CASE REPORT

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Fatal and Nonfatal Poisoning by Hydrogen Sulfide at an Industrial Waste Site

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ABSTRACT: An adult man (A) entered a pit to collect seepage at an industrial waste site in Japan. As he suddenly lost consciousness, three colleagues (B, C, D) entered the pit to rescue him. All of these men lost consciousness in the pit. Two workers (A and B) died soon after the accident, one worker (C) died 22 days after the accident, and one worker (D) survived. Since hydrogen sulfide gas was detected in the atmosphere of the pit, gas poisoning was suspected. Toxicological analyses of sulfide and thiosulfate, a metabolite of sulfide, in blood and urine of the victims were made using the extractive alkylation technique combined with gas chromatography/mass spectrometry (GC/MS).

Sulfide was detected in the blood of A and B at levels of 0.13 and 0.11 mg/L, respectively, somewhat higher than in healthy persons. Thiosulfate was detected in whole blood of deceased victims A and B, in the plasma of deceased victim C, at concentrations of 10.53, 4.59, and 4.14 mg/L, respectively. These values were similar to those found in fatal cases of hydrogen sulfide poisoning. Thiosulfate was not detected in the plasma of survivor D. With respect to urine samples, thiosulfate was the highest in the non-acute death victim C (137.20 mg/L), followed by that in the survivor D (29.34 mg/L), and low (0.90 mg/L) and not detected in the acute death victims, A and B, respectively.

Based on these results, all four patients were victims of hydrogen sulfide poisoning. The concentrations of thiosulfate in blood and urine were more useful than that for sulfide for determining hydrogen sulfide poisoning. Thiosulfate in urine was the only indicator of hydrogen sulfide poisoning in the non-fatal victim.

KEYWORDS: forensic science, forensic toxicology, hydrogen sulfide, thiosulfate, metabolite, analysis, gas chromatography/mass spectrometry

Hydrogen sulfide poisoning is observed by forensic scientists. While identification of sulfide can be made in cases of fatal poisoning (1–8), this compound is unstable and is rapidly metabolized within the human body (9–11). Therefore it is difficult to detect sulfide from human samples in cases of a nonfatal poisoning. There are also problems related to postmortem production of sulfide as blood and tissues putrefy (12). We reported the usefulness of analyzing thiosulfate, a sulfide metabolite, in blood and urine when we confirmed hydrogen sulfide poisoning in nonfatal and fatal cases (7,8,13).

We describe here fatal and nonfatal hydrogen sulfide gas poisoning at a site where stable industry waste was being dumped. The sulfide and thiosulfate concentrations in blood and urine of four victims were determined.

Case Report

Gas poisoning occurred at a site used to dump industrial waste. Only stable waste was gathered to this plant. The seepage from the waste had flowed into a pool at the site up to the day before the accident. On the day of the accident, the seepage was introduced into a pit (length 1.5 m, width 1.3 m, depth 5.5 m) through a pipe placed 38 cm deep. The seepage in the pit was pumped out for the waste water treatment, and was left at a depth of 14 cm. Figure 1 is a diagram of the pit. The seepage was analyzed daily for pH and sulfide concentration, and other pollutants. An adult man (A) entered the pit to collect the seepage. As he suddenly lost consciousness, three colleagues (B, C, D) entered the pit one after another to rescue him. Worker B entered the pit first, then Worker C, then Worker D. All lost consciousness in the pit, and were sent to two hospitals. Two workers (A and B) soon died, one worker (C) died 22 days after the accident, and one worker (D) survived.

Concentrations of hydrogen sulfide and oxygen in the pit were measured 6 h after the accident, using the same conditions as those used at the time of the accident. A gas detector tube (Gastec Type No.4H 10 to 4000 ppm) and XP-302II model gas analyzer (Shin cosmos Electric Japan) were used for hydrogen sulfide and oxygen determination, the values being 1400 ppm and 21%, respectively. Sulfide concentration and pH of the seepage on the previous day were 30 mg/L and 6.5, respectively.

Autopsies on A and B were carried out 7 and 8 h after the accident, respectively. A was 173 cm tall and weighed 68 kg while B was 176 cm tall and weighed 88 kg. Serious damage and disease were not observed in either victim, and whole blood was collected from each cadaver at the time of autopsy. The plasma samples of C and D were collected at the hospital about 2 and 6 h after the acci-

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TABLE 2—*Thiosulfate concentrations in urine samples from four victims (mg/L).*

Compound	Victims			
	A	B	C	D
Thiosulfate	0.90	N.D.*[1]	137.20	29.34
Time of sample collection*[2]	2	2	2	2

N.D. = not detected (*[1]; below 0.3 mg/L). *[2] = hours after the accident.

hydrogen sulfide gas poisoning. It was thus proven that he was in a serious condition when plasma and urine samples were collected, 2 h after the accident.

