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TECHNICAL NOTE

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Sudden death after isobutane sniffing: a report of two forensic cases

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Abstract The intentional inhalation of a volatile substance ("sniffing") causing euphoria and hallucinations is an under-recognised form of substance abuse in children and adolescents with a high morbidity and mortality. Sudden death can be caused by cardiac arrhythmia, asphyxia or trauma. Two fatal cases of isobutane sniffing of cigarette lighter refill containing isobutane are reported. Toxicological investigations revealed the presence of isobutane in the heart blood and brain tissue of both cases (case 1: heart blood 0.1 μg/g, brain tissue 2.3 μg/g; case 2: heart blood 4.6 μg/g, brain tissue 17.4 μg/g) and the presence of its metabolite 2-methyl-2-propanol in the heart blood of both cases (0.5 and 1.8 μg/g, respectively). The histological investigations of the inner organs showed similar results in both victims. Autopsy findings, results of the histological and immunohistochemical investigations, toxicological findings and analytical procedures are discussed.

Keywords Isobutane · Inhalant abuse · Forensic toxicology · Sudden death

Introduction

There has been a steady increase in the number of deaths resulting from inhalation of volatile substances [1]. Fatal outcome of inhalant abuse has been discussed due to several mechanisms: suffocation, trauma after dangerous behaviour, vagal inhibition, respiratory depression and the "sudden-sniffing death syndrome" following cardiac arrhythmia [3, 7, 16, 17]. However, the reason of sudden death related to volatile sniffing is rarely clear even after autopsy [18]. In most cases, reported aerosol propellants, *n*-propane or *n*-butane or mixtures of *n*-propane, *n*-butane and isobutane are involved [2, 4, 11, 14, 18]. Reports on fatal cases after the inhalation of isobutane are rare [14]. Also quantitative data on volatile alkanes in blood and brain of such cases are rare until now (propane and butane [20] and propane isobutane and *n*-butane [4, 15]).

In this paper, we report on two cases of sudden death after sniffing of cigarette lighter refill containing solely isobutane (2-methylpropane). As isobutane is metabolized to 2-methyl-2-propanol [19], quantitative determination of isobutane and 2-methyl-2-propanol was performed. Head space–gas chromatography with flame ionization detector (HS-GC FID) was employed. Although other techniques, like HS-GC–mass spectroscopy (MS) [4] or solid-phase micro-extraction coupled to GC-MS [11], have successfully been applied for the determination of lower molecular hydrocarbons, this paper shows that the most important requirements for an accurate analysis are the pre-analytical conditions. A new calibration technique was developed using isobutane-saturated cream. In contrast to other case reports [14, 18], histological and immunohistochemical investigations were carried out, and both cases exhibited very similar pathological changes in the heart muscle and in the lung parenchyma, which could possibly explain the danger of this type of abuse and the lethal outcome.

Case reports

Case 1

A 13-year-old boy collapsed in a supermarket parking place. After buying a cigarette lighter refill containing isobutane, he sniffed the content of the refill using a plastic bag. His friend, who did not sniff, described that he was first staging and laughing. After sniffing a second bag filled with the gas, he collapsed immediately. The friend called an emergency doctor but resuscitation for more than 30 min remained unsuccessful.

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Case 2

A 20-year-old man was found by his father lying dead on the floor of his room. On a table near the deceased, a lighter refill containing isobutane and a plastic bag were placed. Resuscitation was not performed because rigor mortis and livores had already developed.

Autopsy findings

In both cases, petechial conjunctival and facial dermal haemorrhages were found. Further findings in both cases were the following: diffuse, marked, doughy and pale myocardium, oedema of the lungs with acute marginal emphysema and subpleural haemorrhages, oedema of the brain, acute congestion of all inner organs and terminal vomit aspiration. Other pathological changes were not diagnosed. In the first case, the weight of the right lung was 600 g and the left lung was 557 g. In the second case, the weight of the right lung was 980 g and the left lung was 930 g.

