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Volatile Lipophilic Substances Management In Case Of Fatal Sniffing

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Introduction and background

Inhalants' abuse in order to "get high", now commonly referred to as volatile substance abuse (VSA) or volatile substance misuse (VSM) has become increasingly popular among young people and represents a social problem in many countries worldwide. Propane and n-butane are two volatile alkanes having respectively 3 and 4 carbon atoms in line. They are both easily distributed to lipid-rich tissues such as the brain and have sedative effect on the central nervous system. Propane is less toxic than n-butane or isobutane, having a weaker anesthetic effect [1]. Hypoxia of the tissues by oxygen depletion can therefore be the mechanism of death of victims of such gases [2]. Butane has adverse effects on the central nervous system, and butane-associated encephalopathy has been reported. The cause of death due to butane inhalation is primarily presumed to be cardiac arrhythmia, even though butane is also known to cause other organs, including the central nervous system, and the lungs to fail [3]. Moreover butane is reported to sensitize the heart to circulating catecholamines, such that sudden alarm or exercise (for example fright or running) may precipitate sudden death [4]. In Italy, one of the most common forms of VSA consists in sniffing gas directly from camping gas refill cylinders, which is a widespread phenomenon among the prison population that is allowed to use camping gas stoves.

There are reported cases of ventricular fibrillation in patients misusing butane [5], and both propane and butane are reported to precipitate fatal arrhythmias through the stimulation of the vagus nerve [6]. Moreover several fatal intoxications due to butane [7], propane [6] or mixtures containing both propane and butane [8-9] are reported.

Authors want to give their contribution by reporting for the first time accurate values of both butane and propane in a large set of post-mortem biological samples (peripheral blood, heart blood, vitreous humor, liver, lung, heart, brain/cerebral cortex, fat tissue, kidney). Starting from a lethal case occurred in a prison, where a 29-year-old prisoner was found dead by his cellmate, we describe the forensic investigation that led us to determine the cause of death.

Case report

Summary

A 29-year-old man, well nourished, was found unconscious by his cellmate on the floor of the bathroom of his cell. The cellmate, awakened by the strong smell of gas, rushed into the bathroom and found the man unconscious next to a camping gas stove with gas escaping (Fig. 1). The Emergency Medical Technicians attempted cardiac massage, but the man was declared dead shortly after and the body was moved to the morgue.

Site survey

The prison cell was examined in cooperation with scene of crime officers (SOCOs), two camping stoves were found, one in the bathroom and the other on the table of the cell. These proofs, jointly to a white glue pack, two capsules and one circular tablet were collected, recording the site of their retrieval in the scene of crime notes, for further forensic examination and chemical analysis.

Autopsy findings

External examination

The deceased was a male, 185 cm in height and 84.5 kg in weight; he was transferred to the morgue after being declared dead at the prison cell, where the clothes were removed during resuscitation. The autopsy was performed 26 hours after the death.

External examination showed no evidence of injuries and the presence of subepidermal petechial hemorrhages on the rear region of the chest.

Internal examination

The internal examination of the head showed an intense passive congestion of the meningeal vessels. Specimens were collected from the brain for histological and toxicological analysis. In order to collect gas samples from the trachea and the bronchi limiting contamination, the dissection of the neck was carried out skeletonizing the larynx and the trachea *in situ* without manipulating the respiratory tract below the larynx. The dissection of the thorax allowed the exposure of the main bronchi *in situ*, as well. The gas contained in the trachea and

the two main bronchi was drawn before any discontinuation of the airways occurred. In particular, the gas content of the right bronchus was collected using a 10 ml plastic disposable syringe while a Hamilton gas-tight syringe was used to draw air from the left bronchus (Fig. 2); the collected syringes were wrapped in Parafilm® M (Pechiney Plastic Packaging Company, Menasha, WI, USA) and further submitted to toxicological examination.

Proceeding with the autopsy, the entire surface of the right lung showed emphysematous bubbles, mostly gathered on the inferior lobe. Evidence of rarer and smaller bubbles was visible on the left lung. The exploration of the tracheobronchial mucosa revealed intense hyperemia with mucous material mixed with air bubbles. Given the young age of the victim the macroscopic examination of the heart was deferred until complete formalin fixation of the eviscerated organ, only a small specimen of the heart was immediately collected in a gas tight container and stored at -20 °C for further toxicological examination.

