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Title

Postmortem diffusion of *n*-butane and *i*-butane used for anticontagious plugging spray

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Abstract

Blood and tissue samples from a forensic autopsy of a man in his late 60s, who developed dementia and died of multiple head traumas due to a fall from a moving vehicle, contained certain amounts of n-butane and i-butane. The concentration of n-butane was in the range of 0.48 – 70.5 µL/g, which would be considered as toxic or lethal levels. We had to distinguish whether the cause of his unexplained behavior was due to his pre-existing condition (dementia), or from a confused state induced by butane abuse. No traces of butane use were found at the scene. Police investigation revealed that a propellant used in an anticontagious plugging spray had been administered to him during a postmortem treatment in the emergency hospital. In order to prove the postmortem butane diffusion had resulted from the spray administration and to estimate the diffused concentration, experimental simulation was conducted by using rats. As a result of postmortem treatment with the spray, n-butane at concentrations of 0.54 – 15.5 µL/mL or g were found in the rat blood and tissues. In this case, we provided further evidence that the postmortem butane diffusion, caused by using the anticontagious

plugging spray containing butane gas as a propellant administered to a cadaver during a

postmortem procedure prior to forensic autopsy, should be distinguished from cases of

actual butane poisoning.

1. Introduction

Butanes refers to the two isomers of butane, *n*-butane and *i*-butane, which are highly flammable, colorless gases having an unpleasant smell, and which are easily liquefied. These petroleum gases are commonly utilized as lighter fluid, canned fuel, aerosol propellant and so forth. In recent years, unintended butane poisoning has become a major concern due to its general diversity of uses and its easy accessibility. In addition, some people, mainly in the young generation, inhale these vaporized butanes from a plastic bag to get 'high' as an alternative to paint-thinner or the other organic solvents which are controlled by laws, such as the Poisonous and Deleterious Substances Control Act, in Japan. Inhalation of butanes causes fatal arrhythmia, respiratory depression, asphyxia, and vagal collapse possibly followed by sudden death. There are several reports of sudden death from intentional butane inhalation, and the phenomenon is called as Sudden Sniffing Death Syndrome (SSDS) [1–9]. Even if it does not result in death, users are likely to suffer from damage to neural systems and other harmful conditions in which compete recover is less likely [10–12].

The purpose of this study is to verify the conclusion of an autopsy where lethal concentrations of butanes was detected in the blood and other body tissues, in spite of no traces of butane use before a fatal traffic accident. According to the police

investigation, the body was suspected to have been contaminated with butanes from an anticontagious plugging spray used in postmortem treatment at the emergency hospital prior to forensic autopsy. Classically, the method of plugging cottons has been used to prevent body fluids leaking from the mouth, ears, nose and anus. However, it is becoming common to use superabsorbent polymers as substitute for cotton in postmortem treatments in many hospitals. There are two kinds of plugs with superabsorbent polymers in Japan: one is the applicator-type and the other is the spray-type. In the applicator-type plugging kit, the polymer slurry is prefilled in two different plastic syringes which are used for the pharynges and anus, respectively. In the spray-type plugging kit, the polymer is filled in a spray-can fitted with a plastic nozzle-tube to facilitate propellant delivery at and into various locations. The spray-type plugging kit was used in this case, and we investigated if the detected butanes were contaminants derived from spray propellant. In order to confirm postmortem diffusion from butanes administered to the cadaver after death, we performed an *in vivo* experiment using rats by simulating the conditions of the postmortem treatment in the hospital before autopsy, and compared the tissue distribution and concentration of butanes between the autopsy case and the   experimental simulation.

2. Case history

A Japanese man in his late 60s, 164 cm in height and 46.5 kg in weight, suddenly jumped from the backseat of a running vehicle moving at a speed of 60 km/hr, driven by his brother. He died on arrival by ambulance at a hospital. The deceased was known to have developed severe dementia two years earlier.

A forensic autopsy was performed two days after the time of death reported in the emergency hospital. The autopsy revealed multiple head injuries, such as abrasions and bruises of head skin, subcutaneous bleeding, hemorrhages on bilateral temporal muscles, calvarial bone fracture and transverse basilar fracture, subarachinoid hemorrhage, and cerebral contusion. There were slight abrasions and bruises on the face and four limbs. In addition, traces of superabsorbent polymer were found extending from the pharynges to the esophagus/trachea, and through the external acoustic meatus, as well as at the basal brain (Fig. 1). There was no evidence of lethal disease.

