William T. Lowry,[1] Ph.D., D.A.B.F.T.; Beryl Gamse,[2] Ph.D., P.E.; Andrew T. Armstrong,[3] Ph.D., C.P.C.; John M. Corn,[3] M.S.; Louis Juarez,[4] B.S.; Jean L. McDowell,[2] B.S.; and Roger Owens,[2] B.S.E.E., M.E.E., P.E.

Toxicological Investigation of Liquid Petroleum Gas Explosion: Human Model for Propane/Ethyl Mercaptan Exposures

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ABSTRACT: Four individuals died as the result of a propane explosion. As with many propane explosions, the question was raised as to the adequacy of the product's odorization after the autopsy studies had been conducted. In most cases, this question leads to litigation. Ethyl mercaptan is a widely used odorant for propane and was used in this instance. Three of the four victims had blood available at autopsy for study. Quantitative analyses of the victims' blood, obtained during autopsy, were performed using gas chromatography/mass spectrometry, without subjecting the samples to hydrolysis. These analyses determined the relative amounts of propane and ethyl mercaptan in the blood to be 90, 63, and 175 mL/m^3 headspace, and 0.36, 0.34, and 0.77 μg/L blood, respectively.

Since mercaptans have been reported in human blood as products of metabolism, modeling studies were conducted to establish the validity of the autopsy data and to develop an autopsy toxicology protocol for investigating explosion deaths. When subjects were not exposed to an atmosphere containing ethyl mercaptan, dimethylsulfide was the only mercaptan detectable in their blood without severe hydrolysis prior to analysis. Metabolic ethyl mercaptan is sufficiently bound to be undetectable by the methods used without hydrolysis. Human subjects were exposed to a flammable mixture of air and propane odorized with ethyl mercaptan. The analyses of the blood from these subjects produced results which were comparable with those for the explosion victims, establishing that the question of odorant adequacy can be addressed at the autopsy of propane explosion victims. It is extremely important that the pathologist and toxicologist investigating gas explosion deaths recognize the valuable evidence existing in the victim's blood.

KEYWORDS: toxicology, propane, ethyl mercaptan, blood

An explosion occurred, resulting in the deaths of four individuals. Propane [liquid petroleum gas (LPGas)] was suspected to be the fuel gas for the explosion. As with many

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[1]Toxicologist, William T. Lowry, Ph.D., Inc., Arlington, TX.

[2]Engineer, fire scientist/president, and engineer/vice president, respectively, McDowell Owens Engineering, Inc., Kingwood, TX.

[3]Certified chemist/president and senior chemist, respectively, Armstrong Forensic Laboratory, Inc., Arlington, TX.

[4]Environmental chemist, Institute of Forensic Sciences, Dallas, TX.

propane explosions, the question was raised as to the adequacy of the products's odorization. However, this question was not raised until autopsy studies had already been conducted.

Since propane is an odorless gas, odorants are used to produce a smell for warning purposes. Without adequate warning, a potential liability exists. Ethyl mercaptan is a widely used odorant for propane and had been used in this instance. After an explosion occurs, there is very little evidence of the gas remaining to determine the adequacy of odorization. However, if victims are involved, evidence of the atmosphere which they were breathing remains in the blood. In this case, at autopsy, three of the four victims had blood available for toxicology studies.

The purpose of this study was to confirm the autopsy findings and to develop an autopsy toxicology protocol for investigation of gas explosion deaths.

Autopsy Toxicology

Toxicology studies of the blood received from autopsy produced the results set forth in Table 1. The autopsy data are represented in Fig. 1.

TABLE 1—*Results of blood toxicology studies.*

Subject	Component	Concentration
Victim 1	propane	90 mL/m^3 headspace
	ethyl mercaptan	0.36 μg/L blood
Victim 2	propane	63 mL/m^3 headspace
	ethyl mercaptan	0.34 μg/L blood
Victim 3	propane	175 mL/m^3 headspace
	ethyl mercaptan	0.77 μg/L blood