HS-GC determination of isobutane and 2-methyl-2-propanol

Determination of isobutane

For the preparation of calibrators, 10 g of cream (fat content of 30%, ultra-high temperature treated) was filled into an injection vial (20 ml; Müller and Müller, Holzminden, Germany) and slowly saturated with isobutane (commercially available cigarette lighter refill cartridge). To avoid cracking of the emulsion, it was important to avoid local freezing of the cream during the saturation procedure. The vial was sealed, gently shaken and opened again to evaporate undissolved isobutane. The isobutane content of the spiked cream was determined by weighing, and it was 1.76 mg/g. This cream was diluted with fresh tap water to final concentrations of 0.35, 0.88, 1.76, 3.52, 8.80, 17.60 and 35.20 μg/g. Fresh tap water also served as blank sample.

For case 1, 0.2 g of the calibrators or samples from autopsy was filled into injection vials. For case 2, individual calibration curves were recorded for each sample, according to the sample amount taken during autopsy. The calibrators were always measured in duplicate. No internal standard (ISTD) was used to avoid reopening of the samples which were filled directly into the injection vials during the autopsy. The sealed vials were heated up to 60°C for 15 min. The injection from the HS into the GC system was carried out by pressure generation in the sample vial.

A PerkinElmer F 45 HS-GC consisting of an HS autosampler (60°C), a packed column (2 m×1/4 in., packed with 0.2% Carbowax 1500 on Carbopack 80/100 mesh, Supelco, Bellefonte, PA, USA) and an FID was used. The needle temperature was 150°C, the injector temperature was 130°C, the GC oven was 100°C isotherm and the detector temperature was 250°C. The total runtime for one chromatogram was 10 min. The system was aligned with a PE Nelson 900 series interface. Data evaluation was performed using PerkinElmer Turbochrom Workstation Version 6.1.2.0.1. Calibration curves were generated using a least-squares linear regression and found to be linear over

Table 1 Concentrations of isobutane and its metabolite 2-methyl-2-propanol

	Case 1		Case 2	
	Isobutane (μg/g)	2-Methyl-2-propanol (μg/g)	Isobutane (μg/g)	2-Methyl-2-propanol (μg/g)
Heart blood	~0.1*	0.5	4.6	1.8
Brain tissue	2.3	ND	17.4	ND
Lung tissue	ND	ND	0.8	ND

ND Not detectable
*below LOQ

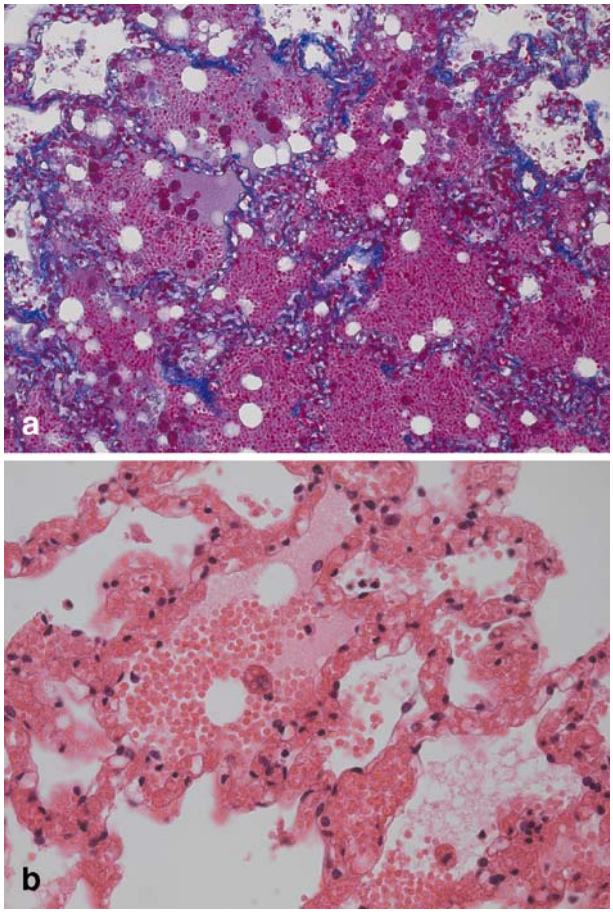

Fig. 1 Lungs: haemorrhagic intra-alveolar oedema with activation of macrophages and subtotal anaemia of the alveolar walls with abundant unstained "blebs". **a** case 1, azan, ×200; **b** case 2, H & E, ×200