Thiosulfate was not detected in the plasma of D (Table 1). The concentration of thiosulfate in the urine of D was 29.34 mg/L (Table 2), nine times higher than the level in healthy persons (17). The thiosulfate concentration of D was similar to those noted in non-fatal cases (7). Therefore, the cause of unconsciousness of D was attributed to hydrogen sulfide gas poisoning. Thiosulfate was stable in plasma samples as well as in whole blood samples.

In conclusion, all four victims were diagnosed as cases of hydrogen sulfide poisoning. Hydrogen sulfide in the atmosphere of the pit proved to be derived from sulfide in seepage of the stable industry waste. Thiosulfate in the blood and urine is more useful than sulfide when attempting to prove hydrogen sulfide poisoning in cases where some degree of survival time has occurred. Even in a case of gas poisoning where hydrogen sulfide was not demonstrated in the atmosphere, it is possible to use thiosulfate concentrations alone as evidence of hydrogen sulfide poisoning.

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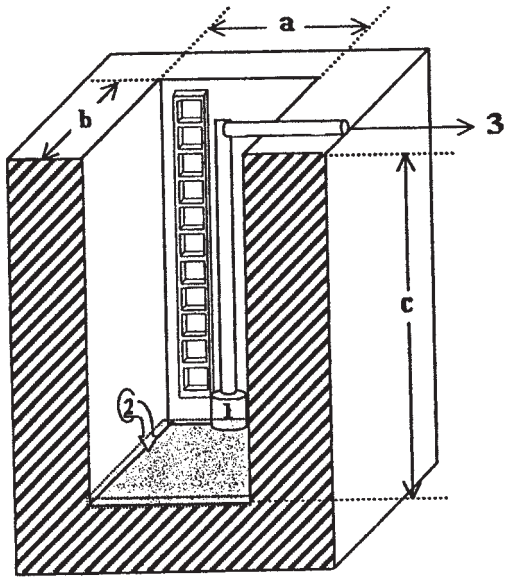

FIG. 1—*Diagram of the pit in an industrial waste site where the gas poisoning occurred.1 = pump, 2 = pipe, 3 = waste water treatment. a(width) = 1.3 m, b(length) = 1.5 m, c(depth) = 5.5 m.*

dent, respectively. Urine samples for the four victims were collected at the hospital about 2 h after the accident. All the samples were sent to the analytical department at the police laboratory and kept at -20°C until analysis.

Generation of Hydrogen Sulfide Gas

Since sulfide was detected in seepage on the day before the accident at the level of 30 mg/L, production of hydrogen sulfide gas from the seepage was suspected. We examined the influence of pH (5.5–7.9), sulfide concentration (0–50 mg/L) and volume of solution (100–400 mL; 10–40% of bottle volume) on generation of hydrogen sulfide gas. We used a 1 L reagent bottle as follows: water containing sulfide was put into the reagent bottle, the bottle was sealed and the solution was mixed at 900 rpm with a magnetic stirrer. Sulfide was vaporized as hydrogen sulfide gas. The gas was collected using a syringe, and was absorbed into an absorbent solution containing zinc sulfate, sodium hydroxide, and ammonium sulfate (14). The sulfide concentration in the absorbent solution was analyzed using the methylene blue method (14), and the concentration of hydrogen sulfide gas in the atmosphere of the bottle was calculated from the thus obtained value.

Toxicological Analysis

Procedure Used to Analyze Sulfide

Sulfide in blood was determined using our method (15). Sulfide was detected as bis(pentafluorobenzyl)sulfide ($C_6F_5CH_2SCH_2C_6F_5$), as follows: 0.2 mL of the sample was added to the mixture of 0.5 mL of 20 mM pentafluorobenzyl bromide (PFBBr) solution in ethylacetate, 2.0 mL of internal standard (I.S.) solution (10μM 1,3,5-tribromobenzene (TBB) in ethyl acetate) and 0.8 mL of 5 mM tetradecyldimethylbenzylammonium chloride solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1 min., and 0.1 g of potassium dihydrogenphosphate was added to the mixture as a buffer to prevent excessive alkylation by tissue protein. The preparation was again vortexed for 10 s and centrifuged at 2500 rpm for 10 min. An aliquot of the organic phase was injected onto a GC/MS apparatus.