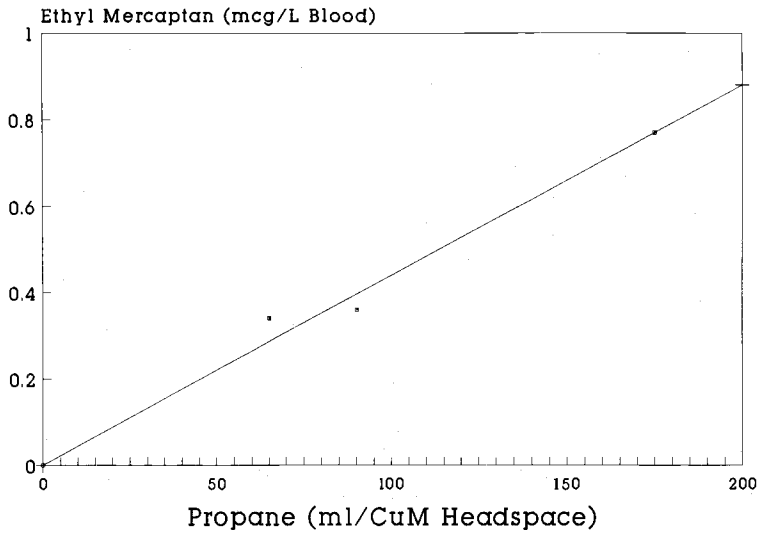

FIG. 1—*Autopsy data for propane and ethyl mercaptan.*

Propane

Propane is an odorless, colorless, and flammable gas. Ethyl mercaptan is used as an odorant for propane gas in commercial use. The flammable limits of propane range from 2.15 to 9.6% [*1*].

Propane is a simple anesthetic and is nonirritant to skin and eyes. At air concentration levels below 1000 ppm, propane exerts very little physiological action. At very high levels (that is, >80%), propane has narcotic and asphyxiating properties. Cases of sudden death have been reported in which propane and propylene were identified in blood, urine, and cerebrospinal fluid.

Animal inhalation studies indicate that a gas concentration of 89% (890 000 ppm) is below the anesthetic level but sufficient to depress the blood pressure of cats. Studies with guinea pigs produced sniffing and chewing movement at 2.2 to 5.5%, with a rapidly reversible effect after cessation of exposure. A 1% concentration causes hemodynamic changes in dogs. Levels of 3.3% decrease inotropism of the heart, which produces a decrease in mean aortic pressure, stroke volume, and cardiac output and an increase in pulmonary vascular resistance. In the primate, 10% propane induces some myocardial effects, and 20% produces aggravation of these parameters and respiratory depression. Ten percent propane in the mouse and 15% in the dog appear to produce no arrhythmia but weak cardiac sensitization [*1*].

In order to prepare a human model study of exposure to propane, the authors developed criteria which allowed exposure above the lower explosive limit but below the level which would cause cardiotoxic effects. The health safety level chosen was the range of 2.3 to 5.0%, with a nominal safety exposure level of 2.5%.

Experimental Procedure

Exposure Protocol

Studies of human exposure to propane gas, under controlled conditions, were conducted utilizing six volunteer subjects, five males and one female. Blood specimens were collected into 10-mL red-top Vacutainer tubes. The pulse and respiration rates of each subject were recorded throughout the study. Blood samples were collected before the exposure for controls and after 5 min of exposure but before removal of the mask. The blood samples were placed in a water/ice bath and transported to the laboratory. All samples were taken in duplicate.

Exposure Apparatus

A propane/air mixture was delivered to the subjects using a Heidbrink Kinet-O-Meter anesthesia machine fitted with a Trimar nose/mouth mask with an inflatable edge seal, both manufactured by Ohio Medical Products. The recirculation system of the anesthesia machine was bypassed so that a fresh gas mixture was always supplied to the subjects. The exhaled gases were exhausted directly to the atmosphere.

The propane concentration in the breathing air was maintained at a constant level, determined by an Explosimeter Model 2A combustible gas indicator, manufactured by Mine Safety Appliances Co. The air flow was set at 21.3 L/min, and minor adjustments were made to the propane flow to maintain an indication of 100% of the lower explosive limit on the combustible gas indicator. The actual exposure concentration was determined by sample collection and analysis. The average propane exposure concentration was 3.5% by volume. The average ethyl mercaptan exposure concentration indicated was at least 0.123 ppm by volume.

The propane was obtained from Scott's Speciality Gases and odorized with ethyl mercaptan at a concentration of 24.5 ppm by weight (17.3 ppm by volume). It was stored in a 15.7-L-capacity aluminum cylinder in the liquid phase and taken from the cylinder headspace in a gaseous phase. As liquid propane odorized with ethyl mercaptan is vaporized, the concentration of the odorant increases because of the different vaporization characteristics of the two materials. The effect is considered negligible for these tests since only about 1% of the propane supply was used, which would result in an approximate 0.8% increase in the odorant concentration. The air supply was a steel cylinder of compressed air conforming to the requirements described in the American National Standards Institute Commodity Specifications for Air Type 1, Grade D (ANSI Z86.1).