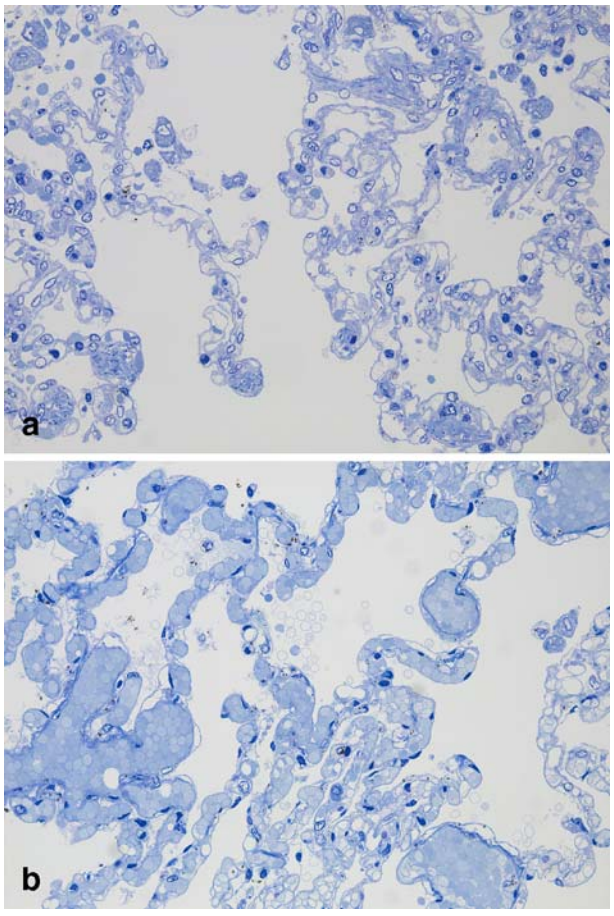

Fig. 2 Lungs: sectional total anaemia of the microcirculation. Formation of multiple intra-capillary and endothelial "blebs" with total obstruction of the capillary lumen. **a** case 1, semi-thin slice, toluidine blue, ×400. Sectional total anaemia of the alveolar walls beside a massive congestion of the microcirculation. **b** case 2, semi-thin slice, toluidine blue, ×400

the range investigated. The limit of detection (LOD) was estimated to be 0.1 μg/g; the limit of quantitation (LOQ) was 0.4 μg/g. For the results of the cases, see Table 1.

Determination of 2-methyl-2-propanol

Two hundred sixty microliters of 2-methyl-2-propanol (Merck, Darmstadt, Germany) was added to 100 ml of fresh tap water to come up with a stock solution of 2 mg/g. From this stock solution, calibrators were prepared by dilution with fresh tap water at 0.25, 0.5, 1.0, 2.5 and 5.0 μg/g. Fresh tap water served also as blank sample. As ISTD, an aqueous solution of 2-pentanol (Merck) was diluted with fresh tap water to come up with a concentration of 120 μg/g. Aqueous calibrators (0.5 g) or biological samples from autopsy were filled into the injection vials. After addition of 50 μl ISTD solution, the vials were sealed. The injection from the HS into the GC system was carried out by pressure generation in the sample vial. A PerkinElmer Autosystem XL GC (PE WAX column, 60 m×0.53 mm, isotherm 80°C, injector temperature 120°C, FID 250°C) equipped with HS 40 HS sampler (sample temperature 60°C, needle temperature 65°C, transfer line 110°C) was used. The system was aligned with a PE Nelson 900 series interface. Data evaluation was performed using PerkinElmer Turbochrom Workstation Version 6.1.2.0.1. The calibration curve was generated using a least-squares linear regression and found to be linear over the range investigated. The LOD was estimated to be 0.1 μg/g; the LOQ was 0.25 μg/g. For the results of the cases, see Table 1.

