CASE REPORT

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A Fatal Case of Trichlorofluoromethane (Freon 11) Poisoning. Tissue Distribution Study by Gas Chromatography-Mass Spectrometry

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ABSTRACT: A case of lethal poisoning due to trichlorofluoromethane (FC11) inhalation is described. The fluorocarbon was determined in biological tissues by headspace gas chromatography-mass spectrometry. FC11 was detected in all the examined tissues, with decreasing levels in heart, lung, brain, liver, blood, kidney, and spleen. The highest concentration measured in heart could be related to the mode of toxic action of fluorocarbons postulated by many authors, characterized by the sensitization of the myocardium to the catecholamines producing arrhythmia and cardiac arrest. Nevertheless the aspecific picture of the anatomo-pathological and histological findings does not exclude that the described accidental fatality may have been caused by the combination of direct freon toxicity with hypoxemic asphyxiation, due to the saturation of the atmosphere by FC11 in the closed environment in which the intoxication occurred.

KEYWORDS: toxicology trichlorofluoromethane, fatal poisoning, tissue distribution, headspace gas chromatography-mass spectrometry

Fluorocarbons were introduced in the 1940s as refrigerants and aerosol propellants because of their "inertness" compared to sulfur dioxide, ammonia, carbon tetrachloride, and chloroform [*1*]. They are a group of synthetic halogen-substituted methane and ethane derivatives, of which trichlorofluoromethane (FC11) is the most popular because of its vapor depressant effect and its solvent and flame retardant action.

A number of fatal cases associated with inhalation of fluorocarbons have been reported in the literature [*2–8*]. However, though some of them were due to mixtures of FC11

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with dichlorodifluoromethane (FC12), none involved FC11 alone as in the described case.

Analysis of fluorocarbons in biological specimens is generally performed by headspace gas chromatography with flame ionization [*2,4,5,9*], electron capture [*3,6,9*], or mass spectrometric detection [*6,10,11*]. Gas chromatography-mass spectrometry (GC/MS) with direct blood injection [*12*] or headspace MS without separation [*13*] have also been used.

The authors describe a fatal case of occupational poisoning in which FC11 tissue distribution was accomplished by headspace GC/MS using dichloromethane as internal standard. Instrumental analysis was carefully planned to achieve remarkable selectivity and sensitivity in fluorocarbon analysis.

Case Report

A 33-year-old man, who was employed in an expanded polyurethane factory where FC11 was used as a foaming agent, was found dead on the floor of an about 4 m^3 room where the delivery system of a FC11 reservoir was placed. The man went into the room a few minutes before to replace the blank flange of a pipe coming from the reservoir with a manual valve. A subsequent inspection revealed that the installation of the valve was not completed.

At the time of autopsy, performed 72 hours later (the body was preserved at −2°C), no signs of trauma or other significant injuries were revealed at the external examination. Autopsy findings included hyperemia and edema of the brain; congestion of the esophagus mucosa; marked dilatation of the right cardiac chamber filled with fluid darkred blood, and dilatation of the conus arteriosus. The lungs appeared considerably hyperemic and edematous. Histological findings revealed a steplike fragmentation of smooth muscular cells and scattered areas of hyperemia in the myocardium; hyperemia of capillary vessels, edematous fluid in the alveoli, intra-alveolar microhemorragiae, macrophagic alveolitis and atelectatic areas in the lungs.

Biological specimens including blood, urine, brain, liver, kidney, lung, heart, and spleen were collected and stored at −20°C until the toxicological analyses. Samples of the liquid contained in the FC11 reservoir, and of the air surrounding the valve were also collected in gas-tight glass vials during the inspection of the factory.

Toxicological Analyses

The identification and quantitation of FC11 were accomplished according to the following procedure.

Materials

FC11 (purity higher than 99%) was purchased from S.L.O. (Pavia, Italy), dichloromethane and n-butanol, both of analytical grade, from C. Erba (Milan, Italy).

