and bronchi clampings or according to the lung or lobe immersion as above after mechanical pressure on the sampled biopsy. Various He concentrations are also obtained in the Table 1 concerning these anatomical sampling sites. Left pulmonary gas is around 0.3 µmol/mL HS (n=2), right pulmonary gas around 0.5 µmol/mL HS (n=2) and taking into consideration the cases where the anatomical position of lung was not reported, He pulmonary concentration range was from not detected to 2.4 µmol/mL (n=6). Other publications have detected He levels from 0.02 µmol/mL HS in expired air from lungs [31] to 34 µmol/mL HS in intratracheal gas [39]. As tracheal gas, pulmonary gases could constitute an useful alternative to lung biopsy.

Choice of samples: gastric gas

During gas inhalation, most of the gas is directed to lung airways. However, perimortem phenomena (through antemortem gasping or post-mortem diffusion) can be responsible for a random He volume presence in the gastric gas. This concentration is sometimes more important than in lungs biopsy [42]. This gaseous sample can be easily taken during autopsy after oesophageal and duodenal clamping [37]. In the results presented in Table 1, He concentration in gastric gas ranges from not detected to 12 µmol/mL HS (n=6). As result, gastric gas should be also considered as a biological matrix of interest to be sampled in case of fatal He exposure.

Choice of samples: brain and other biopsies

According to the He exposure conditions, the amount of inhaled He may be important and sufficient to be detected in brain biopsies, as this organ is highly vascularised. He was put in evidence in only one brain biopsy out of the three brain samples taken for He monitoring (Case 4 in Table 1). Other studies have also reported positive He screening in brain [32] but when

quantified, the He concentration was at trace levels (0.1 %) [31]. Consequently, He can be put in evidence in brain but this matrix should not be considered as the priority sample.

As He is not metabolised, it may be detected as itself in organs through passive diffusion. Therefore, He potential concentration in body organs is directly linked to He exposure conditions. As death by He asphyxiation occurs rapidly in a close system (maximum 5 minutes), the delay of gaseous exchanges is very short, that is the reason why airways samples appear more relevant, followed by brain and cardiac blood. He has never been detected in other matrices such as urine [32], liver, kidney or fat matter (Table 1). These samples should be considered as not useful for He measurement because the expected amount are too low to be detected.

Choice of samples: peripheral / cardiac blood biopsies - intracardiac gas

As mentioned before, after airways and brain samples, cardiac area is the last interesting anatomical site where He has been detected after He exposure. He concentrations of 0.02 and 0.03 µmol/g have been measured in cardiac blood (Table 1) and they are quite below the concentrations found in airways samples. However, in case of body decomposition or when airways matrices sampling is not possible, cardiac blood/fluid could be an alternative sample to put in evidence He traces.

Concerning peripheral blood, although one analysis has led to a positive concentration (1.10^{-3} µmol/g in Table 1), due to the lack of time for gas exchanges from the beginning of He exposure to death, it is unlikely to detect positive He concentrations in this matrix [31]. Cardiac blood should be preferred to peripheral blood but the sampling should be done fastly to prevent He leak or release.

Recently, intracardiac gas has been also proposed as an alternative sample and He gas embolism has been investigated as a potential cause of death [43]. Intracardiac gas volumes were noticeable by postmortem computed tomography (PMCT) as displayed in Figure 1. They were attributed to He despite of biases such as the absence of postmortem delay, the absence of intracardiac gaseous composition and scene of death details (estimated pressure in airways if the tanks were still open). This was hypothesized to come from the high pressure of He tank transferred to airways and lungs, increasing intrathoracic pressure and the level of He diffusion through alveolar-capillary membrane and into circulation, leading to gas embolism [43]. Another group has supported this observation by *Macklin effect* [44], defined by the rupture of terminal alveoli caused by an increase of intrathoracic pressure, and subsequent dissection of