Prior to exposure of the subjects, the propane and air flow rates were set. This mixture was allowed to flow continuously for approximately 1.5 h, during which time all six subjects were exposed. Only minor adjustments were made to the propane flow rate to maintain the indicated gas mixture. Each subject breathed the propane/air mixture while standing beside the anesthesia machine and holding the mask firmly over his or her nose and mouth.

Subject No. 4 set up the anesthesia machine, calibrated the gases, and monitored the instruments throughout the test. Extra exposure was possible.

Analytical Procedure

Whole blood samples were collected in red top Vacutainer blood collection tubes and chilled to 4°C. The samples were kept cool at all times, but not frozen, to prevent loss of blood gases. One set of samples was analyzed within 24 h of collection (initial testing). Another set of blood samples was analyzed 7 days later for ethyl mercaptan and 10 days later for propane (delayed testing).

Blood Propane—A 10-mL portion of each sample was transferred to a 20-mL volatile organic vapor vial and heated at 40°C for 30 min to allow headspace equilibration prior to transferring 1000 μL by gas-tight syringe to the gas chromatograph. The gas chromatograph was a Tracor 540 with a flame ionization (FID) detector. The carrier gas was helium with a linear velocity of 2.3 cm/s into a Supelco, SPB-1 column (100 m by 0.25 mm by 0.5 p). The conditions were isothermal, at 40°C, with a split ratio of 50:1, and range 1. The data system was a Nelson 2600. The water bath was Blue M, MW1110A1. Scott's Speciality Gases were utilized as standards, including Mix 1 (nominal, 10 ppm), Mix 220 (nominal, 100 ppm), and Mix 224 (nominal, 1000 ppm).

The results are reported as millilitres of propane per cubic metre of headspace (that is, parts per million of headspace volume). The detection limit for propane in the headspace was 1.5 mL/m^3.

Breathing Gas Propane—Tedlar bag samples of the breathing gases were analyzed for C_1 through C_4 hydrocarbons by direct injection using the same analytical conditions described for the blood propane procedure. The results are reported as millilitres of propane per cubic metre of gas sample (that is, parts per million by volume). The detection limit for propane in the gas sample was 1.5 mL/m^3.

Blood Ethyl Mercaptan—The samples were analyzed using a Techmar LSC-2 purge and trap concentration apparatus connected to a Hewlett-Packard 5890 gas chromatograph/5970 mass selective detector system. Data were collected on a Hewlett-Packard Chem Station.

Two millilitres of mixed whole blood and 3 mL of deionized water were transferred to the purge-and-trap sparger. The sample was purged with helium at a rate of 30 mL/min for 11 min on a trap. The trap, an 8 by ⅛ in. (20 cm by 3 mm) stainless steel tube

packed with 60/80 mesh Chromasorb 104 (Johns Manville), was held at ambient temperature during the purging process. The trap was thermally desorbed for 2.0 min at 200°C, then baked out for 5 min at 250°C.

The gas chromatograph column was a 30-m J&W DB-624 fused silica capillary column with a 0.53-mm film thickness. The column pressure was 4 lbf/in^2 (27.58 kPa). The column temperature was programmed from 30 to 250°C at a rate of 8°C/min, with an initial hold of 2.0 min.

Under the above analytical condition, ethyl mercaptan dimerizes to diethyl disulfide and elutes at 15.1 min. Data were collected and quantified by single ion monitoring of Masses 66 and 122. The results are reported as micrograms of ethyl mercaptan per litre of blood (that is, parts per billion by weight of blood). The detection limit for ethyl mercaptan in the blood was 0.1 μg/L.

Breathing Gas Ethyl Mercaptan—Mercaptan analysis of the breathing air was carried out utilizing Occupational Safety and Health Administration (OSHA) Method 26, which proceeds via absorption of the gas on mercuric acetate filters with subsequent quantification by a Perkin-Elmer 2000 series gas chromatograph with a flame photometric detector. The concentrations are reported in micrograms of ethyl mercaptan per cubic metre of gas sample and in parts per million by volume of the gas sample. The detection limit for ethyl mercaptan in the gas sample was 0.05 ppm.