3. Materials and Methods

3.1. Chemicals and animals

Standard gasses, such as 9.08% *n*-butane, 5.97% *i*-butane, and 99.5% propane, were purchased from GL Sciences (Tokyo, Japan). Other chemicals used were of the highest

quality commercially available.

Male Wistar/ST rats (8 weeks of age) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animal studies were approved by the Animal Laboratory for Medical Research, Center for Advanced Research and Education, Asahikawa Medical University, and the animals were handled according to the institutional guidelines and regulations.

3.2. Sample preparation

A fluid sample (0.5 mL) and 0.5 g of thin sliced frozen tissue respectively from a cadaver and rats were placed in separate 20-mL GC vials before adding 0.5 mL of 0.05% acetonitrile to each vial as an internal standard. The vials were tightly sealed and subjected to GC analysis. Standard curves were plotted for the authentic gasses, 9.08% *n*-butane and 5.97% *i*-butane in duplicate. The gasses were injected by an electric microsyringe into a tight-sealed vial to which control blood and internal standard samples were previously added. The injection volumes were set as 0, 40, 60, 80, 100 μL to make the final contents 0, 7.26, 10.90, 14.53, 18.16 μL/mL for *n*-butane, 0, 4.78, 7.16, 9.55, 11.94 μL/mL for *i*-butane, respectively.

3.3. GC/FID and GC/MS conditions for butanes determination

Analyses of butanes were also performed by headspace GC under the following conditions: *GC System*, column: SUPEL-Q PLOT 30 m x 0.32 mm i.d., SPELCO; carrier: He, 1 mL/min; oven: 60°C to 150°C at 10°C/min then hold 1 min; injection: volatiles interface, 150°C, split 5: 1; detector temperature: 250°C; *headspace sampler*, loop size: 1 mL; oven temperature: 55°C, loop temperature: 70°C; transfer line temperature: 80°C; vial pressure: 10.2; equilibration time: 15 min.

Identification of butanes was performed using GC/MS (Shimadzu GC/MS-QP2010 Ultra) under the following conditions: *GC System*, column: DB-5MS 30 m x 0.25 mm i.d., J&W; carrier: He, 0.47 mL/min; oven: 40°C (6 min); injection: volatiles interface, 150°C, split 15: 1; detector temperature: 200°C.

3.4. Animal Experiment

Four rats were euthanized by dry ice-derived carbon dioxide gas before being sprayed with postmortem plugging in the mouth, ears and anus in the same manner as the hospital postmortem treatment to the corpse. In order to simulate the actual conditions, carcasses were placed in a plastic bag and allowed to stand for two days in a refrigerator before dissection. Tissues and blood from abdominal aorta were carefully

isolated and immediately frozen at –80°C until examination.

4. Results

As is routine in forensic autopsy, we determined the blood concentrations of ethanol, n-propanol and acetone using gas chromatography (GC), and we unexpectedly found high blood levels of n-butane and i-butane (Fig. 2). In an effort to confirm whether the butanes detected were contaminants from the spray, the GC settings were optimized and a quantitative determination was performed using the plug-samples (Fig. 3). Butanes and propane were detected in the spray-type plug, while the applicator-type plug did not contain major volatile ingredients. The ratios of n-butane to i-butane, and n-butane to propane detected in the spray approximated well to those detected in blood samples.

Standard curves, obtained by spiking standard gasses in a tight-sealed vial to which control blood and internal standard were previously added, were linear in the range of 0-18.16 µL/mL (y=0.1531x-0.0065) and 0-11.94 µL/mL (y=0.1611x+0.0393) with relevant correlation coefficients of r=0.997 and 0.996 for n-butane and i-butane, respectively. A quantitative determination of propane was not attempted, because only high concentrations of propane could be obtained, and exact dilution of the gas was

considered impractical. All samples were analyzed on the same day which is standard

curves were obtained.

