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A case of drug-facilitated sexual assault leading to death by chloroform poisoning

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Abstract The purpose of this investigation was to determine the cause of death of a 13-year-old girl, where none was immediately evident. Our analysis showed it to be a very unusual case of a drug-facilitated sexual assault (DFSA), which led to the tragic death of the young rape victim and then to the suicide of the rapist. The incapacitating agent used was chloroform. The post-mortem analysis revealed a blood concentration of 833.9 mg/l for the girl, whereas the quantitation of chloroform in various fluids and viscera of the rapist proved that he had recently been handling the solvent (with concentrations in fat tissues 20 times higher than in his blood). This case draws attention to the need for broad searches for volatile substances in such investigations.

Keywords Chloroform · Drug-facilitated sexual assault · Death · Forensic science · Headspace gas chromatography/mass spectrometry

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Case report

A 13-year-old girl, 1.56 m tall and weighing 37 kg, was found dead in her bed at 17.00. The district attorney ordered an autopsy, but the forensic scientists noted no injuries that could explain her death: no signs of strangulation or suffocation, no signs of violence, including anal and vaginal (the hymen was intact), nor any visceral congestion that would have raised the suspicion of a toxic cause of death. Blood, bile, urine and hair samples were taken for toxicological analysis, whereas vaginal swabs were analysed for the presence of sperm. Unfortunately, no other fluids or tissues were taken from the victim (if a sample of lung tissue had been taken, we could have stated categorically the way the solvent was administered), so the analytical data were limited to the above-mentioned fluids. The two forensic scientists who carried out the autopsy did not note the characteristic odor of chloroform (or any other solvent) when opening the body, nor did they note any corrosive effects on the tissues. The biological fluid samples were removed and transferred in 30-ml borosilicate glass flasks with self-crimping screw tops. They were taken to the laboratory within 24 h and analysed there the same day.

At 19.45 the same evening, the corpse of a 36-year-old man was found in a town 60 km away from the discovery of the girl's body. He was known to the girl's family and had frequent close contact with her. Apparently, he had committed suicide at his workplace by throwing himself off a storage tank after telling his brother on the telephone that he had "done something stupid". There was no evidence of chloroform at the scene. The man was 1.86 m tall and weighed 100 kg. He had a higher technical diploma in chemistry, specializing in toxicology. He was not known to be undergoing any psychiatric treatment or to have any previous criminal or psychiatric history. The fact that the girl had been raped was only established by the analysis of swabs and the DNA comparison between the rapist's blood and the girl's vaginal samples. This hypothesis was only put forward, and subsequently verified, because the laboratory concluded that