air along the perivascular and peribronchial fascial sheaths into the mediastinum, thoracic cavity, and/or connective tissue of the neck and subcutaneous tissue, and even retroperitoneal space. However, significant high pressure of He is required to cause such an effect and if it is theoretically possible, to our knowledge, He tanks on this kind of scene of death are frequently found in close position. Moreover, the plastic bags used for this kind of suicide are not designed to support the commercial range (1 600 – 2 700 kPa) described. As result, these explanations are not very relevant to explain He gas embolism as potential cause of death. Another explanation for He presence in intracardiac gas is linked to the natural He diffusion/release from organs in airtight cardiac site, the postmortem interval and the generation of decomposition gas [38]. Indeed, decomposition gas generation is easily noticeable with PMCT as postmortem delay increases. In He exposure case and similarly to O_2 embolism, He dissolved in the body (cardiac blood, airways, etc.) can diffuse in the airtight cardiac cavity [45]. This hypothesis is strongly supported by the result of Case 3 in Table 1, where a He concentration of 0.64 µmol/mL HS was found in intracardiac gas, simultaneously with H_2 (0.60 µmol/mL HS) confirming the beginning alteration after 40 hours of postmortem delay. However, even if the He role in the cause of death is still under discussion, the presence of He is noticeable in intracardiac gas and makes it an interesting alternative sample like gastric gas, especially in case of altered bodies and when other traditional samples (mostly airways samples) cannot be taken.

New autopsy procedures for sampling

Considering the diversity of protocols, the relevance of obtained conclusions concerning fatal He exposure and the forensic management of this kind of cases, it appears mandatory to propose a standardization of autopsy guidelines.

This requirement is driven by the need to obtain homogenous samples, to minimize the loss of He and to reduce the risk not to detect it. For example, some of results in Table 1 are displayed as “not detected”, which does not mean that He was not present at sampling time, but that He is not noticeable at analysis time. Moreover, the absence of He in the samples can be explained by body handling and forensic circumstances (long post-mortem delay, a short and weak exposure to He but sufficient to have caused the death) but also inappropriate samplings and storage. As result, He is falsely not detected because of the lack of representativeness of biological sample due to insufficient sampling selection and management.

In the light of the most recent studies about fatal He exposure, new autopsy procedures for sampling are presented in Figure 2. Additionally to the usual DNA samplings (to exclude the presence of an unexpected individual who could have touched the body or the material) and other evidences collection by the police, biological samplings to be performed by the forensic pathologist are mandatory. Firstly, a sample of the residual gas contained in the tanks should be taken to confirm the gaseous composition (He). Secondly, solid biological biopsies must be taken during autopsy. It concerns obviously samples of each lung lobes in a sufficient amount (not less than 10 g, transferred in usual HS vial of 20 mL of capacity). The autopsy should begin the shortest possible, preferably as soon as the body is received at the forensic centre in order to reduce the post-mortem delay from the body discovery to the samplings. A cold chain maintenance to prevent natural He post-mortem diffusion from the body should be kept until sample collection. Brain sample (not less than 10 g) and cardiac blood (not less than 15 mL) may be also taken and transferred in usual HS vial of 20 mL. They should be stored in freezer (-20°C) with lung biopsies until analysis but analysed only if lung biopsies are He negative. HS vials are really the best container for this kind of biopsies. Indeed, the solid plastic seal of other containers would oblige the operator to transfer the sample in a HS vial with a rubber seal, allowing the insertion of syringe needle for GC injection. HS vial is the only solution to avoid subsampling which can lead to He leak/loss and lack of representativeness [31].

Alternatively and especially if no autopsy is required by the prosecutor, gaseous samples can be taken. Indeed, due to the minimal traumatic gestures for gas samplings, these samples can be collected even during an external examination. For more precision, the tracheal, gastric and cardiac gaseous samplings can be done under laser guidance during PMCT [38]. However, as pulmonary gas samplings require mechanical pressure of the lungs lobes, they must be taken during autopsy. However, tracheal and gastric gases can be easily sampled during conventional autopsy too. Cardiac gas sampling is recommended especially in case of decomposition. It can be sampled during the autopsy if the volume is sufficient but it could be easier to sample it under laser guidance during PMCT. For gaseous samplings, the sampling set-up consists in a needle mounted on a three-way tap in closed position. A 20 mL syringe connected with Luer-Lock fitting was screwed on the tap which has to be turned in open position. The gas is withdrawn according to the available intracadaveric site volume and the tap is closed. The needle is then inserted through the rubber seal of a HS vial previously fully filled with stabilised water, while another needle is also inserted in the rubber seal. The tap is open and the gas is manually transferred in the HS vial, taking the place of water evacuated by the other needle.