Samples of heart blood, peripheral blood, gastric content, urine, bile, vitreous humor, in addition to specimens of subepidermal fat tissue, lungs, liver, kidneys were also collected in separate gas-tight containers, stored at -20 °C for the same purpose.

Macroscopic examination of the body did not reveal any other elements of interest. Specimens of the lungs including the areas showing macroscopic evidence of emphysema, specimens of the liver, kidneys, spleen and pancreas were collected for further histological analysis.

The macroscopic examination of the heart after formalin fixation identified a bilateral dilatation of ventricular cavities (3.5 cm left and 3 cm right), a diffuse opacification of the endocardium and focal fibrosis of the myocardial tissue with pervious coronary arteries; specimens of the heart including the coronary arteries were collected for histological analysis.

Histological findings

A routine microscopic histopathological study was performed on specimens fixed by using buffered formalin 10%, embedded in paraffin and stained with hematoxylin/eosin. All specimens were examined with a light microscope (DMLB 100T Leica, Wetzlar) connected with a photo-camera computer system (DFC 320 Leica, Heerbrugg); all the images were saved using IrfanView v. 4.22.

The observation of the specimens showed the following significant elements. The heart sections revealed stretched and wavy myocells consistent with dilation of the ventricular chambers; the nuclei of the myocells appeared enlarged and the intramural arterioles showed thickening of the tunica media consistently with hypertrophy. Focal areas of perivascular fibrosis are also detected.

The observation of the lung sections revealed subpleural blebs and peripheral emphysematous areas. The alveolar septa showed marked congestion and the alveolar lumens appeared enlarged and distended by the presence of mucoid material interspersed with air bubbles which tent to push the mucoid material to the alveolar margins. The same mucoid material mixed with cellular debris and partially lysed blood cells was also observed inside the lumen of the primary and secondary bronchi. Edema was also observed (Figs. 3-4). Intensive congestion was detected in the liver and kidney sections.

Toxicological findings

Screening test performed with immunochemical technique on urine and blood allowed the detection of benzodiazepines. Generic qualitative analysis carried out with gas chromatography coupled to mass spectrometry (GC-MS) on peripheral blood identified caffeine. The analysis carried out by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) confirmed the presence of benzodiazepines on peripheral blood in the following quantities: diazepam (68 μg/L), nordiazepam (110 μg/L), oxazepam (17 μg/L), temazepam (6.2 μg/L). Diazepam, nordiazepam, and oxazepam were also identified in the sample of vitreous humor. No ethanol was detected in urine, peripheral and heart blood.

The volatile alkanes were analyzed using an Agilent 6890N GC, combined with a headspace gas autosampler, and coupled with an Agilent 5973 mass spectrometer, operating in the electron ionization mode at 70 eV. The column was an Agilent Select Permanent Gases, made of two capillary columns set in parallel: a molecular sieve 5 Å PLOT capillary column and a PoraBOND Q. The temperature program was as follows: 100°C, held for 2 min, and raised at 10°C/min to 250°C; the injector (splitless mode) set to 100°C and the interface MS temperature to 230°C. Injections were done manually with airtight gas syringes [10-11]. Internal standard was 13C02. Both butane and propane were identified in

all samples (Fig. 5 and Fig. 6), including the air samples collected from the bronchus and from emphysematous bubbles. Quantitative results are reported in Table 1.