Instrumentation

FC11 analyses were performed on a Hewlett-Packard 5890A gas chromatograph coupled with a Hewlett-Packard 5970 mass selective detector. An HP-5 Ultra 2 cross-linked 5% phenyl, methylsilicone fused-silica capillary column (12 m × 0.2 mm I.D. × 0.33 μm film thickness) was used. The operating temperatures were: injector (split ratio 1:70), 150°C; oven, 30°C; transfer line, 280°C. Carrier gas was helium (1 psi). Under the described conditions the retention times of FC11 and dichloromethane (internal standard)

were 0.73 and 0.86 min, respectively. The identification of FC11 in the reservoir, in the air surrounding the valve, and in blood was performed by scanning the mass range 30–150 u every 0.2 s. Quantitative analyses on tissues were carried out by monitoring the ions at *m/z* 66, 101, and 103 for FC11, and *m/z* 84 and 86 for the internal standard.

Sample Preparation

The whole procedure, including the preparation of standard solutions, was carried out at −20°C. FC11 and dichloromethane solutions in n-butanol (1.5 mg/L and 5.2 mg/L, respectively) were prepared. Aliquots of the frozen samples (weight range 1 to 1.5 g) were placed in preweighed vials immediately sealed with a teflon-rubber stopper and weighed again. Aliquots of the corresponding blank samples were prepared in a similar mode and spiked with the FC11 solution introduced through the stopper using a microsyringe. The amount of FC11 added was based on a preliminary analysis of each sample; an equal volume of the internal standard solution was added to the samples and to the corresponding spiked tissues. The vials were placed in a water bath and maintained at 40°C for 30 min. A 100 μL volume of the headspaces was then withdrawn with a gas-tight syringe and injected in the gas chromatograph.

The biological fluids were also tested for the presence of ethyl alcohol, carbon monoxide, sedative-hypnotics, barbiturates, phenothiazines, antidepressants, pyrazolone analgesics, benzodiazepines, opiates, cocaine, amphetamines, and tetrahydrocannabinol metabolites. None of these compounds was found.

Results and Discussion

The presence of FC11 in the reservoir, in the athmosphere surrounding the valve, and in blood of the deceased subject was confirmed by the GC/MS analysis. No other fluorocarbon was detected. Figure 1 shows the total ion chromatogram obtained for blood and the mass spectrum of the peak at 0.73 min. The large peak observable in the chromatogram at 0.53 min is due to air.

Figure 2 gives the ion chromatograms (*m/z* 101 and 86 added) obtained for a blank blood (*A*), the blank blood spiked with 60 mg/kg of FC11 (*B*), and blood (*C*) and brain (*D*) samples from the deceased subject.

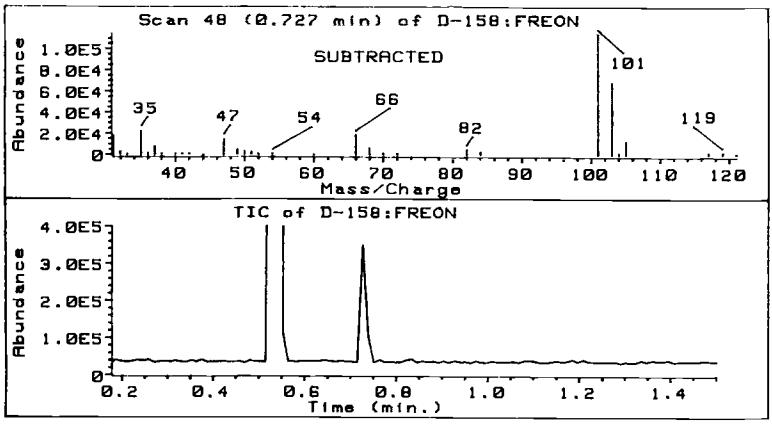

FIG. 1—*Total ion chromatogram obtained for the blood sample, and mass spectrum of the peak at 0.73 min. The large peak at 0.53 min is due to air.*