The HS vials containing the intracadaveric samples are stored in fridge upside down until analysis.

Consequently, all these solid and gaseous samples have to be carefully and rapidly taken to guarantee the possibility to detect He at least in one of these matrices. The HS vials (with solid or gaseous samples) should be kept at freezer or fridge until analysis and analysed according to their relevance to the case and the first results concerning He concentrations in lungs biopsies.

Optimal detection strategy

The HS vials received at the laboratory should be managed in respect with the cold chain and must be stored in conformity to their state (fridge for gaseous samples, freezer for solid samples) until their analysis.

Before analysis, gaseous samples must be left on the table to reach an equilibrium with ambient temperature. Solid biopsies can be submitted to a heating step of 10 min at 80°C. Open systems for GC injection such as CombiPAL should be avoided regarding to the diffusivity of He. Closed HS system or manual airtight gas syringe injections should be preferred.

Considering the most recent literature on analytical He measurement, the best sensitivity and selectivity is obtained using GC-MS with H_2 as carrier gas. He signal is commonly acquired in Single Ion Monitoring (SIM) mode at m/z = 4. The use of a standard is a clever option in order to guarantee the quantification. As He stable isotope is not available, other gases such as CH_4, CO or N_2O can be used and co-sampled in the airtight gas syringe and simultaneously injected in the GC with the sample of interest.

This analytical procedure has been applied to the cases presented in Table 1 and only the He concentrations ranges identified according to their anatomical site are summarized in Figure 3, in order to ease the interpretation of the results. The concentrations ranges are mainly observational values obtained on a weak amount of cases and investigations on a more important number of cases are required. However, this work constitutes the first collaborative data study in order to increase the toxicological interpretation in fatal He exposure cases.

Conclusion

Due to the increasing amount of fatalities by inert gas asphyxia, particularly He, it becomes urgent to propose optimised procedure regarding the sampling and analysis techniques. Lungs

biopsies of each lobe constitute the best samples to diagnose He exposure. Considering the pathophysiology of He and the results of this study, brain and cardiac blood have been proposed as secondary matrices of interest but He could only be present in traces. He concentrations in solid/liquid samples are reported up to 28 µmol/g (lungs) and 0.03 µmol/g (cardiac blood). Gaseous biological alternative matrices have therefore been presented as very informative such as tracheal gas, pulmonary gases from bronchi after mechanical pressure of lungs, gastric gas and cardiac gas. The two last ones may be especially useful in case of decomposed bodies where biological solid samples may be not be relevant. In this study, He concentrations in gaseous samples are reported up to 6.0 µmol/mL (tracheal gas), 2.4 µmol/mL (pulmonary gas), 0.64 µmol/mL (cardiac gas) and 12 µmol/mL (gastric gas). All the samples should be transferred in HS vial defined as the gold standard container, closed hermetically with aluminium cap and rubber seal. He should be analysed by GC-MS using H_2 as carrier gas to obtain the best signal. Due to many uncertainties during exposure (duration, breathing volume/rate, body mass index of the deceased, etc.), sampling and storage until analysis, and with respect to efforts and costs, a qualitative confirmation of helium in the body seems to be sufficient in routine casework. The proposed order of biopsies to be sampled should be followed to maximize He detection. Consequently, this management for fatal He exposure could be extended to other fatalities implying inert gas exposure such as argon or nitrogen.

Conflict of interest

The authors declare no conflict of interest.