Blood and tissue concentrations of butanes in autopsy of the deceased (Table 1) showed the blood concentration of n-butane was 4.14 µL/mL in blood collected from the descending aorta, and 3.39 µL/mL in blood from the right heart. These values were in the range of 0.11-15.3 µL/mL: i.e, levels reported in previous cases of butane abuse (Table 2).

In order to simulate the postmortem butane diffusion and to estimate the diffused concentration in the victim's body, a model experiment was conducted using rats. The measured blood concentration of n-butane in rat was 6.97 µL/mL (Table 3). This value was close to the concentrations in case of autopsy, and blood collected from the descending aorta (4.14 µL/mL) and the right heart (3.39 µL/mL). Tissue concentrations detected ranged from 0.54 to 15.5 µL/g (Table 3), and distributions were similar to that of the autopsy samples, although concentrations in some tissues were different. The amounts of detected i-butane were around a half of n-butane level as was the case with autopsy.

5. Discussion

A drug and poison screening procedure is necessary to determine the cause of death in a forensic autopsy. We perform the tests for alcohol by GC/FID, cyanide and paraquat by indicator tubes, drugs of abuse by means of immunoassay kits, and target- and non-target-screening of forensic toxicology related drugs and poisons by LC-MS/MS as routine processes as far as possible. In this case, using the routine alcohol test, we unexpectedly detected a considerable amount (to a level considered toxic or lethal), of butane from a patient died of multiple traumas in a traffic accident (Table 1).

Police investigation revealed a propellant used in an anticontagious plugging spray had been administered to him during a postmortem treatment in the emergency hospital. In order to prove the postmortem butane diffusion was from the spray and to estimate the diffused concentration, an experimental case simulation was conducted by using rats. Based on the results, similar tissue distributions of butanes were observed in both the autopsy and experimental cases. Considering the relative concentration vs blood concentration, the brain and heart levels showed more than 20-fold difference in the two cases. The brain concentrations were obviously low compared to other tissues in rat. This may be because the rats had neither blood flow nor basilar fractures. And the reason why the brain concentration in autopsy case was not low like rat case is because

the polymer had penetrated the skull from a basilar fracture, and butane gas had direct contact with the brain itself. The substantial concentration difference in the heart was probably caused by diffusion from the polymer in the esophagus (Fig. 1). The diffusion must have made the heart concentration much higher than other tissues in the autopsy case.

According to relevant literature [8], components of the gas samples include: refill can for oven (71% n-butane, 28% isobutane, and 1% propane), a lighter refill (54% n-butane, 20% isobutane, and 26% propane), and LPG (97.8% propane, 1.5% isobutane, 0.1% n-butane, 0.2% propylene, and 0.4% other gases). The canned fuel and lighter fuel are used for butane abuse especially. The propellant composition we analyzed in this study corresponded approximately to that of the contaminants in the autopsy blood sample (Table 4). We could conclude that the reason the victim jumped from the moving car was not butane abuse but a consequence of his dementia. We might be able to identify the origin of butane gas if a database of the relevant composition difference was constructed. However, the gas composition/concentration in the experimental rat blood resembled more of lighter fuel. We cannot fully explain this discrepancy and yet it is to be noted that detectable gasses would vary depending on body fat contents and storage circumstances such as duration, temperature, sealing, etc.

Conclusions

We were presented with an autopsy case with detectable butanes in his blood even though the deceased had no contact with butanes while he was alive. Mortalities attributable to SSDS are occasionally associated with butane abuse. Determining the cause of death may be more efficient and straightforward if evidence of canned fuel or lighter fluid is known at the scene. Otherwise, medical experts in legal medicine must be careful when considering butanes detected in cadavers, because postmortem procedure may confuse determination of the cause of death. In fact, a body treated with spray-type plugging might have actually died of butane abuse. This report describing a rare case where a lethal concentration of a chemical compound was found to have contaminated a corpse after death due to postmortem exposure to the chemical.

Conflict of Interest

The authors report no conflict of interest and declare no financial and personal relationships that could inappropriately influence the submitted manuscript.