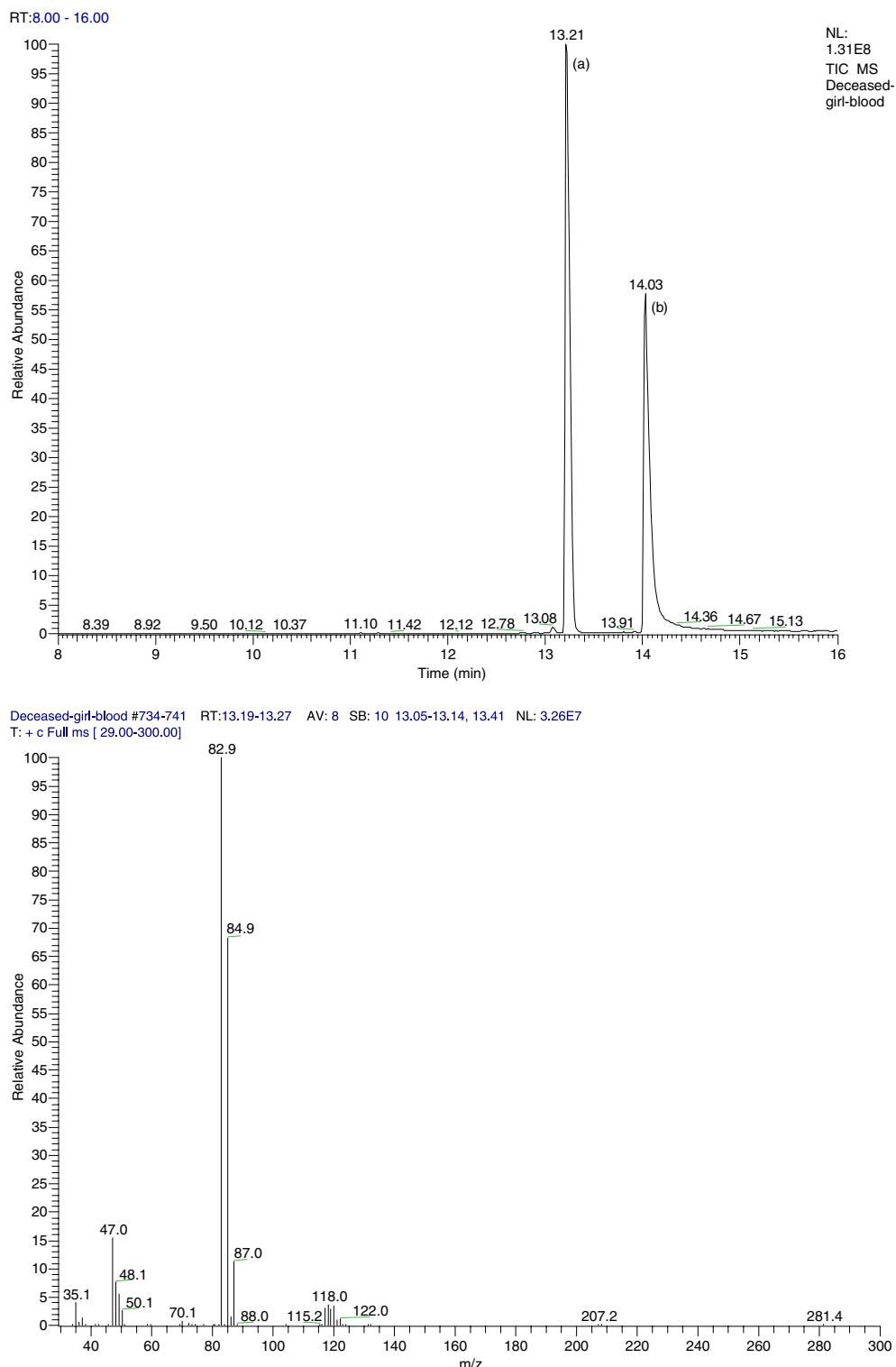
the cause of death was toxic and probably very criminal in origin. The autopsy of the rapist was carried out 6 days after his death (the body had been kept in a cold room since its discovery). The samples were taken to the laboratory within 24 h and analysed there the same day.

Materials and methods

Instrumentation

The gas chromatograph used was a Trace GC (Thermo Electron, Courtaboeuf, France) equipped with an HS2000

Fig. 1 Above, Total ion current chromatogram of 0.5 ml of blood of the young rape victim. Peaks, (a) chloroform (b) internal standard. Below, full scan mass spectrum of peak a chloroform



autoinjector and a DSQ mass spectrometer. The analytical column was a Poraplot Q, 25 m in length, with an internal diameter of 0.25 mm (8 µm film thickness) from Chrompack (Les Ulis, France). Helium was used as carrier gas at a flow rate of 1 ml/min in constant flow mode (i.e. 135 KPa at 50°C). The temperatures were interface 220°C and ion source 200°C. Pulsed injection with surge pressure (1 ml) was carried out at 200°C and 200 KPa for 1 min. The initial oven temperature was 50°C for 2 min and was increased to 230°C at 15°C/min and held for 6 min. The chromatographic run time was 20 min. Repetitive scans in positive mode were acquired from 29 to 300 a.m.u. at a rate of 300 a.m.u. per second from 4.5 to 20 min.

Incubation for headspace (HS) injection was carried out at 90°C for 12 min, whereas the syringe was heated to 100°C, and the injection rate was 3 ml/min.

Sample preparation

One millilitre of deionized water, 0.5 ml of 0.1% butanol in water [used as internal standard (IS)] and 0.5 ml of blood or biological fluid were successively introduced into a 20-ml glass vial for HS.

For the viscera, a piece weighing approximately 1 g was introduced into the HS vial and weighed with a precision balance. After the introduction of 1 ml of deionized water and the internal standard, where the volume was adjusted according to the weight of the viscera, the tube was sealed and treated in an ultrasonic bath for 1 h. It was then placed in the autosampler and analysed as a biological fluid sample.