Results and Discussion

The respiration rate of each subject was determined to allow individual dosage. The individual data are set forth in Table 2. These data were collectively evaluated with the individual's blood propane and ethyl mercaptan concentrations and are represented in Figs. 2 and 3, respectively.

Exposure Data

The results of the initial blood analyses for propane and ethyl mercaptan are set forth in Tables 3 and 4, respectively.

In many cases, toxicology studies cannot be conducted immediately after autopsy. In order to evaluate the storage effect of blood samples on blood gases, a second set of blood samples were collected. The second set was analyzed seven and ten days after collection for ethyl mercaptan and propane. The results of the delayed testing for propane are set forth in Table 5 and those for the ethyl mercaptan data in Table 6.

The blood propane values are shown in Fig. 4 and the blood ethyl mercaptan values in Fig. 5. The data were plotted with the ethyl mercaptan concentration as a function of the propane concentration and are presented in Fig. 6 for initial testing and in Fig. 7 for

TABLE 2—*Individual pulse and respiration data for each subject.*

Subject	Pulse/min	Respiration/min
1	64	12
2	98	22
3	98	16
4	64	11
5	72	25
6	72	20

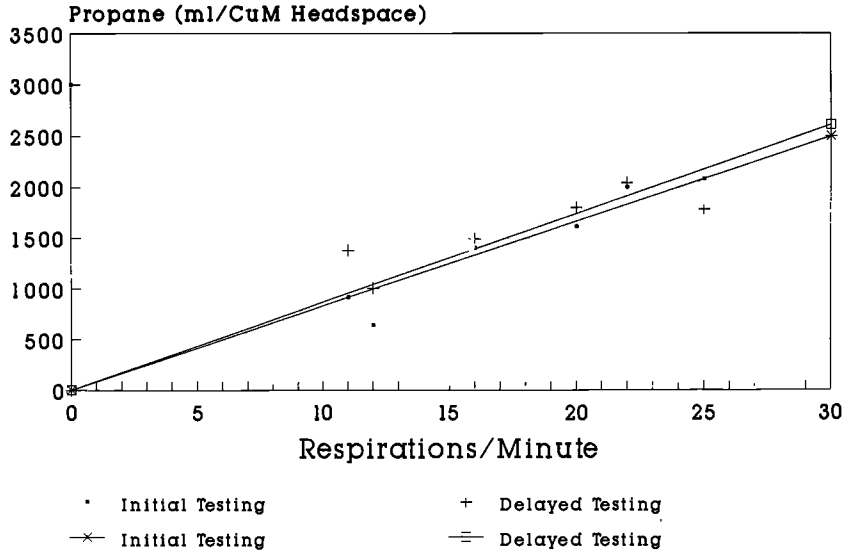

FIG. 2—*Propane blood concentration versus respiration.*

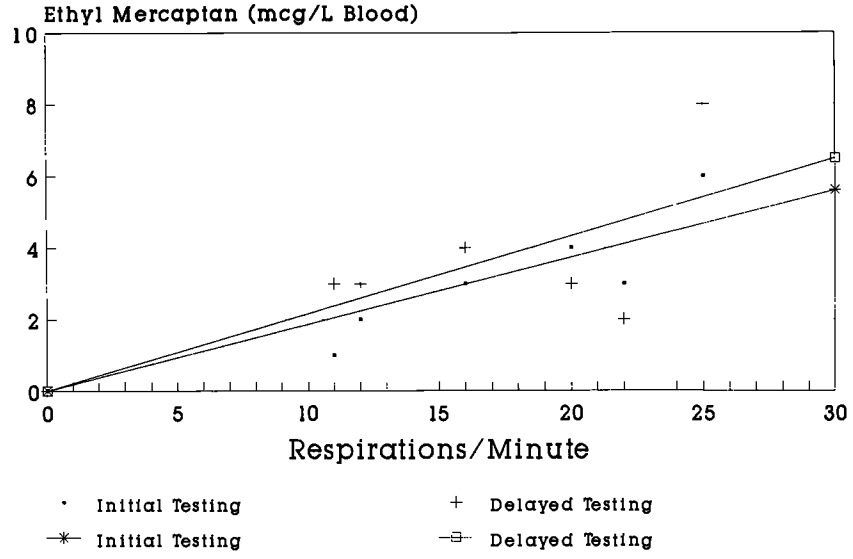

FIG. 3—*Ethyl mercaptan blood concentration versus respiration.*

TABLE 3—*Results of initial blood analyses for propane.*

Subject	Control, mL/m³	Exposure, mL/m³
1	10	639
2	11	2000
3	8	1400
4	29	916
5	10	2080
6	8	1612