Discussion

In Italy, the voluntary inhalation of gas cylinders is a widespread phenomenon in the prison population, where the use of gas stove in the cell is allowed and regulated by law [12-13]. In these cases, the specialists involved in the determination of the cause of death need to properly handle the samples of biologic material to allow the analysis of volatile substances, by using gas-tight containers [14]. The forensic strategy in this kind of death implies different steps: evidences collection such as bottles and canisters, wide sampling of biological matrices during the autopsy. Numerous biological samples should be taken to rebuild the pathway and magnitude of exposure. According to the presence and concentration of substances in the different matrices, acute inhalation can be differentiated from chronic exposure. The most lipophilic organs and tissues should be sampled first, with a specific focus on targeted organs according to the toxicity. Additional analyses should be performed to check the correspondence between the materials found on the scene and the substances found in the samples. In our case we obtained significant concentration of butane from many samples. The result of butane concentration in the brain, corresponding to 2.9 µg/g, allow us to consider that oxygen depletion, especially from the brain, played a significant role in causing the death. This measured value is included in the concentration range of lethal cases where butane was identified as the unique compound in the gaseous mixture and responsible of the death. Moreover, the butane concentration values in the liver (0.43 µg/g), in the lung (2.3 µg/g), and in the kidney (1.2 µg/g) were also within in the concentration range of cases where butane was the only responsible for the death [10]. The highest butane concentrations were found in the brain, lungs, heart and kidney, showing a complete distribution inside the body. The concentrations in fat tissue and blood are lower. As result, this distribution seems to be consistent with a consequent time of gas exposure as already shown with propane [11], rather than a sudden death with limited distribution.

Propane, found at lower concentration than butane both in the container found on the scene and in the biological samples, and benzodiazepines are expected to further depress the activity of the central nervous system, but it is important to emphasize that "the mechanism of sudden death directly related to volatile abuse is seldom clear but suggested to include cardiac arrhythmia, hypoxia, and respiratory depression" [1]. In our case, despite the histological findings in the heart specimens are consistent with VSA induced damage, they cannot be considered enough to affirm that the death was due to cardiac arrhythmia, while there is evidence of cellular asphyxia. More precisely, the generalized hypoxia leading to the cardiac and respiratory arrest was the consequence of the combination of the central and peripheral effects of the volatile gases which, on one side act as neurodepressant agents on the bulbo-pontine respiratory unit while simultaneously substitute the oxygen in the lungs. Nevertheless the possible outbreak of a cardiac arrhythmia, secondary to focal myocardial fibrosis, cannot be excluded, but it was not possible to find any post-mortem sign or information related to a cardiac cause of death such as fibrillation.

It has also to be noted that the quantitative results found in biological samples had poor correspondence with the results from the camping gas stove found on the scene. Indeed, propane was found as the main component whereas the concentrations in biological samples were weaker than butane. This distribution can be explained by a lower hydrophobicity and a higher boiling point of propane compared with butane. Propane could be more rapidly excreted and released from the body. Kinetic gas releases from food matrices sprayed with propane/butane mixtures have already been reported [15]. However, the main source of variation of alkanes concentrations in the biological samples are the interindividual differences and the sniffing conditions (duration of exposure, magnitude of breath, etc.).

Parafilm® M did not guarantee a gas tight closure, and did not avoid leaks. Alkanes measurements of biological gaseous samples must be therefore considered as qualitative. However, it has permitted to link remaining volatile alkanes presence in airways with their quantitative concentrations in the body. If Hamilton syringes are not available and it is not possible to wait, the use of 10 ml plastic disposable syringes wrapped in Parafilm® allows at least the forensic identification of volatile substances. A more effective gas sampling protocol

based on Luer-Lock syringes equipped with three-way valve or introducing volatile compounds into gas-tight sealed vials containing stabilized water was recently published and its use is strongly supported for future cases [16-18].

Conclusions

In this case of a 29-year-old man, found unconscious by his cellmate on the floor of the bathroom of his cell, the forensic pathologist's timely intervention on the scene of crime, the early involvement of the forensic toxicologist and his active participation at autopsy were fundamental for the resolution of the case. The sampling approach adopted by forensic pathologists and forensic toxicologists and the analyses by GC-MS played a key role to obtain the forensic identification of butane and propane in biological samples and meaningful quantitative data, allowing to determine the cause of death. A methodology for the collection of samples has to be followed to perform an efficient analytical strategy of such cases. International consensus on volatile lipophilic substances management is become mandatory. The approach adopted in this case report brings objective and valuable information for this outcome.

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Figure legends

Fig. 1: Camping gas stove found on the floor close to the victim.