Quantification

Quantitation was performed using a multi-point calibration table based on the peak area ratio between analyte and IS. Because the concentration of chloroform in the victim's blood was very high compared to that of the offender, two different quadratic calibration curves were used: from 0.5 to 10 mg/l for the suspected rapist and from 2 to 1,000 mg/l for the victim.

The ranges of calibration were set up with blood that had tested negatively for chloroform beforehand.

Relative standard deviation (%) were respectively equal to 6.8, 6.1, 5.2 and 4.5 for 0.5, 2, 10, and 1,000 mg/l ($n=6$). Limit of detection was calculated from an analysis of the blanks (with $n=30$) using the following mathematical formula: $LD=m_{blanks}+3 SD_{blanks}$ and was found to be 0.04 mg/l (m_{blanks} , mean of the blanks; SD_{blanks} , standard deviation of the blanks).

Table 1 Concentrations of chloroform in the girl

Biological fluid	Concentration (mg/l)
Peripheral blood	833.9
Bile	148.6
Urine	9.7

Analytical results

For the girl

The test for sperm in the vaginal swabs was positive. The DNA from the seminal fluid was later identified as that of the suspected rapist.

A complete toxicological analysis was conducted, which included immunoassay techniques, carbon monoxide and cyanide detection, as well as gas chromatography/mass spectrometry (GC-MS), liquid chromatography/photodiode array detection (LC-PDA), HS-GC-MS and LC-MS-MS screenings. Headspace analysis for volatiles was carried out not because of the circumstantial evidence, but because it is systematically performed in all investigations into the cause of death of sexual assault cases. Analysis by HS-GC-MS revealed the presence of chloroform (see Fig. 1) in concentrations listed in Table 1. All other techniques returned negative.

For the suspected rapist

The same type of analysis was conducted on the various samples taken during the autopsy. Only chloroform was detected, but in much weaker concentrations than in the girl. The concentration levels according to tissue type are listed in Table 2.

In the course of the investigation, an opened 2.5-l bottle of chloroform was found at the company where the suspected rapist worked. Although it had not been used for several years, this solvent had not been destroyed and

Table 2 Concentrations of chloroform in samples from the rapist

Biological tissues	Concentrations
Cardiac blood	0.25 mg/l
Urine	0.26 mg/l
Bile	0.38 mg/l
Gastric liquid	0.36 mg/l
Liver	0.06 mg/kg
Kidney	0.34 mg/kg
Lung	0.30 mg/kg
Subcutaneous fat	5.44 mg/kg
Intestinal fat	5.18 mg/kg

would have been easily accessible to a man with his training and position in the company. No chloroform was found at his home.

Discussion

Chloroform is a chlorinated solvent that was widely used in the past as an anesthetizing agent but has been considered obsolete for this application for several decades. It was also used as an additive in certain pharmaceutical preparations, but since several studies have proved its carcinogenic properties in animals, its use on humans or animals has been banned. Chloroform is still used in the chemical industry as a synthesis intermediate and as an extraction solvent in laboratories, but its use is decreasing as more and more laboratories move towards less toxic solvents [1, 2].

Post-mortem concentrations in the literature and interpretation of the victim's results

In nine patients who died during surgery when chloroform was used as an anesthetic (but whose death was not due to chloroform poisoning), the post-mortem concentrations were between 60 and 182 µg/g in the brain, between 22 and 145 µg/g in the lungs and between 22 and 88 µg/g in the liver [3].

In six cases of forced or intentional acute chloroform poisoning (suicide or homicide), the concentrations found are listed in Table 3 [4–11].

The analytical table for the rape victim thus fits the pattern for someone who died of a massive overdose of chloroform, which the coroner concluded was the direct cause of death. The concentrations measured in the girl were high compared with those in the scientific literature. We have no particular explanation for this. It was not possible to verify the route of penetration (oral or pulmonary).

Concentrations among recreational users and interpretation of the suspected rapist's results

A single oral dose of 500 mg produces blood concentrations of between 1 and 5 mg/l after 1 h and has an apparent half-life of 1.5 h.

