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Quantitative determination of *n*-butane metabolites in three cases of butane sniffing death

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ABSTRACT

Butane is an addictive volatile substance like toluene. We report three forensic autopsy cases of sudden death that occurred while sniffing *n*-butane and isobutane from portable gas cartridges. *n*-Butane and isobutane were detected in all three cases. In cases 1–3, *n*-butane concentrations in heart blood were 54.3, 25.5, and 30.7 μg/mL, respectively. These concentrations were considered fatal according to the previous reports. In addition, *n*-butane metabolites (2-butanol and 2-butanone) were detected in cases 1 and 3 but not in case 2. Blood levels of 2-butanol and 2-butanone were 6.5 and 1.8 μg/mL, respectively, in case 1, and 6.3 and 5.6 μg/mL, respectively, in case 3. According to the police investigation, the decedent in case 1 had misused butane gas for more than 6 months in the period leading up to death. The decedent in case 3 also had a history of chronic misuse of butane gas. There was no history of chronic misuse of butane gas by the decedent in case 2. It was suspected that he attempted suicide via inhalation of butane gas using a plastic bag, leading to a rapid death. The presence or absence of *n*-butane metabolites might reflect the way of butane inhalation, such as the frequency and duration. Although additional experimental and case studies are necessary to establish the forensic applications of *n*-butane metabolite detection, it may be a useful method to understand the decedents' pattern of butane sniffing before death.

1. Introduction

Volatile substance misuse, especially among the youth, is an alarming social problem in many countries [1]. It is reported that among the misused volatile substances, butane misuse has the highest fatality rate [2]. In the UK, 211 fatalities from volatile substance misuse were reported from 2003 to 2007, and most of these deaths were associated with misuse of gas fuels such as butane [3]. Butane gas is found in cigarette lighter refills and in gas cartridges for use in portable gas stoves; thus, it is easily obtained. There are no data on the prevalence of butane gas misuse in Japan. However, the number of fatalities due to butane inhalation has been reported to be about 10 persons per year since 2000 [4]. Considering that the number of fatalities due to toluene inhalation was about 15 persons per year, butane misuse is also clearly of grave concern for public health.

Metabolism of *n*-butane has been studied in animals [5]. The main metabolites of *n*-butane are 2-butanol and 2-butanone. Walker et al. [6] reported that these metabolites were detected in a blood sample from an individual who had died due to butane misuse. However, no quantitative data are available on the concentrations of butane metabolites in individuals who died due to butane misuse. Here, we report three forensic autopsy cases of sudden death that occurred while sniffing butane gas. Blood and tissue concentrations of *n*-butane and isobutane, as well as concentrations of the metabolites of *n*-butane (2-butanol and 2-butanone), were determined. We discuss the importance of metabolite detection to understand the way of butane sniffing before death.

2. Materials and methods

2.1. Case history and autopsy findings (Table 1)

2.1.1. Case 1

An 18-year-old woman was found dead in her room. The previous night, she had consumed alcohol and inhaled gases from a gas cartridge used for a portable gas stove, which contained *n*-butane

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Table 1
Case summaries of the three cases.

Case no.	1	2	3
Age (years)	18	28	34
Gender	F	M	M
Previous history of drug misuse	Toluene, butane	Toluene,[a] butane[a]	Toluene, butane
History of psychiatric disease	Depression	Depression	Not recorded
Resuscitation	No	No	No
Postmortem interval	12 h	1.5 days	3 days
Ethanol in blood	0.08%	0.12%	0.00%
Detected drugs by GC–MS screening	Phenobarbital, diazepam, clotiazepam	No drugs	No drugs

[a] No known misuse for 10 years.

and isobutane. She had misused butane and toluene for several years before the fatal accident and had consulted a mental hospital to rehabilitate the misuse. A forensic autopsy was performed about 12 h after her death. External examination showed no injuries. Edema of the brain and lungs was noted. Ethanol concentration in femoral vein blood was 0.08%, and toluene was not detected. Blood drug screening by gas chromatography–mass spectrometry (GC–MS) detected phenobarbital, diazepam, and clotiazepam, all of which were below therapeutic levels.

2.1.2. Case 2

A 28-year-old man who was suffering from depression along with suicidal thoughts was found dead in his house. His face was covered in a plastic bag, and butane cartridges were found next to the body. He told his friends that he had misused butane and thinner ten years ago, but that he did not misuse them recently. A forensic autopsy was performed about 1.5 days after his death. External examination showed no injuries. Edema of the lungs was remarkable, and an excessive amount of frothy liquid was found in the mucosa of the bronchi. Ethanol concentration in femoral vein blood was 0.12%. Screening by GC–MS detected no drugs in the blood.