Fig. 2: Air withdrawal from the right bronchus with a 10 mL disposable syringe.

Fig. 3: Sections of bronchi containing mucoid material interspersed with air bubbles (figure **a** 2.5x – figure **b** 5x).

Fig. 4: Lung sections showing congestion of the alveolar septa, edema and unstained “blebs” inside the alveolar spaces (10x).

Fig. 5: Chromatogram of the MS signal of the volatile substances from the liver, showing the analytical identification of propane.

Fig. 6: Chromatogram of the MS signal of the volatile substances from the liver, showing the analytical identification of butane.

Table(s) and caption(s)

Table 1: Quantitative results of propane and butane.

Sample	**Propane**	**Butane**
Peripheral blood	1.7 ng/g	130 ng/g
Heart blood	1.7 ng/g	62 ng/g
Vitreous humor	0.5 ng/g	6.9 ng/g
Liver	7.4 ng/g	430 ng/g
Lung	39 ng/g	2.3 µg/g
Heart	30 ng/g	1.6 µg/g
Brain/cerebral cortex	61 ng/g	2.9 µg/g
Fat tissue	7.3 ng/g	322 ng/g
Kidney	16 ng/g	1.2 µg/g
Air from left bronchus (withdrawn with 500 µL Hamilton syringe)	2.5 nmol/ml	21 nmol/ml
Air from emphysematous bubbles (withdrawn with 10 mL syringe)	2.1 nmol/ml	23 nmol/ml
Air from right bronchus (withdrawn with 10 mL syringe)	25 nmol/ml	140 nmol/ml
Gas cylinder	1.1 µmol/ml	6.1 µmol/ml

