R. W. Urich,[1] *M.D.; P. H. Wittenberg,*[1] *M.D.;*
D. L. Bowerman,[1] *M.D.; J. A. Levisky,*[1] *M.S.; and*
J. L. Pflug,[2] *M.S.*

Electron Impact Mass Spectrometric Detection of Freon® in Biological Specimens

Recently the toxicity and abuse of aerosols containing Freon® have been brought to the attention of the public. Unpublished reports by the Drug Abuse Council indicate increased instances of aerosol addiction among the very poor, disenfranchised, and marginal minority groups. Also, deaths related to aerosols have been known for years. Bass [*1*] described 110 SSDs (Sudden Sniffing Deaths) during the 1960s, 59 of which were Freon-related. Christopoulos and Kirch [*2*] and Baselt and Cravey [*3*] demonstrated the presence of trichlorofluoromethane and dichlorodifluoromethane in postmortem specimens of Freon-induced deaths. The above authors used gas chromatographic (GC) techniques employing electron capture and flame ionization detectors (FID), respectively, for the quantitative analyses of these compounds. Standefer [*4*] also reported FID-GC data on specimens from a death associated with fluorocarbon inhalation.

Toxicity associated with legitimate use of pharmaceutical preparations has also been suggested. Dollery et al [*5*], using GC and mass spectrometry, reported blood fluorocarbon levels of 1.7 μg/ml in patients using preparations containing Freon propellants for the treatment of asthma. Fluorocarbon levels in blood have also been determined by conversion to inorganic fluoride and assayed with a specific ion electrode [*6*]. The physiologic and pathologic effects caused by Freons have been described by Bass [*1*] and others [*7–10*]. The structures of four Freons are shown in Fig. 1.

In this report we describe a simple, sensitive, and specific qualitative mass spectrometric

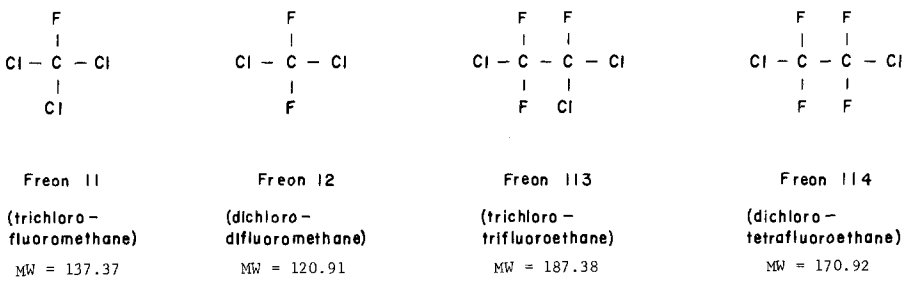


FIG. 1—*Structures of Freons.*

Received for publication 16 Feb. 1976; revised manuscript received 16 April 1976; accepted for publication 22 April 1976.

[1] Toxicologists, Toxicology Laboratory, St. Francis Hospital, Colorado Springs, Colo. 80903.
[2] Toxicologist, Frank J. Seiler Research Laboratory (AFSC), United States Air Force Academy, Colo. 80840.

analytical technique identifying Freons in blood, liver, lung, and brain of a 14-year-old girl who died after inhalation of the frying pan spray PAM®. Quantitation of the fluorocarbons was not done in this case because approximately 2½ h of cardiopulmonary resuscitative efforts occurred prior to death. Half-life studies of fluorocarbons in blood [*4,11–13*] indicate that approximately 1% of peak value remains after 100 min.

Methods and Materials

Blood, liver, brain, and lung specimens were obtained during autopsy. Approximately 3 g of each specimen were placed in evacuated blood collections tubes (15 ml) containing no additive. The tubes were stoppered, sealed, and frozen immediately and maintained in that state until analyzed to minimize the loss of the volatile compounds through evaporation.

