CASE REPORT

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Concentrations of Mustard Gas [Bis(2-Chloroethyl)Sulfide] in the Tissues of a Victim of a Vesicant Exposure

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ABSTRACT: An Iranian soldier died at a toxicological intensive care unit at Munich seven days after a vesicant exposure. At the autopsy the typical symptoms of mustard gas intoxication were found. The vesicant was detected qualitatively by gas chromatography-mass spectrometry (GC-MS) in the abdominal fat and quantified in the tissues and in the body fluids by the following method: (1) extraction by dichloromethane, (2) cleanup of the extracts by thin-layer chromatography (TLC) on silica plates, (3) extractive derivatization with gold-chloride, and (4) quantitative determination by electrothermal atomic absorption spectrometry (ET-AAS). The equal extracts, after heating, served for blanks. The following concentrations were found (milligrams of mustard gas/kilograms of tissue wet weight): brain 10.7, cerebrospinal fluid 1.9, liver 2.4, kidney 5.6; spleen 1.5, lung 0.8, muscle 3.9, fat 15.1, skin 8.4, skin with subcutaneous fatty tissue 11.8, liquid from a skin blister: below detection limit, blood 1.1, and urine: below detection limit.

KEYWORDS: pathology and biology, toxicology, mustard gas, tissues (biology)

In 1985 an Iranian soldier (reported age: 24) died—ultimately of pneumonia—at the toxicological intensive care unit of the Technische Universität in Munich, 7 days after a vesicant exposure. The findings of the autopsy (performed at the Institute of Forensic Medicine at Munich) confirmed the clinical diagnosis of mustard gas [bis(2-chloroethyl)sulfide, Yperite, S-Lost] intoxication and are reported in detail elsewhere [*1*]. In this paper the analytical efforts to detect and quantify the vesicant in the tissues and in the body fluids of the deceased will be discussed.

Samples

The tissues and the body fluids were taken at the autopsy and stored in polyethylene bags and bottles at −20°C until they were analyzed.

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Experimental Procedure

Heyndrickx et al. [2] have reported several methods (thin-layer chromatography [TLC], gas chromatography [GC], gas chromatography-mass spectrometry [GC/MS], and atomic absorption spectrometry [AAS]) for the analysis of mustard gas in different matrices. To detect this agent in human tissues, we have optimized, combined, and modified these methods by many investigations on spiked samples before the analyses of the case specimens.

General Remarks

At all analytical steps, special care was taken that the temperature never exceeded 20°C. In any case, the total analytical procedure was performed uninterrupted to avoid losses.

Qualitative Detection

For this purpose an aliquot from freshly defrosted abdominal fat was homogenized, the homogenate extracted with the threefold quantity of dichloromethane, and the organic layer injected immediately into the GC-MS system without any evaporative process. The GC and the MS equipment and conditions are summarized in Table 1. Figure 1 shows the trace of the total ion current, the molecular ion 158 *m/e*, and the base peak 109 *m/e* of mustard gas, as received from this case. The GC retention times of this peak and that of a mustard gas standard (obtained from the Deutsche Bundeswehr) were the same. Figure 2 shows the complete, original mass spectra, as obtained from the fat sample of the deceased. This spectrum is identical to a mustard gas reference.

Quantitative Detection

Extraction Method—A sample of the tissue was homogenized, and 1 to 7 g of the homogenate or of a body fluid (see Table 2) were mixed thoroughly with an agitator with a fivefold quantity of dichloromethane, subsequently shaken mechanically for 15 min, and then centrifuged. The dichloromethane layer was separated. This extraction procedure was repeated twice with the body fluids and three times with the other tissues, respectively. The combined organic layers were evaporated to dryness at room temperature and reduced pressure in a rotary evaporator.