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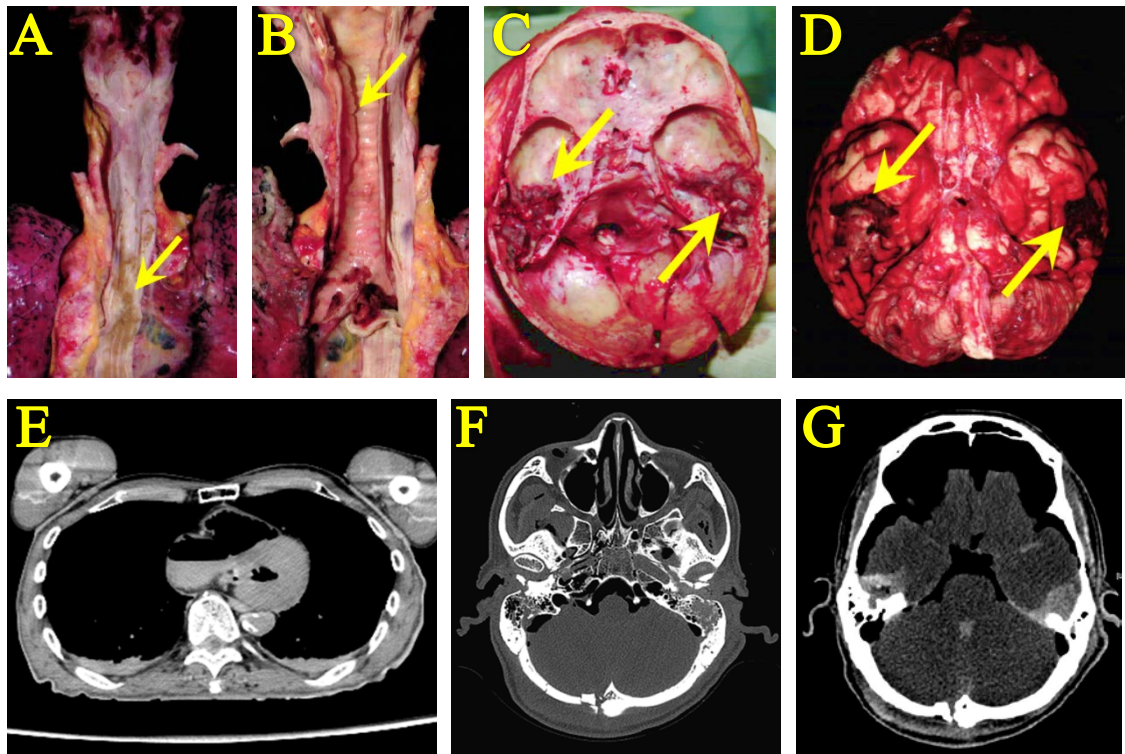