Figure 1

Figure 2

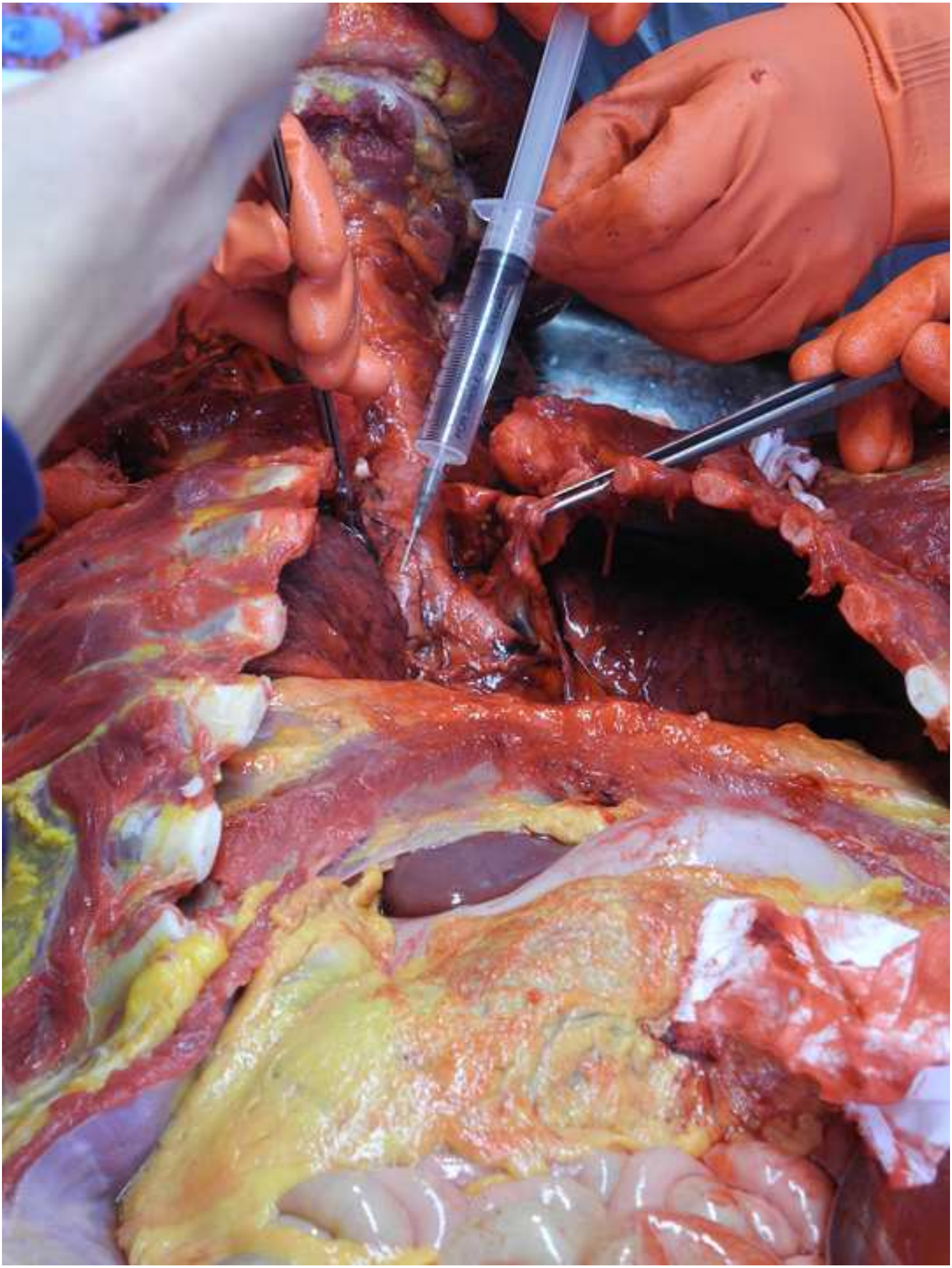
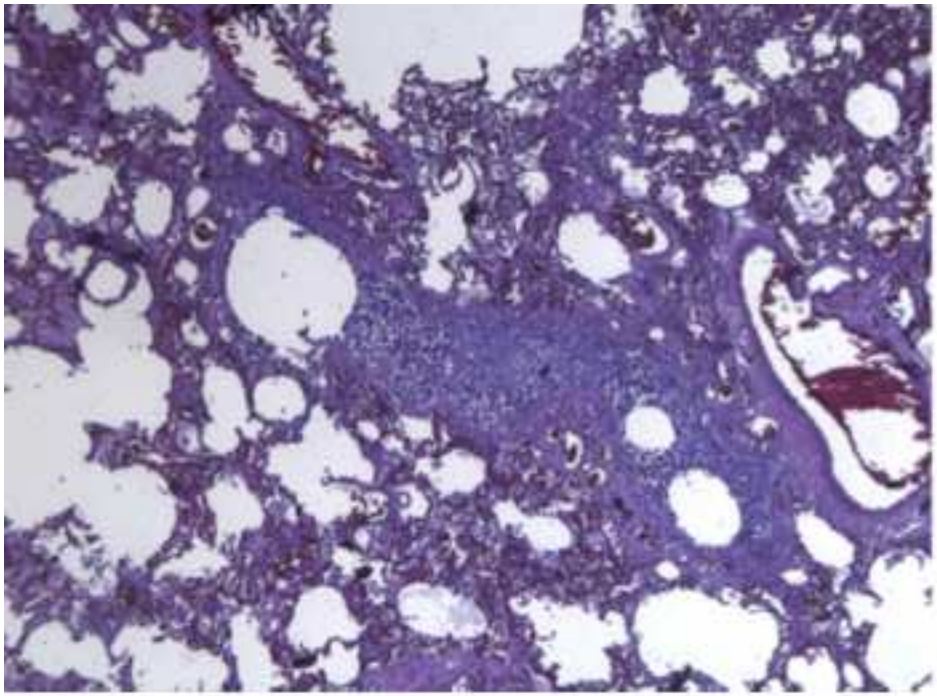