Mass Spectra of Specimens

Just prior to analysis, the tube containing the specimen was removed from the freezer and immersed in a boiling water bath for 5 min. A closed system was maintained at all times. A portion of the gas (approximately 2.5 ml) from above the specimen was removed with a 10-ml Hamilton Gas-Tight syringe #1010, (Hamilton Co., Whittier, Calif.) and added through the septum of the gas sampling system to the small, 2.5-ml volume of the manifold system of the DuPont 21–491 double focusing mass spectrometer. This volume was allowed to enter the analyzer through the molecular leak while the pressure gage was monitored to maintain a pressure below 1×10^{-6} torr (1.3×10^{-4} Pa). The source temperature was 200°C, with the oven and transfer line at 145°C. The ionizing potential was 70 eV and the ionizing current 250 to 300 μA. The accelerating potential was 1100 V, coupled with an electric sector voltage of 100 V. The mass spectra were recorded on a CEC 5-124A recording oscillograph at a chart speed of 1 in./s (25 mm/s). A mass scan rate of 1000 s/decade in the linear scan mode was employed to produce the spectra. Peak assignment was made by using the DuPont digital mass marker which had been calibrated over the perfluorokerosene mass range.

Mass Spectra of Controls

To obtain the spectrum of the mixed Freons of PAM, a portion of the contents of the control PAM was sprayed into a blood collection tube and sealed. The vapors (approximately 2.5 ml) were introduced into the 2-litre expansion volume of the mass spectrometer and the spectrum was recorded (Fig. 2). The concentration of the mixed Freons in the expansion volume was lowered by pumping out a portion of the sample. Spectra were recorded at each successive reduction in concentration until one was obtained that closely matched that of the specimens. The background for the mass spectrometer was checked with the same operating conditions as those used for sample analysis. Also, the background between samples was checked and no peaks above $m/e = 44$ (CO_2 from air) were observed.

Results and Discussion

The molecular structures of the various Freon propellants used in many pressurized aerosols are shown in Fig. 1. The frying pan spray PAM is known to contain both Freon 11 and Freon 12 (trichlorofluoromethane and dichlorodifluoromethane). The mass spectrum of the mixed Freons obtained from a PAM control is shown in Fig. 2. The group of peaks at $m/e = 117$, 119, and 121 caused by the fragment ion $(CCl_3)^+$

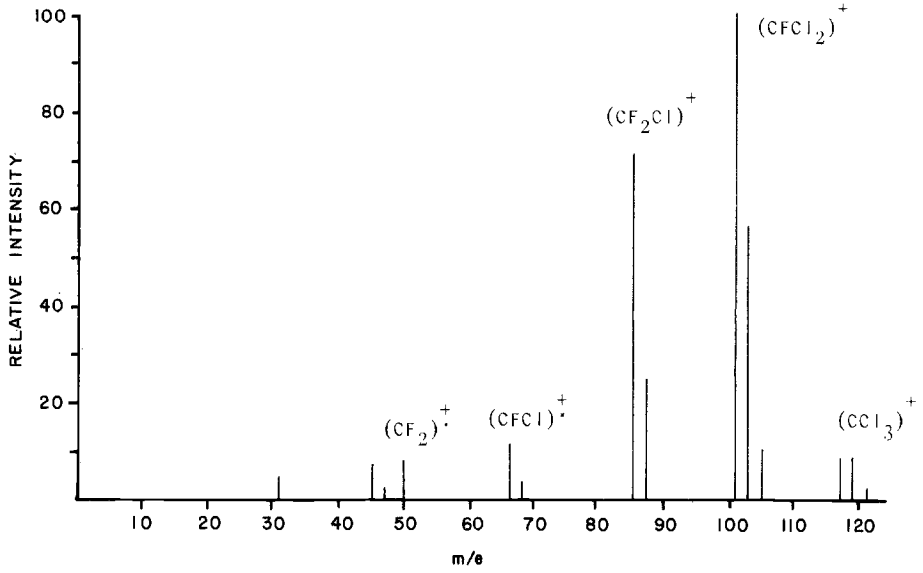
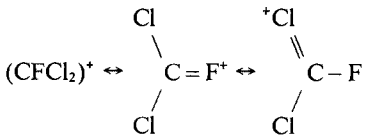