Table 3 Concentration of chloroform in fatal cases [6–10]

	Blood (mg/l)	Brain (mg/kg)	Liver (mg/kg)	Kidney (mg/kg)	Urine (mg/l)
Average concentration	64	133	82	52	21
Extreme values	10–194	50–310	6–201	16–124	0–70

A person who inhaled chloroform vapor in a container for 7 s (until he fell unconscious) developed a peak blood concentration of 4 mg/l after 20 min [10].

According to the data in the international scientific literature, a concentration level of around 0.30 mg/kg in the kidneys and lungs, such as that measured in the suspected rapist, shows that he was in contact with chloroform vapor a short time before his death. This is consistent with the level of 0.25 mg/l measured in the blood and is compatible with the hypothesis that he inhaled a small amount of chloroform while chloroforming his victim, thus corroborating the police investigators' version of events.

Analysis of his fatty tissue, however, showed a level of concentration 20 times higher than in other areas. This considerable difference cannot be explained simply by a single exposure 2 or 3 h before his death.

The more likely hypothesis is that the suspected rapist had already been exposed to blood concentrations of around 5 mg/l in the recent past, which the literature shows is the level required to cause loss of consciousness. There are several possible secondary hypotheses. We are aware that these hypotheses are speculative, inasmuch as the investigators were unable to confirm them through the facts of the case or by questioning the rapist's close relatives. Their presentation in writing, however, should heighten the reader's awareness if a similar case comes up in the future.

1. The perpetrator was addicted to chloroform (bearing in mind that this solvent is a particularly effective intoxicant).
2. He attempted, unsuccessfully, to commit suicide using chloroform (however, it was not found at the scene, and this hypothesis is unconvincing).
3. In planning his crime, he tried to measure the effect that chloroform would have on his victim by using it on himself beforehand. In this case, it is possible that he did not take into account the considerable difference in build and weight between his victim and himself (100 vs. 37 kg). If he neglected to adjust the dosage accordingly, this would probably have caused the girl's death.
4. A phenomenon of post-mortem redistribution could account for the non-homogeneous distribution. This hypothesis is also unconvincing inasmuch as a non-homogeneous distribution implies an active transfer, whereas in phenomena of post-mortem redistribution, the concentrations tend to move towards the same levels in all parts of the organism.

Place of solvents in chemical submission (involving DFSA cases but also robberies and extortions)

The Centre de Pharmacovigilance (drugs monitoring center) in Lyon registered 20 cases of chemical submission in 2004. Of these 20 cases, four reported the use of ethanol alone, and two mentioned the use of a volatile substance (the present chloroform case and a robbery involving the

forced inhalation of diesel oil to obtain the combination of a safe).

It is important to note that these two cases were brought to the fore analytically by the same laboratory. It is thus extremely likely that cases of chemical submission using a volatile substance are underestimated in France, inasmuch as few laboratories systematically use a sufficiently effective and sensitive HS-GC-MS technique.

And yet there are numerous volatile substances that can be used in this way, although it is true that they are not always easily accessible to the general public. This is the case with chloroform in particular, but also with trichloroethylene, ether, carbon tetrachloride, bromoform, acetone and also freons and other perfluorocarbons. There are also pesticides such as chloromethane and bromomethane, and of course anesthetizing gases such as enflurane, halothane, isoflurane and sevoflurane (although these are normally only available in the hospital sector). On the other hand, some chemicals, such as motor fuels and household stain removers, are very accessible. As an example, we could mention the increasingly common practice among drug addicts in the French West Indies of adding around 10 to 15 ml of gasoline to a can of beer.

Conclusions

We have reported on a very unusual case of drug-facilitated sexual assault (DFSA), which tragically led to the death of the young rape victim and then to the suicide of the rapist. Whereas the scientific literature shows the widespread use of alcohol, cannabis, cocaine, benzodiazepines, zolpidem or GHB for DFSA, this paper calls attention to the use of chloroform, a drug that may be old-fashioned but is alarmingly effective.

We cannot overemphasize the importance in analytical toxicology of systematic search methods that include

obligatory broad searches for volatile substances, irrespective of the original direction of the investigation.

Lastly, this case draws attention to the importance of sub-adipose or peri-intestinal tissue when looking for solvents. In many cases, analysis of the hair is recommended to document the administration of a hypnotic drug after the event, but this would be ineffective if the incapacitating agent was a volatile substance, because its fixation in hair, nails and teeth is nonexistent. In such cases, the analysis of adipose tissue is especially important.

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