Figure 3



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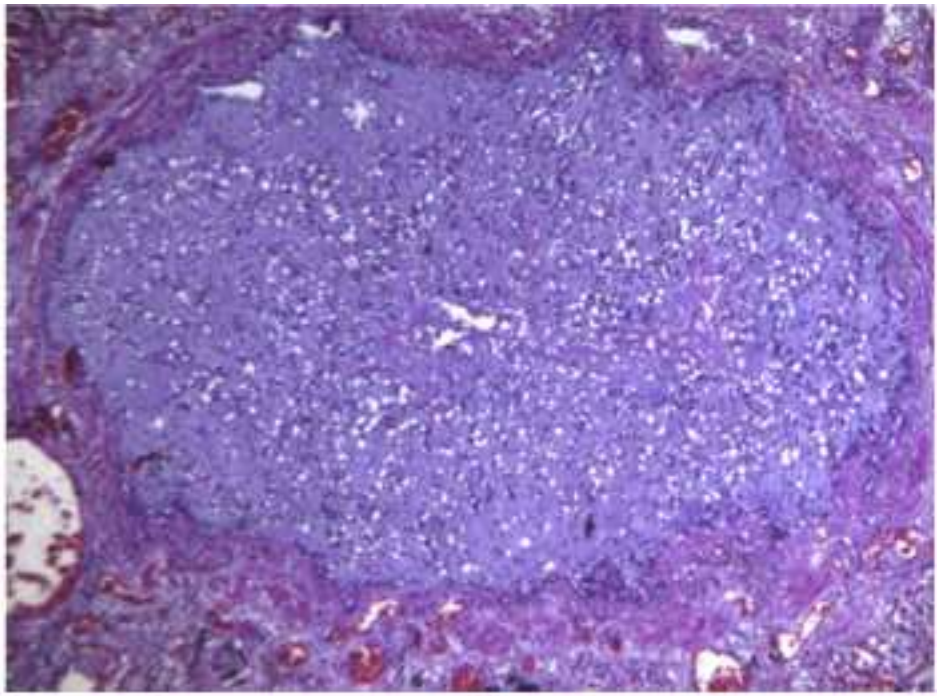