FIG. 2—*Mixed mass spectra of the Freons of PAM.*

arises from loss of fluorine from the molecular ion of trichlorofluoromethane. The isotope abundance ratio indicates that the ions giving rise to these peaks contain three chlorine atoms. The intensity of the fourth peak of this isotopic grouping, *m/e* = 123, is too low to be detected. The second group of peaks in the spectrum at *m/e* = 101, 103, and 105 is due to the ion $(CFCl_2)^+$ and arises from the fragmentation of the molecular ion of trichlorofluoromethane and dichlorodifluoromethane. Loss of chlorine from $(CCl_3F)^{\cdot+}$ and loss of fluorine from $(CCl_2F_2)^{\cdot+}$ result in the formation of this ion. A third group of peaks is detected at *m/e* = 85 and 87.

Loss of chlorine from $(CCl_2F_2)^{\cdot+}$ results in formation of the fragment ion $(CClF_2)^+$. Other *m/e* peaks are noted at 66 and 68 from $(CFCl)^{\cdot+}$; at 50, from $(CF_2)^{\cdot+}$; at 47 and 45, from $(CCl)^+$; and at 31, from $(CF)^+$. The absence of the molecular ions of both Freons in the spectrum is not surprising. Halogenated hydrocarbons do not exhibit significant molecular ions [*14*]. Loss of a halogen atom is a facile process under electron impact, and the resulting positive ions are stabilized by back donation of a nonbonding electron pair from a remaining halogen atom.



The fragmentation pathway leading to the observed *m/e* peaks of the spectrum of the mixed Freons of control PAM is presented in Fig. 3.

A qualitative comparison between known control PAM and qualitatively identified components from the specimens was made. Figure 4 shows this comparison. Figure 4*a* is a reproduction of the most sensitive galvanometer tracing of the mass spectrum of a very low concentration of the mixed Freons of the PAM control. As shown, the spectrum of the reduced concentration still exhibits the characteristic groups of peaks at *m/e* values of 101, 103, and 105 and at *m/e* = 85 and 87. The higher and less intense *m/e* peaks of