Further analysis

Blood alcohol (femoral blood), analysed using HS-GC, was not detectable in both cases. Immunological drug screening (urine) was performed with micro-plate enzyme immunoassay (Bio-Rad) and was negative for amphetamine, methamphetamine, cannabinoids, cocaine (benzoylecgonine) and opiates in both cases.

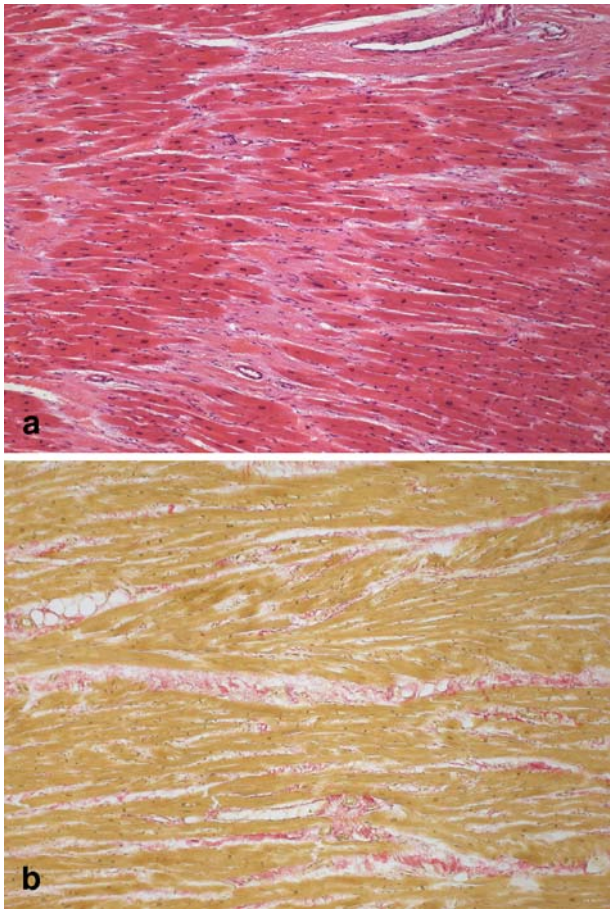

Fig. 3 Myocardium: diffuse myocardial fibrosis. **a** case 1, H & E, ×100; **b** case 2, sirius red, ×100

Histology

Lungs In both cases, areas with an intra-alveolar haemorrhagic oedema with activated macrophages and erythrophagia were spread over wide areas of empty alveoli (Fig. 1a,b). A vesicularization of the capillary endothelia in the alveolar walls—distributed over the whole-section plane—causing in sections a total anaemia of the alveolar walls was remarkable (Fig. 2a,b). In contrast to case 1, in case 2, beside a sectional alveolar wall anaemia, there existed a massive congestion of the alveolar wall capillaries (Fig. 2b).

Myocardium Diffuse interstitial fibrosis of the myocardium showing a negative Berlin blue reaction was accompanied by an inconspicuous intramural coronary arterial system (Fig. 3a,b). In contrast to case 1, in case 2, the development of fibrosis was less intensive. Fresh necroses of single or groups of myocardial fibres could be demonstrated by immunohistochemistry in both cases using the markers fibronectin (Fig. 4a,b) and troponin C (Fig. 5a,b) according to Ortmann et al. [10].