TABLE 4—*Results of initial blood analyses for ethyl mercaptan.*

Subject	Control, μg/L	Exposure, μg/L
1	<0.1	2
2	<0.1	3
3	<0.1	3
4	<0.1	1
5	<0.1	6
6	<0.1	4

TABLE 5—*Results of delayed testing for propane.*

Subject	Control, mL/m³	Exposure, mL/m³
1	8	1007
2	8	2045
3	7	1494
4	30	1380
5	8	1784
6	81	1803

TABLE 6—*Results of delayed testing for ethyl mercaptan.*

Subject	Control, μg/L	Exposure, μg/L
1	<0.1	3
2	<0.1	2
3	<0.1	4
4	<0.1	3
5	<0.1	8
6	<0.1	3

delayed testing. A comparison of the ethyl mercaptan concentration as a function of the propane concentration for the autopsy, initial testing, and delayed testing data is made in Fig. 8.

It is quite obvious that the collection and storage of blood samples will not affect the results of analyses for propane and ethyl mercaptan.

Autopsy Control Studies

Blood analyses of ten randomly selected autopsy cases were conducted for the presence of ethyl mercaptan as a secondary control. Ethyl mercaptan was not detected in these control samples. Dimethyl sulfide was detected in each sample.

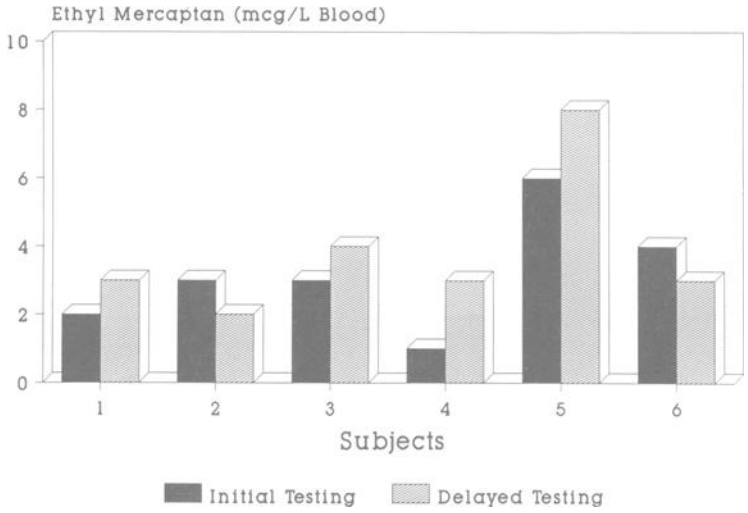

FIG. 4—*Propane blood concentrations.*

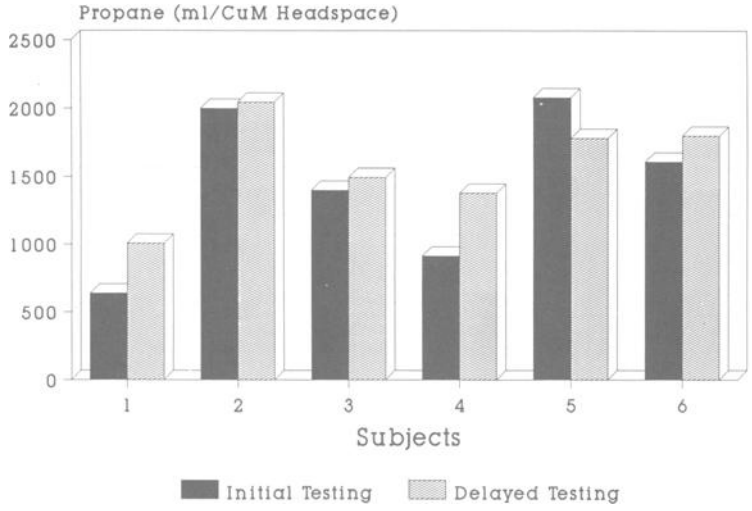

FIG. 5—*Ethyl mercaptan blood concentrations.*

Blood Concentrations of Natural Mercaptans

Blood concentrations of mercaptans have been studied in relation to their role in hepatic encephalopathy [*2–4*]. The concentrations of blood of the mercaptans methanethiol, ethanethiol (ethyl mercaptan), and dimethyl sulfide in patients with different grades of hepatic coma were evaluated and compared with values in rats in coma after an intraperitoneal injection of methanethiol [*2*]. Also, blood mercaptan concentrations in patients