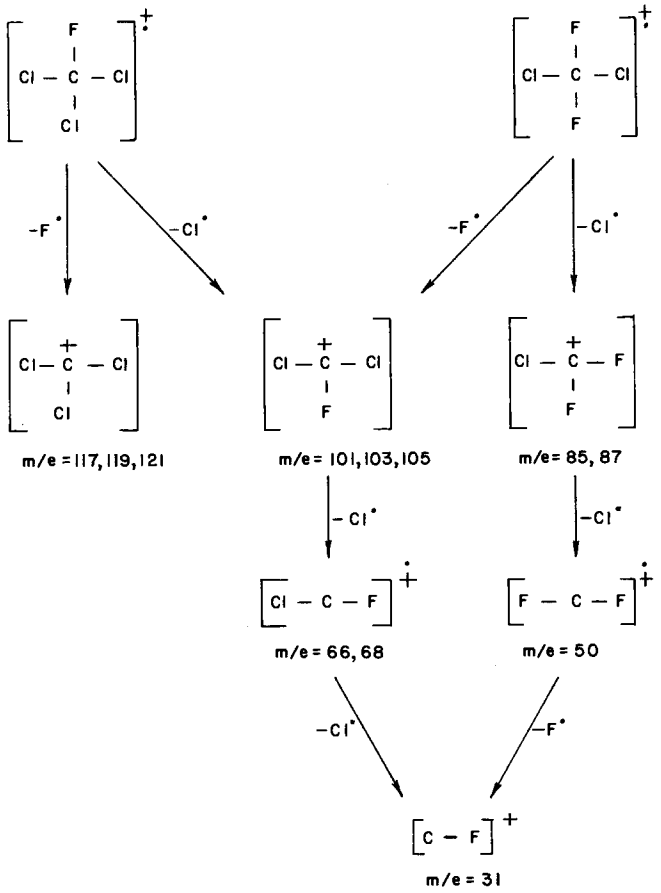


FIG. 3—*Fragmentation pathway for the Freons of PAM.*

Freon 11 fragmentation are absent. The mass spectra of the postmortem specimens of blood, liver, brain, and lung are displayed in Figs. 4*b–e*. Comparison of PAM with the test specimens reveals the same characteristic groups of peaks. The fluorocarbon *m/e* peaks in the spectra of the specimens account for approximately 0.2% of the total ion current. Peaks from moisture and air account for the remainder of the ion current.

As noted in the fragmentation pathway (Fig. 3), the *m/e* peaks at 101, 103, and 105 can arise from fragmentation of both Freon 11 and 12 or from only Freon 12. We interpret the peaks in the spectra of the specimens as arising from fragmentation of both Freon species. The reported mass spectrum of Freon 12 [*15*] gives *m/e* = 85 as the base peak. The spectra of the specimens show the 101 peak as the base. Therefore, it follows that fragmentation from two Freon species contributes to the intensity of the peak at 101 rather than fragmentation resulting from only Freon 12. Freons 113 and 114 fragment to form the same *m/e* peaks [*15*], but these fluorocarbons are absent in the frying pan spray PAM.

The isotope abundance ratio for the group of peaks at 101, 103, and 105 in the spectra of the specimens differs slightly from the calculated ratio. In practice, the measured isotope peaks are usually slightly different from the calculated contributions because of incomplete resolution, bimolecular collisions, or impurities [*16*].

The increased incidence of abuse of Freon as well as other volatiles has prompted us

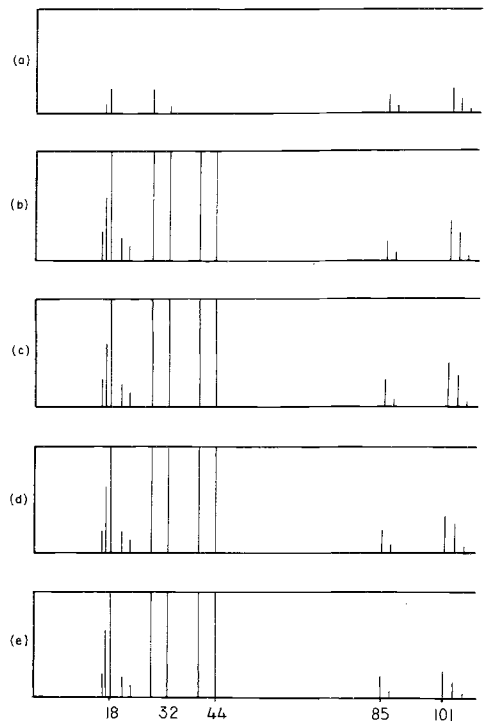


FIG. 4—*Reproductions of the mass spectra of* (a) *low concentrations of control PAM;* (b) *volatiles from blood*; (c) *volatiles from liver*; (d) *volatiles from brain*: *and* (e) *volatiles from lung.*

to screen for this class of compounds as part of a toxicologic examination where postmortem anatomical findings do not establish the cause of death. The headspace mass spectrometric analytical procedure described is not limited to the detection of fluorocarbons. Many volatile compounds in biological specimens can be detected. Unpublished data from our laboratory show volatiles such as low molecular weight halogenated hydrocarbons, benzene, toluene, diethylether, methylethylketone, and acetonitrile at concentrations of 1 to 10 ppm in blood-enriched samples can readily be detected. Diethylether and toluene have been detected, identified, and confirmed in postmortem specimens using this analytical technique.