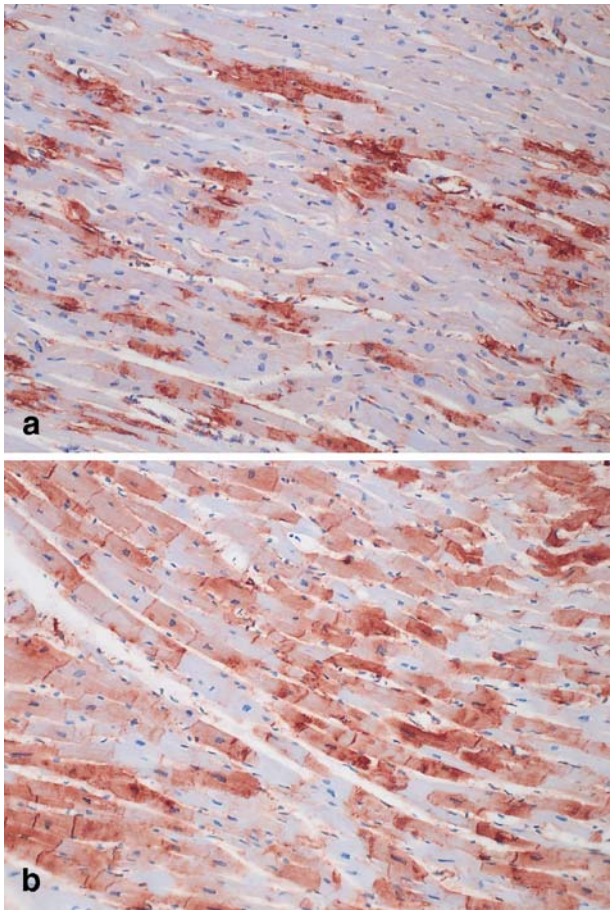

Fig. 4 Immunohistochemical investigation of the myocardium using the marker fibronectin: fresh myocardial fibre necroses demonstrated by intra-sarcolemmal accumulation of fibronectin. **a** case 1, ×200; **b** case 2, ×200)

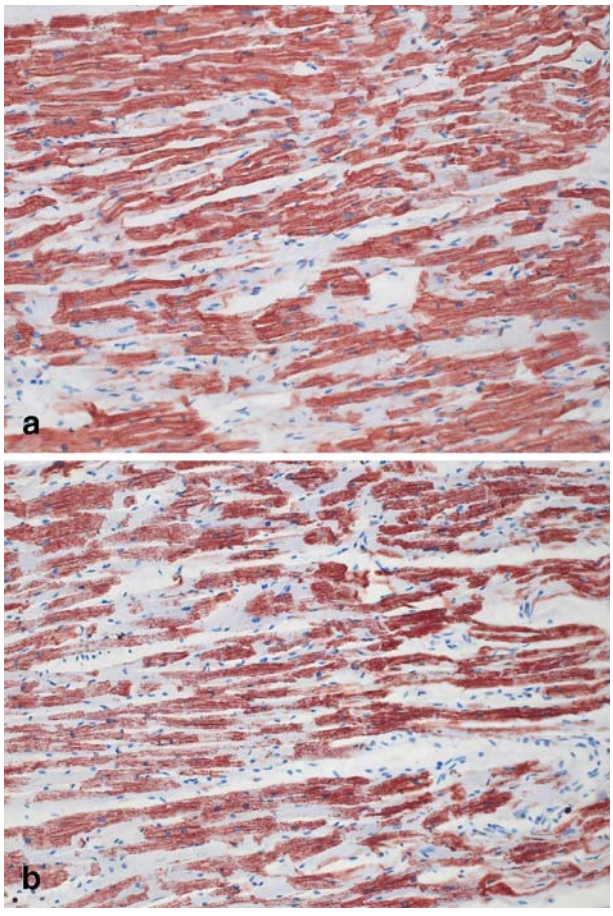

Fig. 5 Immunohistochemical investigation of the myocardium using the marker troponin C: fresh necroses of single myocardiocytes demonstrated by the intracellular loss of the cardiac antigen troponin C. **a** case 1, ×200; **b** case 2, ×200)