TABLE 1—*GC/MS conditions.*

Equipment: GC/MS 4515 Finnigan		
Gas liquid chromatography:		
Column: SE 54, 15 m by 0.25 mm		
Fused silica, film thickness: 0.25 μm		
Carrier gas: Helium, 45 cm/s		
Splitless injection		
Temperatures:	injector:	250°C
	oven: 2 min. isotherm	55°C
	10°C/min. to	120°C
	interface:	270°C
	transfer line:	270°C
Mass spectrometry:		
Ionization mode: electron impact, 70 eV		
Mass range: 50 to 200 amu; scan time: 0.5 s		
Ion source: Temperature 120°C, pressure 9×10^{-7} torr		

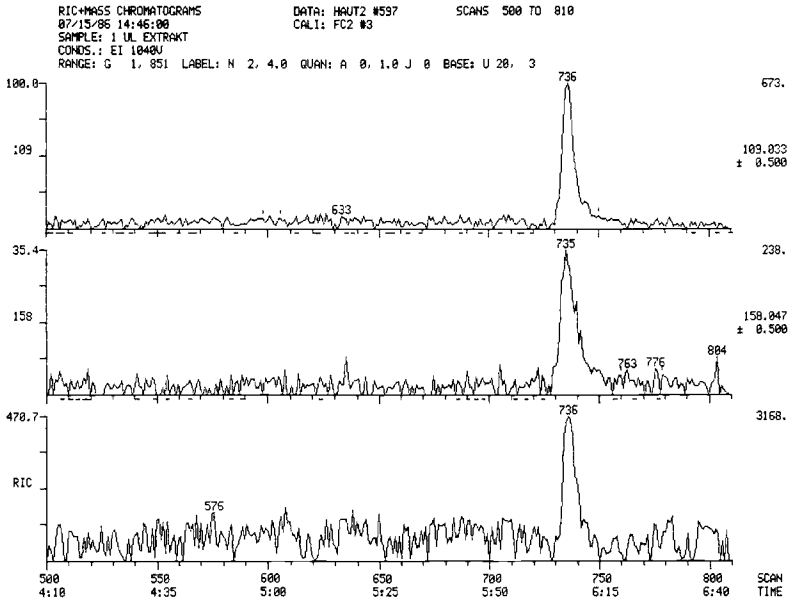

FIG. 1—*Mass chromatogram (upper track: base peak 109* m/e *of mustard gas; middle track: molecular ion 158* m/e *of mustard gas; lower track: total ion current), obtained from an abdominal fat sample of the deceased Iranian soldier.*

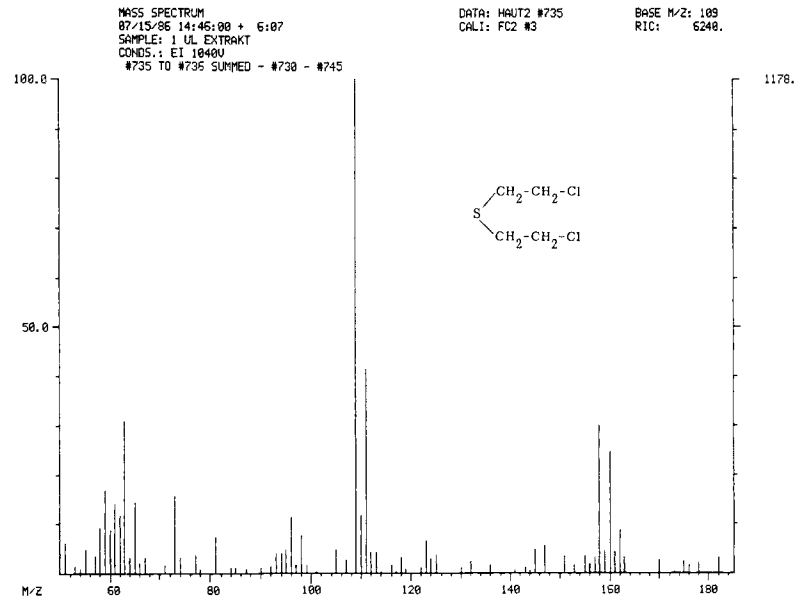

FIG. 2—*Complete mass spectra (identical to a mustard gas standard), obtained from an abdominal fat sample of the deceased Iranian soldier.*