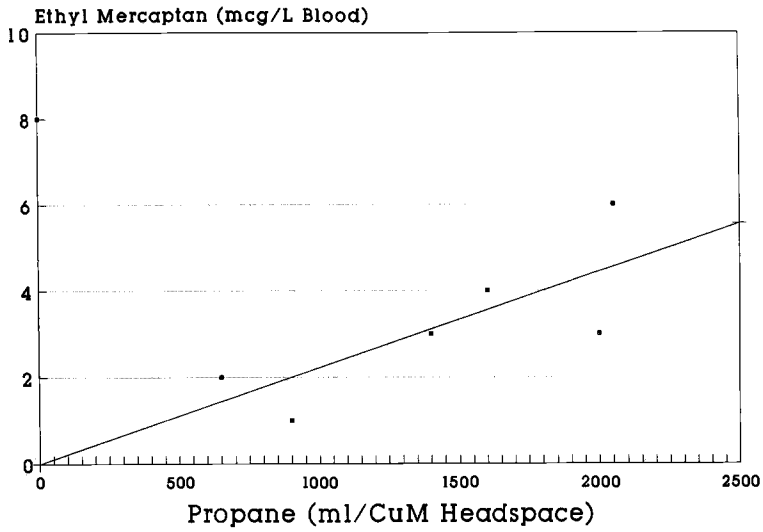

FIG. 6—*Propane versus ethyl mercaptan—initial testing results.*

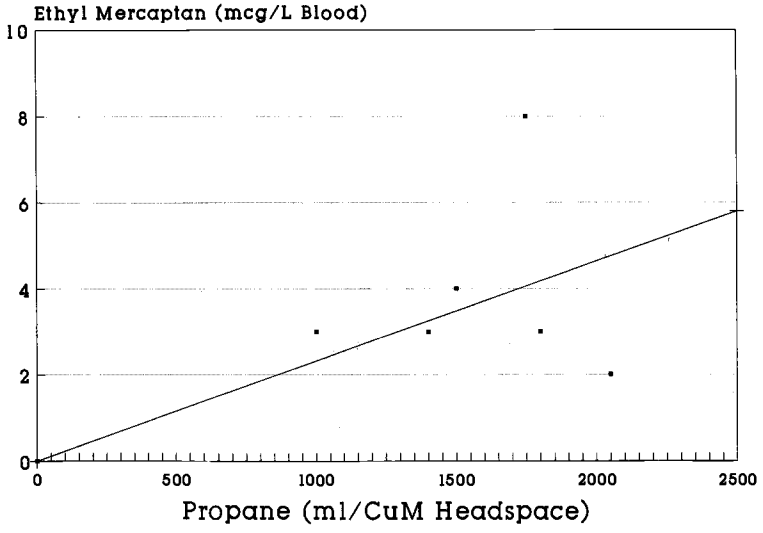

FIG. 7—*Propane versus ethyl mercaptan—delayed testing results.*

with liver disease were divided into those exhibiting hepatic encephalopathy and nonencephalopathic control subjects. The results were compared with 29 normal subjects [*4*] and are given in Table 7.

The methods for mercaptan determinations reported [*2–4*] required significant hydrolysis utilizing zinc and phosphoric acid with heat (60°C) for 10 min to release the bound mercaptans. Without hydrolysis, ethyl mercaptan concentrations were below the detection limits of the gas chromatograph with a flame ionization detector (FID) [*4*].