Discussion

The HS-GC analysis revealed the inhalation of isobutane in both cases. There were no signs of the inhalation of *n*-propane or *n*-butane. The concentrations of isobutane in case 1 are distinctly lower than in case 2. One reason may be that in case 1, resuscitation efforts were undertaken for 30 min. In case 2, the man was obviously dead when he was found. Resuscitation may have caused a significant decrease in isobutane concentrations, predominantly in the lung and blood. Another reason may be that in case 2, the samples for HS-GC analysis were filled into the injection vials already during the autopsy. The vials were sealed directly and analysed without reopening them. For this procedure, it is important to use clean and pre-weighted vials and to fill them with an appropriate sample amount. For the method described in this paper, about 0.2 to 0.5 g of blood, brain tissue or lung tissue is optimal. For the accurate determination of isobutane concentration, an individual calibration curve was recorded for each sample amount, e.g. for 0.25 g brain tissue, a calibration curve was recorded using 0.25 g of diluted cream calibrators. For

calibration purpose, the saturation of cream with isobutane was advantageous in comparison with the saturation of isobutane-free blood samples, because the latter did not result in measurable weight differences of the blood samples in pre- and post-saturation. This is obviously due to the higher fat content of cream and the lipophilic properties of isobutane.

If intoxication with isobutane is not known at the time of autopsy and no appropriate samples were taken in sealed vials, in our opinion, the brain tissue is the sample of choice for a subsequent detection of the gas. As 2-methyl-2-propanol is less volatile than the parent substance, the pre-analytical conditions play a minor role in the determination of the isobutane metabolite.

The mechanism of the sudden death during or after volatile substance sniffing is still a matter of debate. Specific post-mortem features, either macroscopically or microscopically, have not yet been identified [17]. Furthermore, the mechanism and the kind of cell damage after isobutane sniffing so far have not yet been described. Our intention was to find out if there exist typical histomorphological changes in inner organs after sudden death due to butane sniffing, which could allow us to draw conclusions as to the mechanism of cell damage. Therefore, intensive histological and immunohistochemical investigations were performed. In both cases, a distinct fibrosis of the myocardium was found. Myocardial fibrosis is non-characteristic for young individuals and seems to be induced by chronic sniffing since no sclerosis of the coronary arteries and no signs of myocarditis were found. In both cases, immunohistochemical investigations using fibronectin and troponin C as markers of early ischemic myocardial damage [10] showed an intra-sarcolemmal accumulation of fibronectin and intracellular loss of the cardiac antigen troponin C in isolated myocardial fibres or groups of fibres. Since the acute and chronic myocardial changes were the same in both cases, with differences in their intensity only, the same mechanism seems to be responsible. Sudden-sniffing death was originally described by Bass [3]. The hydrocarbons of inhalants are suspected to sensitize the myocardium to adrenaline, and the sudden surge of this hormone produced by a startle reflex is thought to result in cardiac arrhythmia. However, usually at autopsy, no cause of death had been found [3]. In our two cases, there existed no clue for a startle. Therefore, the mechanism of death has to be discussed. The alveolar wall capillaries showed changes similar to the typical drowning lungs [5] with vesicular transformation of capillary endothelia and development of an obstructive microangiopathy. These changes could be due to the direct toxic effect of the inhalant. As a consequence of the mechanical barrier in the lung circulation, anaemia may develop in the myocardium combined with congestion in the greater circulation. Autopsy findings (petechial haemorrhages and acute congestion of the inner organs) and histomorphological changes in the myocardium could be explained this way. Another explanation for the development of the "blebs" in the alveolar wall capillaries could be the inclusion of the isobutane gas within the capillaries blocking the microcirculation. From lipophilic narcotic agents, we know that they can cause damage of basal membranes in neuronal cells due to the incorporation of the agent into the lipid layer, which may result in swelling of the basal membrane cells. The same mechanism could be assumed for isobutane due to its lipophilicity. Since after isobutane sniffing, the agent first has to pass the alveoli; a similar mechanism of damage of the basal membranes in the alveolar walls could be proposed. This would explain the especial lung histology (Fig. 2a,b). However, here, we investigated two cases only. To be sure for the pathomechanism of sudden death after butane sniffing, similar forensic cases should be centralised retrospectively as well as prospectively and investigated under standardised conditions. In conclusion, in agreement with the literature, e.g. [6, 8, 9, 12, 13], these cases demonstrate again the importance of histological and immunohistochemical investigations in forensic casework.

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