TABLE 2—*Mustard gas concentrations found in the tissues.*

Tissue	Sample Taken	Extract Weight, g	Mustard Gas Concentration
Brain	2.54 g	0.34	10.7 mg/kg
Cerebrospinal fluid	2.10 mL		1.9 mg/L
Liver	6.24 g	0.03	2.4 mg/kg
Kidney	5.14 g	0.16	5.6 mg/kg
Spleen	5.68 g	0.05	1.5 mg/kg
Lung	6.27 g	0.05	0.8 mg/kg
Muscle (thigh)	4.04 g	0.11	3.9 mg/kg
Fat (thigh)	2.79 g	1.37	15.1 mg/kg
Abdominal skin	0.78 g	0.21	8.4 mg/kg
Skin with subcutaneous fat	2.71 g	1.03	11.8 mg/kg
Liquid from a skin blister	4.00 mL	...	below detection limit
Blood	20.00 mL	...	1.1 mg/L
Urine	20.00 mL	...	below detection limit

The residue was weighed and dissolved in 10 mL of *n*-hexane. Half of the sample or—if the total extract weighed more than 100 mg—an aliquot of 50 mg was separated for TLC, while another aliquot of the residue was heated for 1 h to 150°C to get a mustard gas-free blank.

TLC Cleanup Procedure

Equipment and Chemicals—Precoated TLC glass plates 10 by 20 cm, SIL G-25 UV_{254} (Fa. Macherey & Nagel, Düren, F.R.G.) were used.

The solvent was dichloromethane, analytical grade (E. Merck, Darmstadt, F.R.G.).

The spray reagents were palladium chloride ($PdCl_2$) solution (1% in HCl 10%) and gold trichloride ($AuCl_3$) solution (1% in water).

The mustard gas stock solution was 1 mg/mL in *n*-hexane.

Method—Before TLC separation, the silica plates were cleaned by a blank run in the chamber with dichloromethane and dried. A maximum of 50 mg of the residue was dissolved in some 100-μL *n*-hexane, spotted in a band of 8 cm width on a TLC plate, and developed over a distance of 15 cm. For visualization (of test spots only!) a $PdCl_2$ solution or a $AuCl_3$ solution (more sensitive) were sprayed on the plate. The R_f-value of the Mustard Gas was approximately 0.90, distinctly higher than the front of the highest lipid fraction (triglycerides). The silica gel at a height of 12.5 to 15 cm was scraped off the plate.

Formation of the Mustard Gas-Gold Complex

Chemicals—Gold stock solution was 1 mg Au/mL (Alfa Products, Danvers, MA). Toluene was analytical grade (E. Merck, Darmstadt, F.R.G.).

Method—The silica gel was transferred to Teflon® test tubes, 100-μL gold stock solution and 1.0-mL water were added, and the tubes vortexed for 2 min. Then 4 mL of toluene were added and the mixture (water, toluene, silica gel) shaken another 2 min mechanically. Finally, the toluene layer was separated by centrifugation.

Quantitative Determination with ET-AAS

Equipment and Chemicals—The Perkin-Elmer AAS 3030 Zeeman, HGA 600, printer-plotter PR 100 was used. Gold stock solution was used (see above).

TABLE 3—*AAS conditions for the detection of the mustard gas-gold complex.*[a]

Temperature Program			
Step	Temperature, °C	Ramp Time, s	Hold Time, s
Drying	120	20	20
Charring	250	10	10
Atomizing[b]	2400	0	3
Cleaning	2650	1	4

[a]Wave length 242.8 nm; slit 0.7 mm; wall atomization; purge gas: 300-mL argon/min.
[b]Gas stop.

Method—Of the obtained organic layer (see above)—if necessary after dilution with toluene—20 μL were injected in the AAS graphite tube. The AAS conditions are summarized in Table 3.