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Figure 4

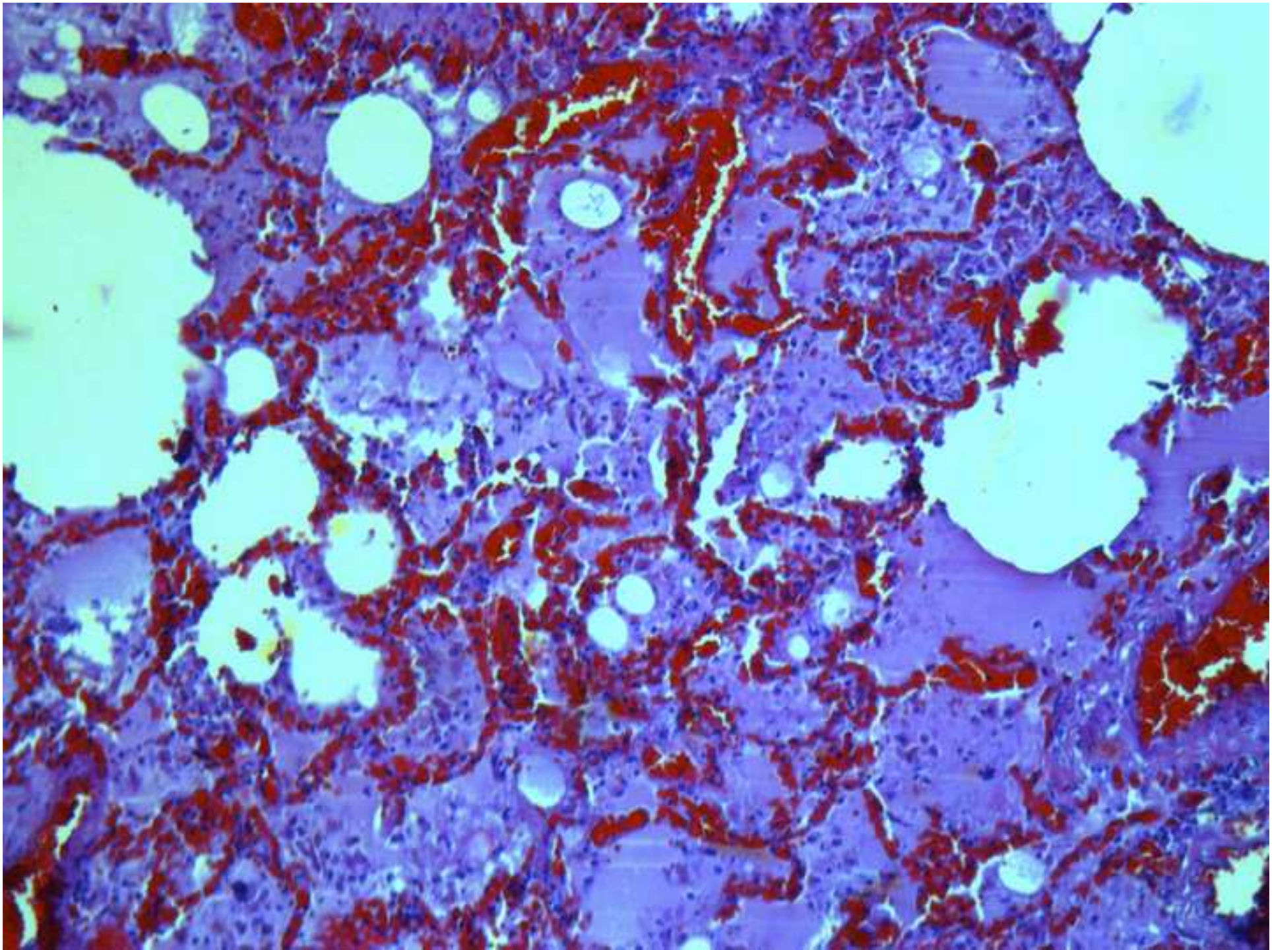

Figure 5

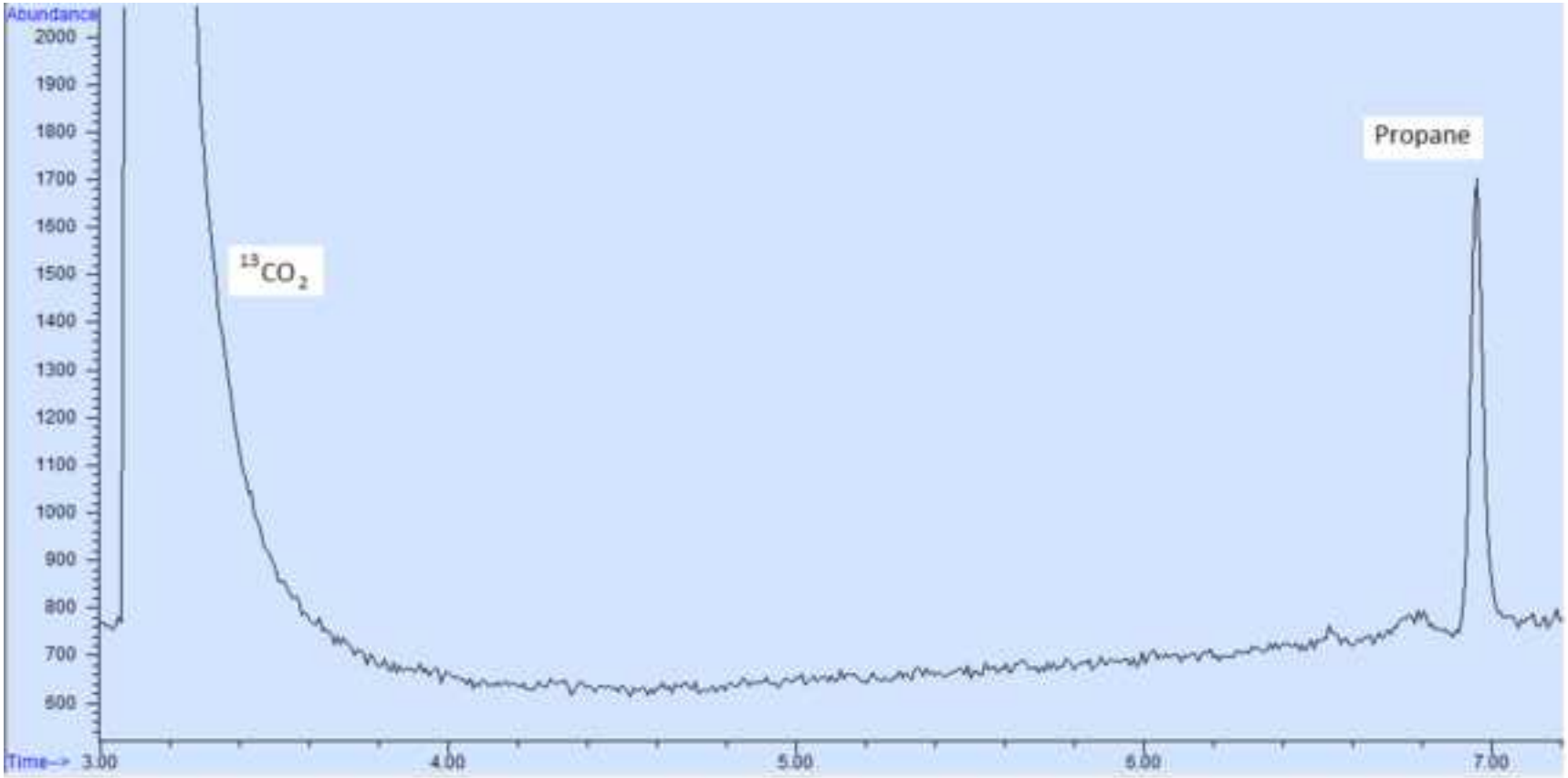