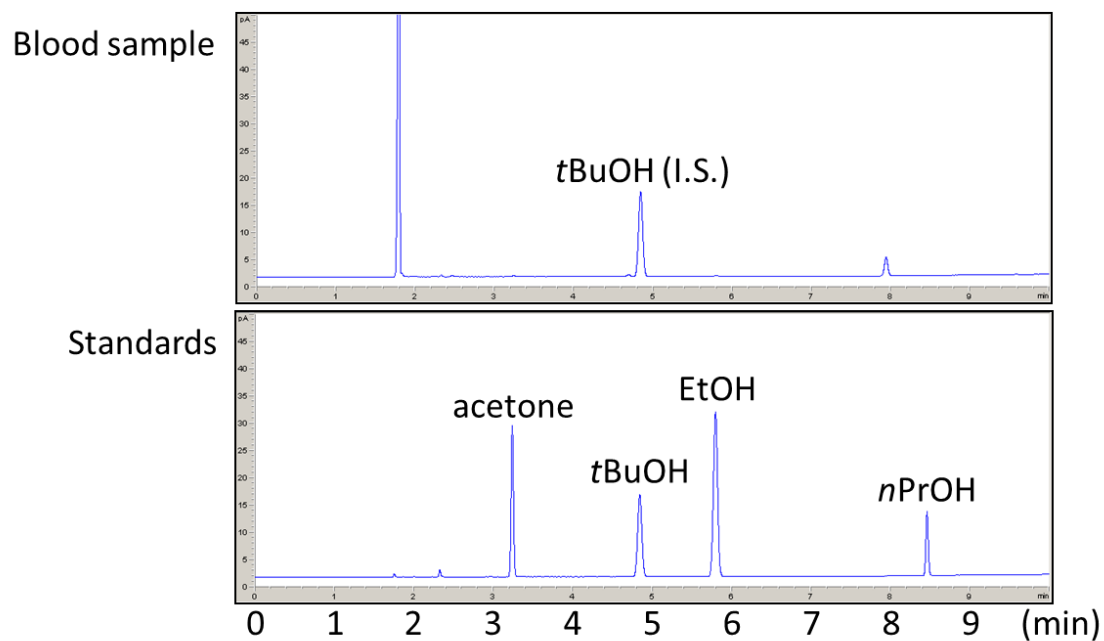