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Case	Country	Tracheal gas (µmol/mL HS)	Pulmonary gas (µmol/mL HS)			Gastric gas (µmol/mL HS)	Cardiac gas (µmol/mL HS)	Lungs biopsies (µmol/g)			Blood (µmol/g)		Brain (µmol/g)	Liver (µmol/g)	Kidney (µmol/g)	Urine (µmol/g)	Fat matter (µmol/g)
			Right	Left	No precision			Right	Left	No precision	Cardiac	Peripheral					
1	Germany (Hamburg)									1.4, 1.8							
2	Germany (Hamburg)									3.8, 6.0		ND				ND	
3	Germany (Frankfurt)						0.64										
4	Germany (Cologne)							Detected (n=2)	Detected		ND	ND	Detected	ND	ND		
5	Germany (Cologne)									ND	ND	ND					
6	Germany (Bremen)									8.1, 20, 28	ND		ND				ND
7	France (Bordeaux)		0.59	0.2		0.44					0.02	ND					
8	France (Bordeaux)	0.09			2.4	0.04					ND	ND					
9	France (Lyon)									3.7, 12	0.03						
10	France (Montpellier)				ND						ND		ND				
11	France (Montpellier)	ND, 6.0			1.3, 2.2	ND					ND	ND					
12	France (Angers)	0.48	0.54	0.34		0.98					ND	1.10^{-3}					
13	Switzerland (Geneva)	0.75			1.1	12		1.5 (SL) < 1.0 (ML) 12 (IL)	1.2 (SL) 14 (IL)								
14	Switzerland (Geneva)	2			1.7	ND		41 (SL) ND (ML) 9.4 (IL)	3.2 (SL) 14 (IL)		ND	ND					

Table 1. Helium concentration in biological samples and intracadaveric cavities

(ND : Not Detected, SL: Superior Lobe, ML, Middle Lobe and IL : Inferior Lobe)

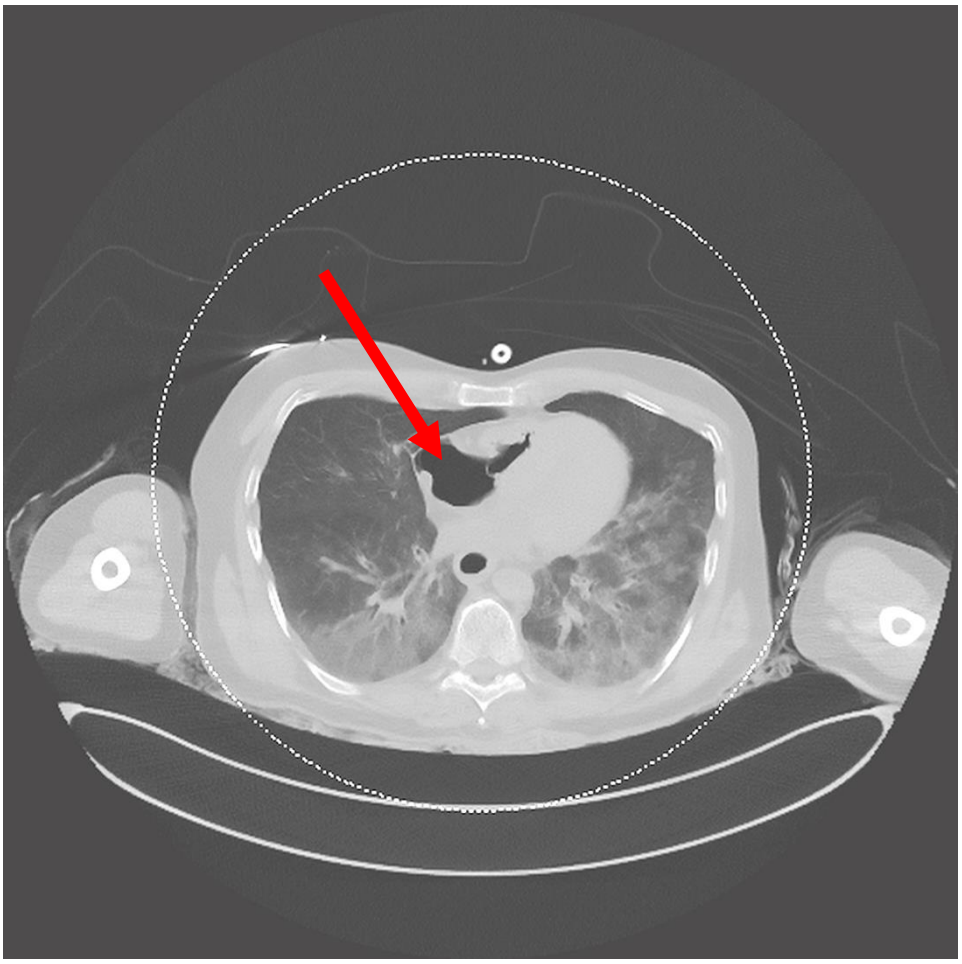

Figure 1. CT Scan of the intracardiac region showing gas sampled in the right ventricle (red arrow).