Summary

Freons from an aerosol spray can were detected in the blood, liver, brain, and lung of a 14-year-old girl who died after intentional inhalation. A headspace mass spectrometric analytical technique was employed to detect the fluorocarbons. The spectra from the specimens showed the presence of *m/e* peaks at 101, 103, and 105 from the ion $(CFCL_2)^+$ which arises from fragmentation of trichlorofluoromethane (Freon 11) and dichlorodifluoromethane (Freon 12), and peaks at 85 and 87 from the ion $(CF_2Cl)^+$ which arises from fragmentation of dichlorodifluoromethane (Freon 12). The technique presented here provides greater specificity than previously reported analytical procedures for the identification of these volatile toxic chemical compounds in biological specimens.

Acknowledgments

We wish to acknowledge the assistance of Mrs. Turza Pflug in preparing the

illustrations. Also special thanks to Ms. Sherry Abeyta and Mrs. Carol Rogers for clerical assistance.

References

[1] Bass, M., "Sudden Sniffing Death," *Journal of the American Medical Association,* Vol. 212, No. 12, 1970, pp. 2075–2079.

[2] Christopoulos, G. N. and Kirch, E. R., "Estimation of Fluoroalkane Propellants," *Journal of Forensic Sciences,* Vol. 19, No. 1, 1974, pp. 168–171.

[3] Baselt, R. C. and Cravey, R. M., "A Fatal Case Involving Trichloromonofluoromethane and Dichlorodifluoromethane," *Journal of Forensic Sciences,* Vol. 13, No. 3, 1968, pp. 407–410.

[4] Standefer, J. C., "Death Associated with Fluorocarbon Inhalation: Report of a Case," *Journal of Forensic Sciences,* Vol. 20, No. 3, 1975, pp. 548–551.

[5] Dollery, C. T., Draffan, G. H., Davies, D. S., Williams, F. M., and Conolly, M. E., "Blood Concentrations in Man of Fluorinated Hydrocarbons After Inhalation of Pressurized Aerosols," *Lancet,* Vol. 2, 1970, pp. 1164–1166.

[6] Stein, T. P., Robbins, W. K., Brooks, H. B., and Wallace, H. W., "Determination of Fluorocarbon in Blood," *Journal of Biomedical Materials Research,* Vol. 9, No. 5, 1975, pp. 479–485.

[7] Taylor, G. J., IV and Harris, W. S., "Cardiac Toxicity of Aerosol Propellants," *Journal of the American Medical Association,* Vol. 214, No. 1, 1970, pp. 81–85.

[8] Taylor, G. V., IV, Harris, W. S., and Bogonoff, M. D., "Ventricular Arrhythmias Induced in Monkeys by Inhalation of Aerosol Propellants," *Journal of Clinical Investigation,* Vol. 50, No. 1, 1972, pp. 33–37.

[10] "Health Hazards of Aerosol Propellants," *FDA Drug Bulletin,* Vol. 5, No. 4, 1975.

[11] Patterson, J. W., Sudlow, M. F., and Walker, S. R., "Blood Levels of Fluorinated Hydrocarbons in Asthmatic Patients After Inhalation of Pressurized Aerosols," *Lancet,* Vol. 2, 1971, pp. 565–568.

[12] McClure, D. A., "Failure of Fluorocarbon Propellants to Alter the Electrocardiogram of Mice and Dogs," *Toxicology and Applied Pharmacology,* Vol. 22, No. 2, pp. 221–230.

[13] Morgan, A., Black, A., Walsh, M., and Belcher, D. F., "Studies on the Absorption of Inhaled Fluorocarbon Propellant Vapors Using ^{38}Cl Tracer Techniques," *British Journal of Radiology,* Vol. 45, No. 525, 1972, p. 630.

[14] Budzikiewicz, H., Djerassi, C., and Williams, D. H., *Interpretation of Mass Spectra of Organic Compounds,* Holden-Day, Inc., San Francisco, 1964, p. 130.

[15] Grasselli, J. G., Ed., *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds,* CRC Press, Cleveland, March 1974.

[16] Silverstein, R. M. and Bassler, C. G., *Spectrometric Identification of Organic Compounds,* 2nd ed., Wiley, New York, 1967, p. 10.

Toxicology Laboratory
St. Francis Hospital
Colorado Springs, Colo. 80903