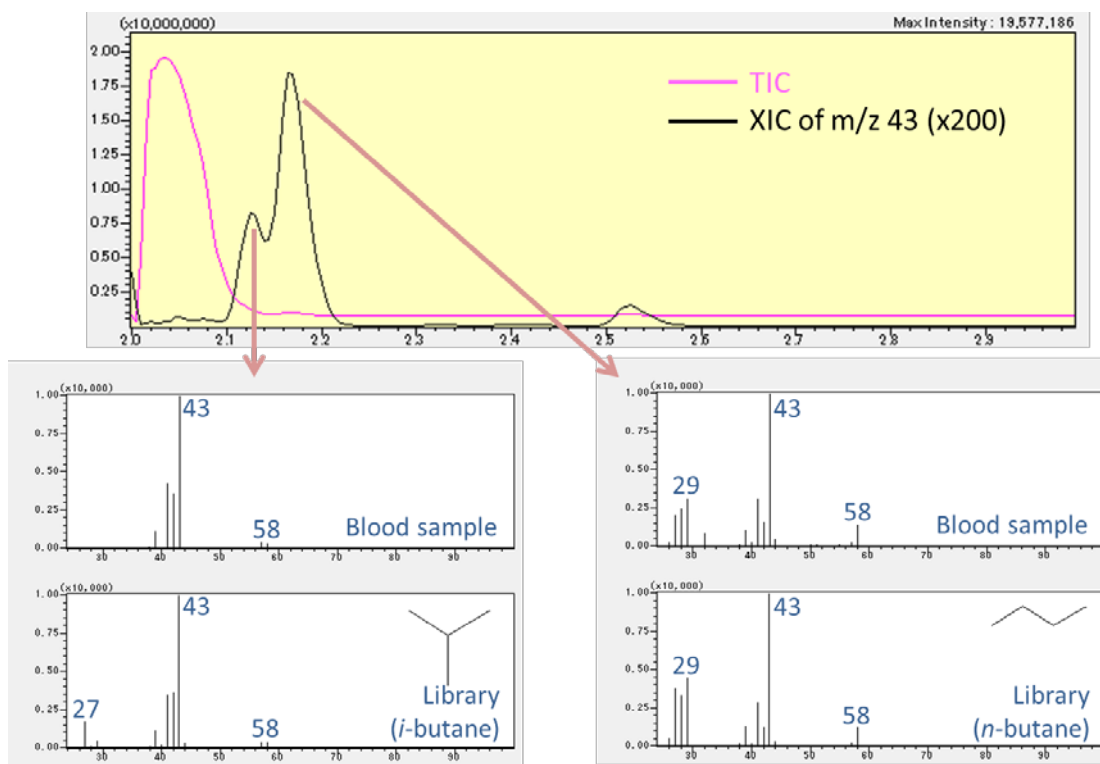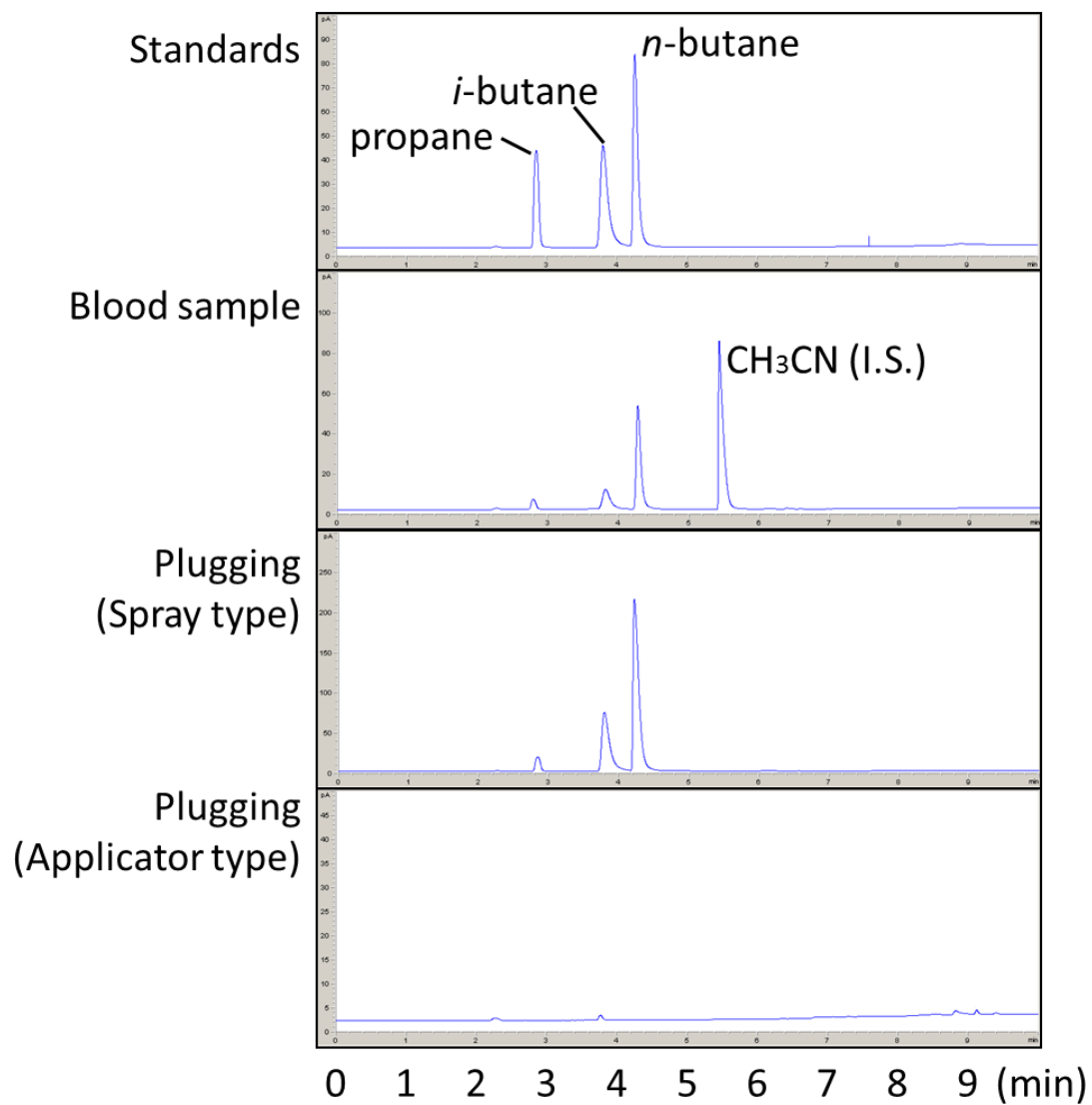
Fig. 1