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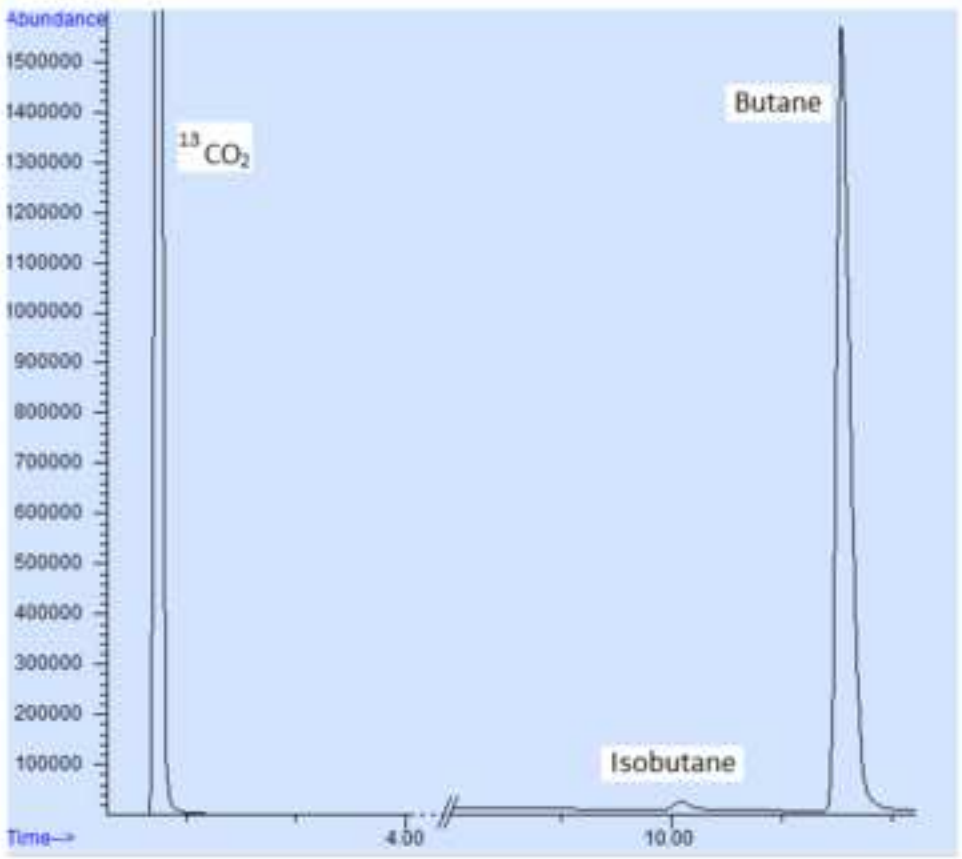
Figure 6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