2.1.3. Case 3

A 34-year-old man who had been a toluene addict was found dead in his house. Many empty butane cartridges were scattered across his room. An autopsy was performed the following day, and the postmortem interval was estimated to be about 3 days. The body had turned slightly putrid, and edema of the lungs was noted. There were no specific findings in the other organs, except for slight putrefaction. Ethanol, toluene, and other drugs were not detected in the blood.

2.2. Toxicological analysis of volatile substances

2.2.1. Chemicals

Standard gas containing 1% *n*-butane and isobutane was purchased from GL Sciences Inc., Tokyo, Japan. All solvents and chemicals were of analytical grade and were procured through local suppliers.

2.2.2. Screening of volatile substances by GC–MS

We screened for volatile substances in the decedents' heart blood by GC–MS. In all three cases, blood was collected in glass vials, and tissue samples were collected in thick plastic bags. These were sealed and immediately stored at −30 °C until toxicological analysis. In each case, 1 mL of defrosted blood and 1 mL of water were sealed in a glass vial with a silicon septum. The vial was warmed at 60 °C on an aluminum block heater (Sibata Scientific Technology Ltd., Saitama, Japan) for 15 min. Aliquots (200 μL) of the headspace gas were collected in a 1-mL gastight glass syringe and injected into GCMS-QP2010/Parvum2 (Shimadzu Corporation, Kyoto, Japan), with a DB-5ms capillary column (30 m × 0.25 mm I.D., 250 nm film thickness, J&W Scientific, Folsom, CA). Helium was used as the carrier gas, and the instrument was operated in constant pressure mode. The temperature of the interface was set at 250 °C. The oven temperature was held at 35 °C for 2 min and then increased to 200 °C at a rate of 10 °C/min. The injector temperature was set at 200 °C. The mass spectrometer was operated in electron ionization (EI) mode with an electron energy of 70 eV. Analysis was performed in scan mode (*m*/*z* 40–200).

2.2.3. Quantitative analysis of n-butane and isobutane by gas chromatography (GC)

We used GC to perform quantitative analysis of *n*-butane and isobutane in heart blood and tissue samples. For each case, 1 mL of defrosted heart blood, or 1 g of sliced frozen tissue and 0.5 mL of 0.04% *tert*-butanol as an internal standard, was sealed in a glass vial with a silicon septum. A preliminary analysis was performed to estimate the butane levels of samples, and calibration curves that spanned the highest concentration of each sample were prepared. A calibration curve from 0 to 225 μg/mL was made for case 1, and from 0 to 50 μg/mL for cases 2 and 3.

For quantitative calibrations, standard gas was transferred into a glass vial by the water displacement method [7]. The exact volume of diluted standard gas was diluted to an appropriate concentration using a gastight syringe and a glass vial. Using gastight syringes, exact volumes of standard gas were transferred into sealed vials containing 1 mL of blank blood. Each sample and calibration vial was prepared in triplicate. For each, the weights of *n*-butane and isobutane were calculated using Avogadro's law.

Headspace GC was performed using a Shimadzu GC-17A equipped with a flame ionization detector (FID, Shimadzu Corporation, Kyoto, Japan) and a PerkinElmer HS-40 headspace sampler (PerkinElmer Japan Co., Ltd., Yokohama, Japan). A Supel-Q™ PLOT capillary column (30 m × 0.32 mm I. D., 250 nm film thickness) was used. The oven temperature was increased from 35 °C to 250 °C at a rate of 16 °C/min. The injection port temperature was set at 110 °C, and the detector temperature was set at 250 °C. Helium was used as the carrier gas at constant pressure mode (50 kPa). The sample heating temperature was 80 °C, the needle temperature was 100 °C, and the transfer temperature was 110 °C.

2.2.4. Quantitative analysis of 2-butanol and 2-butanone by GC–MS

We performed quantitative analysis of 2-butanol and 2-butanone in heart blood and tissue samples. Samples were prepared for GC–MS using the same method as that used for quantitative analysis of volatile substances, except that this time, 0.004% *tert*-butanol was used as an internal standard. For calibration, 1 mL of blood containing 2-butanol and 2-butanone (1.25–10 μg/mL) was used. GC–MS conditions were the same as those used for screening the volatile substances. Analysis was performed in scan mode. The quantitative monitoring ions of 2-butanol, 2-butanone, and *tert*-butanol were m/z 45, 43 and 59, respectively.