Fig. 2

A

B

Fig. 3

Table 1. Blood and tissue concentrations of butanes in autopsy samples

| Tissues | Concentration[a] (μL/mL or g) | | | | | | | |
	n-butane (relative amount[b])				*i*-butane (relative amount[b])			
blood (descending aorta)	4.14	±	0.05	(1.22)	1.06	±	0.02	(1.34)
blood (right heart)	3.39	±	0.02	(1)	0.79	±	0.01	(1)
brain	6.18	±	1.62	(1.82)	2.81	±	0.74	(3.54)
lung	1.63	±	0.16	(0.48)	0.40	±	0.07	(0.51)
heart	70.5	±	19.8	(20.8)	36.1	±	10.4	(45.5)
liver	2.47	±	0.30	(0.73)	1.02	±	0.14	(1.29)
spleen	1.57	±	0.13	(0.46)	1.44	±	0.20	(1.81)
kidney	0.48	±	0.10	(0.14)	0.18	±	0.04	(0.23)

[a]Data are expressed as the mean ± SEM of three samples. [b]Relative concentration against blood concentration.

Table 2. Blood and tissue concentrations of butanes in a reported sudden death due to butane abuse

Tissues	case ref No.	1 [1]	2 [2]	3[a] [3]	4[a] [3]	5 [4]	6[a] [7]	7[a] [7]	8 [8]	9 [8]	10 [8]	11 [9]
		n-butane concentration (μL/mL or g)										
blood		2.40	0.94	n.d.	n.d.	15.3	1.63	0.49	0.52	0.11	n.d.	1.22
brain		3.00	3.91	4.30	26.10	13.3			0.39	0.21	0.11	5.44
lung		1.10	0.61	1.10		7.50	0.44	0.19	0.29	0.13	n.d.	1.97
heart			1.15						0.79	0.15	0.08	
liver			2.00			26.6	0.57	0.39		0.38	0.19	4.26
spleen			1.46			27.4						
kidney			2.10	2.90		13.6	0.19	0.36	0.27	0.14	n.d.	

[a]Data are converted from originally described unit (μg/mL or g) under the condition of 25°C and 1 atmosphere. n.d.: not detected.

Table 3. Blood and tissue concentrations of butanes in rat samples

| Tissues | Concentration[a] (μL/mL or g) | | | | | |
	n-butane (relative conc.[b])			i-butane (relative conc.[b])		
blood	6.97 ± 1.31	(1)		2.24 ± 0.62	(1)	
brain	0.54 ± 0.12	(0.08)		0.26 ± 0.03	(0.12)	
lung	15.5 ± 3.24	(2.22)		6.19 ± 1.60	(2.76)	
heart	2.90 ± 0.85	(0.42)		1.37 ± 0.50	(0.61)	
liver	8.44 ± 2.32	(1.21)		3.67 ± 0.95	(1.64)	
spleen	5.14 ± 2.33	(0.74)		2.90 ± 0.78	(1.3)	
kidney	9.75 ± 3.83	(1.4)		5.11 ± 2.00	(2.28)	

[a]Data are expressed as the mean ± SEM of four rats. [b]Relative concentration against blood concentration.

Table 4. Gas compositions detected from samples and commercial liquid petroleum

gases (LPGs)

	ratio (%)		
	n-butane	*i*-butane	propane
spray-type pluggings	63.7	30.1	6.2
autopsy case blood	68.5	22.7	8.8
experimental rat blood	57.1	17.2	25.8
canned fuel [8]	71.0	28.0	1.0
lighter gas [8]	54.0	20.0	26.0
household LPG [8]	0.1	1.5	97.8

Figure Legends

Fig. 1

Autopsy photos and computed tomography (CT) images of the autopsy case. Superabsorbent polymer retained in the esophagus (A), trachea (B), base of skull (C) and bottom of the temporal lobes (D). CT images in the chest level (E; WW250, WL40) and basal skull level (F; WW1300, WL330), and bottom of the temporal lobe level (G; WW140, WL90).

Fig. 2

A: Gas chromatography (GC) analysis of a blood sample and authentic standards. The blood sample (0.2 mL) or authentic standard mixture (ethanol: 1.129 mg/mL, *n*-propanol: 0.145 mg/mL, acetone: 0.133 mg/mL) was placed in a 20-mL GC vial before adding 0.2 mL of 0.02% *t*-butanol to the vial as an internal standard. The vials were tightly sealed and thereafter used for GC analysis.

B: Gas chromatography/mass spectrometry (GC/MS) analysis of a blood sample. The blood sample (0.2 mL) was placed in a 20-mL GC vial before adding 0.2 mL of 0.02% *t*-butanol to the vial as an internal standard. The vial was tightly sealed and thereafter used for GC/MS analysis.

Fig. 3

GC analysis of a blood sample and authentic standards. The blood sample (0.5 mL) or

0.5 g of the polymer extracted from applicator-type plugging was placed in a 20-mL

GC vial before adding 0.5 mL of 0.05% acetonitrile to the vial as an internal standard

(only in blood sample). The amount of standard gasses and spray-type plugging were

adjusted to portray a workable chromatogram. The vials were tightly sealed before GC

analysis.