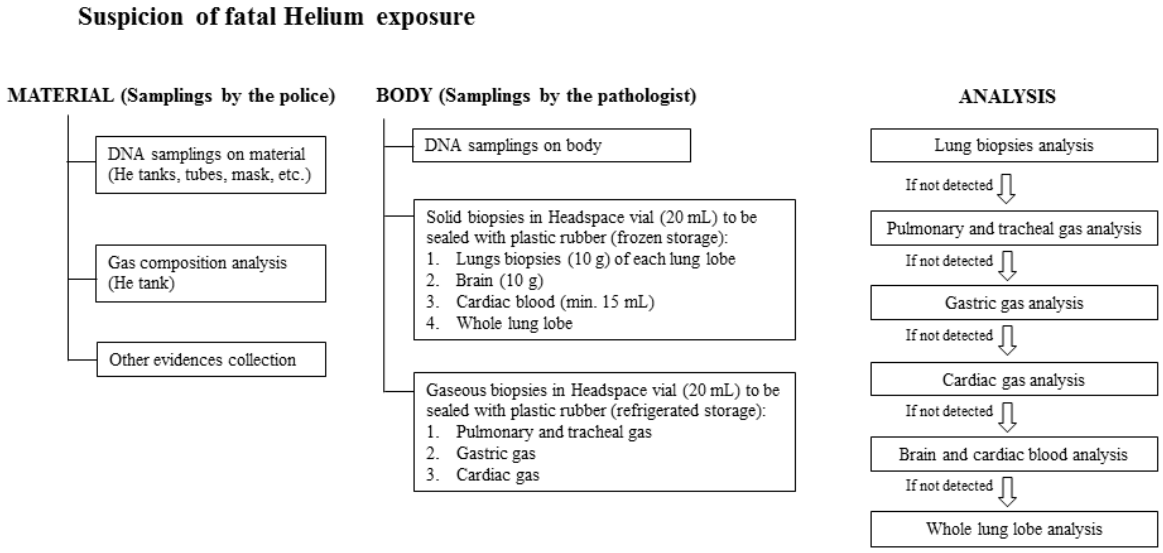

Figure 2. New operations list and biological matrices to be sampled in case of suspicion of fatal helium exposure

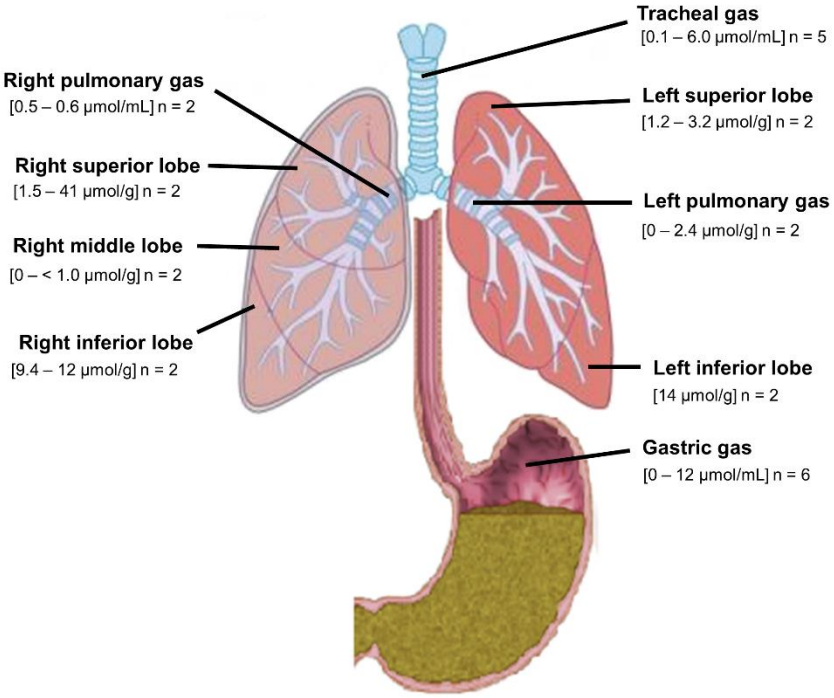

Figure 3. Helium concentrations ranges identified according to their anatomical site