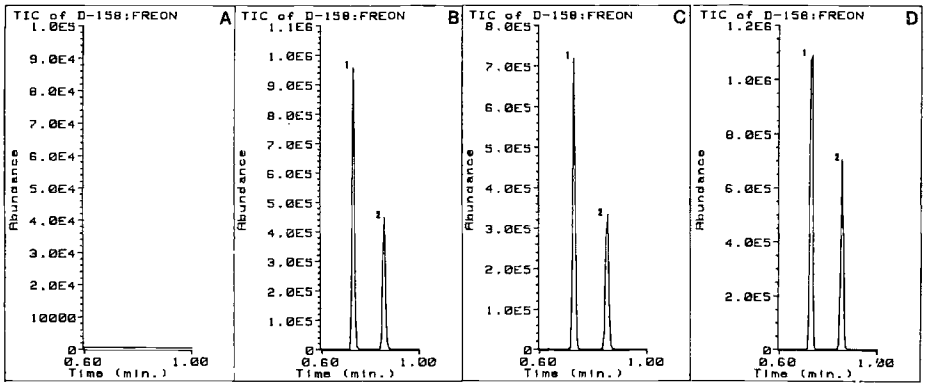

FIG. 2—*Ion chromatogram* (m/z *101 and 86 added) obtained for a blank blood sample* (A), *for the blank blood spiked with 60 mg/L of FC11* (B), *and for the blood* (C) *and brain* (D) *of the deceased subject. Peaks: 1 = FC11; 2 = internal standard.*

The tissue distribution of FC11 is reported in Table 1. High concentrations of the fluorocarbon were measured in all the examined tissues, at comparable levels or even higher than those reported for FC11 in other lethal cases, all due, however, to inhalation of mixtures of FC11 and FC12 [*2,3,7*]. These high levels might be explained by a death occurred when the subject was still exposed to FC11 and to the preservation at −2°C of the dead body until the collection of biological specimens.

The tissue concentrations reflect the distributions previously observed [*2,3,7*], with the highest levels of the fluorocarbon measured in heart, followed by lung, brain, liver, and kidney. FC11 was under the detection limit in urine, while a bile sample was not available.

Fluorocarbons are believed to explicate their toxic action through myocardium sensitization to the circulating catecholamines, resulting in ventricular arrhythmia and cardiac arrest [*7,14–18*]. Interestingly, and possibly related to the postulated specific cardiac toxicity, heart was the tissue where FC11 was found at the highest concentration, from 2.7 to 20 times higher than in the other examined tissues. This observation is in agreement with the results reported by Standefer [*2*] who studied the tissue distribution of FC11 and FC12 in a case of death associated with inhalation of a mixture of the two fluorocarbons (43% and 57%, respectively), while heart was not analyzed in the other reported cases involving FC11.

The cardiac toxicity of FC11, therefore, could be responsible of the observed acci-

TABLE 1—*Distribution of FC11 in biological specimens.*

Tissue	FC11 (mg/kg)
Blood	62.8
Brain	108.9
Lung	149.1
Heart	406.6
Liver	74.1
Kidney	50.2
Spleen	20.6
Urine	≤0.1

dental death, although hypoxemic asphyxiation due to fluorocarbon saturation of the room atmosphere may have been a contributing factor. The anatomo-histo-pathologic picture, showing a generic evidence of acute cardio-respiratory failure, is coherent with the suggested hypothesis, but it does not provide any further indication.

With regard to instrumental analysis, noteworthy is that the analytes are eluted from the column in less than 1 min, with an excellent selectivity. The high resolution and the peak symmetry achieved allowed the use of dichloromethane as internal standard, even if the difference between the retention times of the two peaks is less than 8 s. In the adopted conditions consecutive injections can be performed without switching off the mass detector filament, so that a large number of samples can be analyzed in a short time without any interference from other volatile endogenous or exogenous compounds. Furthermore the short scan range monitored (30 to 150 u) allowed a very high scan rate, so that the complete mass spectrum of FC11 was recorded even with a peakwidth of less than 1 s and an unequivocal identification of the fluorocarbon has been attained.

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