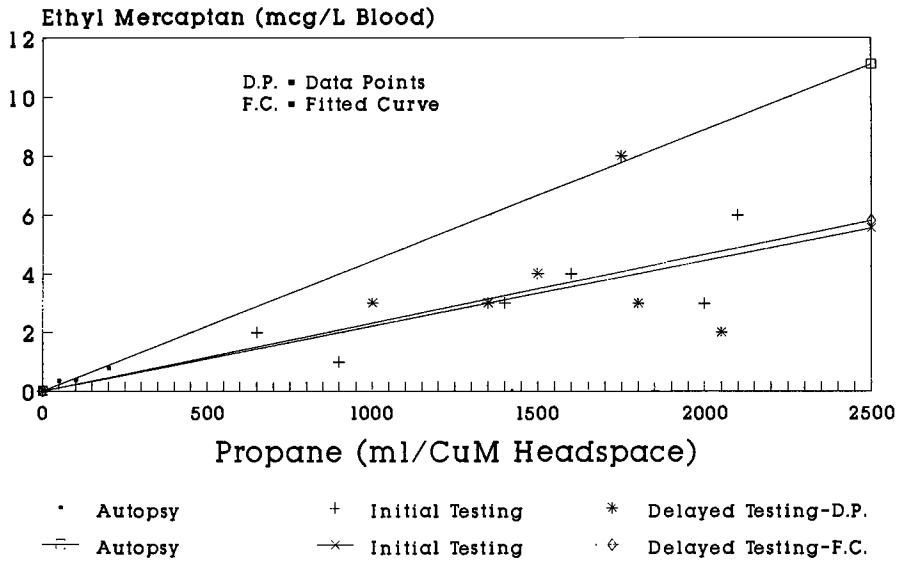

FIG. 8—*Propane versus ethyl mercaptan—combined data.*

TABLE 7—*Comparison of blood mercaptan concentrations for normal subjects and for patients with liver disease.*

Mercaptan	Normal Subjects, μmol/L	Subjects with Liver Disease, μmol/L
Methanethiol	5.7	7.7
Ethanethiol	0.3	0.2
Dimethyl sulfide	0.4	0.6

Dimethyl sulfide is a neutral molecule that is not covalently bound in serum or blood. It exists in the "free" form in blood. It is detected in normal blood and is easily distinguished from the other mercaptans by its R_f value [*3*].

Conclusions

An explosion occurred, resulting in the deaths of four individuals. Autopsy toxicology produced quantitative levels of propane and ethyl mercaptan indicating that the individuals had been exposed to odorized liquid propane gas (LPgas) prior to the explosion.

Controlled studies of human exposure to odorized LPgas produced results within experimental error to confirm the autopsy data. Analysis immediately after exposure did not produce significantly different results from delaying analysis one week, signifying that storage of blood samples will not affect the analytical results.

Mercaptans have been reported in human metabolism, especially in the role of pathogenesis of hepatic coma. However, all reported methods require severe hydrolysis to produce unbound volatile mercaptans in the blood at a limit detectable by gas chromatography [*2–4*]. With hydrolysis, the mean ethyl mercaptan level in normal blood was

0.3 μmol/L (18.6 μg/L) [4]. Without hydrolysis, the ethyl mercaptan concentration was below the detection limit [4].

Autopsy control studies found no detectable ethyl mercaptan, but did identify dimethyl sulfide, as expected.

For the plots of propane and ethyl mercaptan concentrations as a function of respiration rate (Figs. 2 and 3), straight lines through the origin were fitted using the least squares method. The data trends indicate that blood saturation was not reached for either substance for the propane and ethyl mercaptan concentrations used, the 5-min exposure time, and the range of respiration rates experienced.

These results indicate that ethyl mercaptan and propane enter the blood in proportion to their exposure concentrations, as evidenced by the linear relationship established. Straight lines were also fitted to the plots of blood ethyl mercaptan concentration as a function of propane concentration (Figs. 1, 6, and 7). The slope of the line for the autopsy data (Fig. 1) is 0.0044. The slopes of the fitted lines for the initial and delayed testing of the human exposure tests (Figs. 6 and 7) are 0.0022 and 0.0023, respectively.

The results indicate that the ratio of ethyl mercaptan to propane in the atmosphere to which the explosion victims were exposed was at least as great and probably greater than that in the atmosphere to which the experimental subjects were exposed.

The odorization with ethyl mercaptan of the LPgas in the explosive atmospheres to which explosion victims were exposed immediately before death can be determined. This determination may be made at autopsy by blood analyses for volatile propane and ethyl mercaptan without hydrolysis. The LPgas to which the individuals were exposed for a few minutes in the case described herein was adequately odorized with ethyl mercaptan.

It is extremely important that the pathologist and toxicologist investigating gas explosion deaths recognize the valuable evidence existing in the victim's blood.

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Address requests for reprints or additional information to
William T. Lowry
William T. Lowry, Ph.D., Inc.
733 B North Fielder Rd.
Arlington, TX 76012