For each tissue, the individual blank value (see above) was subtracted. The calibration was performed by mustard gas standard solutions, spotted on TLC plates, and worked up simultaneously. The application of a standard addition method, as proposed by Heyndrickx et al. [2], seemed not to be necessary, because we know from several pilot tests with spiked samples that dichloromethane extracts the mustard gas nearly quantitatively (95%) from any human soft tissue, and the applied AAS compensation method (Zeeman effect) avoids a depression of the gold signal by the cleaned up organic matrices or other interferences.

Results

The results of the quantitative detection are summarized in Table 2.

The detection limit is not caused by the method of the detection, the extreme high sensitive ET-AAS, but by the background of the blank value. In this method, a small amount of a lipid soluble gold complex is extracted from an aqueous phase into toluene, while there is still a large excess of gold ions in the water. If some water is brought into the toluene, it carries gold ions in the organic layer too. At pure aqueous solutions of gold-III-chloride this factor can be neglected; but in tissue extracts there are always some emulsifiers, even after the TLC cleanup, which draw traces of the gold ions into the toluene. Therefore, the detection limit depends on the blank value, that is, the value of the heated sample, and this blank value differs individually from tissue to tissue. We decided that the mustard gas concentration in a sample is insufficient for quantification, if the difference between the untreated sample and the heated one is lower than the blank value of the heated sample alone. The detection limits, defined in this way, are in a region of approximately 0.1 mg/kg.

Discussion

From several papers presented at the First [3] and the Second [4] World Congress "New Compounds in Biological and Chemical Warfare" 1984 and 1986 in Ghent the conclusion can be drawn that the main chemical agent applied in the Gulf war has been (and still is) mustard gas. This was confirmed by the symptoms and the reports of the victims and the chemical analysis of the content of a blind bomb from the battle field. Vycudilik [5] reported the finding of traces of mustard gas in urine samples of victims. Conditional on the working up procedure applied by him (treatment of the urines with chloride), he could not differentiate whether the urines primarily contained mustard gas, or the mustard gas was formed

during the working up procedure from compounds like thiodiglycol, a metabolite of mustard gas. The lack of a positive GC-MS detection of mustard gas in samples not treated with chloride argues in favor of the second possibility. Recently Wils et al. [6] have found thiodiglycol by the same method and in the same order of magnitude also in urine samples of a control group (persons without any contact to mustard gas) and concluded that this "excluded the unambiguous verification of the use of mustard gas against the Iranian patients."

We have proved the presence of unmetabolized mustard gas in the tissues of the deceased by two completely independent methods: gas-chromatography/mass spectrometry with complete MS spectra and atomic absorption spectrometry after TLC separation and formation of a mustard gas-gold complex. To our knowledge, this is the first definite verification of mustard gas in the tissues or body fluids of a victim.

Similar to other high lipophilic substances (for example, pesticides, polychlorinated biphenyls [PCBs], or tetrahydrocannabinol [THC]), mustard gas seems to accumulate in the lipid compartments of the human body. The relatively high mustard gas concentrations, found in this case even seven days after the exposure, point out that a redistribution of mustard gas from the lipid depots occurs rather slowly.

As expected for a high lipophilic compound and confirmed by the investigations of Vycudilik [5], the urinary excretion of unmetabolized mustard gas is extremely low; but obviously this is not caused by a rapid disintegration of the agent, as supposed, but by its strong fixation to the lipid compartments of the body.

The chemical stability of mustard gas is reported very contradictorily in the literature [7]. Our investigations do not indicate any considerable chemical liability of this agent at least in the lipid tissues antemortem and postmortem. In the presented case the tissues and the body fluids were stored after autopsy at −20°C for twelve months till they were analyzed.

From our results it can be concluded that the "tissue of choice" for the detection of mustard gas in a corpse is any lipid rich tissue. Especially for the examination of living victims in the future we propose to consider a biopsy of the abdominal fat.

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