Procedure Used to Analyze Thiosulfate

Thiosulfate in blood and urine was determined using our method (16). Thiosulfate was detected as bis(pentafluorobenzyl)disulfide ($C_6F_5CH_2SSCH_2C_6F_5$), as follows: 0.2 mL of the sample was added to the mixture of 0.5 mL of 20 mM PFBBr solution in acetone, 0.05 mL of 200 mM L-ascorbic acid solution and 0.05 mL of 5% sodium chloride. The preparation was vortexed for 1 min., and 2 mL of 25 mM iodine solution in ethyl acetate, 0.5 mL of I.S. solution (40 μM TBB in ethyl acetate) were added to the mixture. The preparation was again vortexed for 30 s and the mixture was centrifuged at 2500 rpm for 15 min. and left to stand for 1 h. An aliquot of the organic phase was injected onto a GC/MS apparatus.

GC/MS Conditions

GC/MS was done using a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA) interfaced to a JEOL Automass 150 mass spectrometer. The column was a J&W Scientific (Folsom, CA) fused-silica capillary tube of DB-225 (30 m × 0.32 mm I.D., 0.25μm film thickness). A splitless injection mode was selected with valve off-time of 1.0 min. The initial temperature of the column was held at 100°C for 2 min, programmed at 10°C/min to 220°C. The injection port, separator, and ion source were kept at 220, 240, and 210°C, respectively. Helium was the carrier gas used at a flow-rate of 2 mL/min. The ionization energy and current were 70 eV and 300μA, respectively. GC/MS in scan mode was used for both identification and quantitation of sulfide and thiosulfate.

Preparation of Calibration Curves

Samples to be tested were prepared by adding the standard solution of sulfide or thiosulfate to whole blood, plasma and urine, which were collected from a healthy volunteer. The samples were prepared to contain sulfide or thiosulfate at concentrations of 0.05–2.0 mg/L, 0.5–100 mg/L, respectively. A calibration curve for sulfide was obtained by plotting the peak area ratio of the molecular ion (m/z 394) of the derivative of sulfide to that (m/z 314) of TBB against the sulfide concentration, using mass chromatography. A calibration curve for thiosulfate was obtained in the same manner using the molecular ion (m/z 426) of the derivative of thiosulfate.

Results and Discussion

Generation of Hydrogen Sulfide Gas

The concentration of hydrogen sulfide in the atmosphere of the bottle was increased with decreasing pH, high concentration of sulfide, and a high volume of water. Figure 2 shows the relationship between hydrogen sulfide concentration (y) in the atmosphere of the bottle and sulfide concentration (x) in the water when pH and volume of water were set as those of the day before the accident (pH 6.5, water volume 10%). The curve presented a straight line through zero with regression of $y = 40.357x$ and a correlation coefficient of 0.997. As shown in Fig.2, the concentration of hydrogen sulfide generated from sulfide in seepage at the level of 30 mg/L was 1200 ppm. Since this level is close to that measured in the pit after the accident (1400 ppm), hydrogen sulfide in the atmosphere of the pit was considered to have generated from sulfide in the seepage.

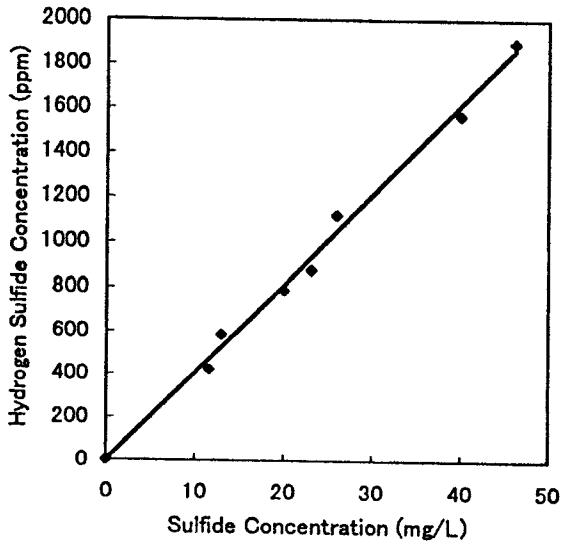

FIG. 2—*Relationship between hydrogen sulfide concentration in the atmosphere and sulfide concentration in the water. PH = 6.5, water volume = 100 mL (10% of the bottle volume).*

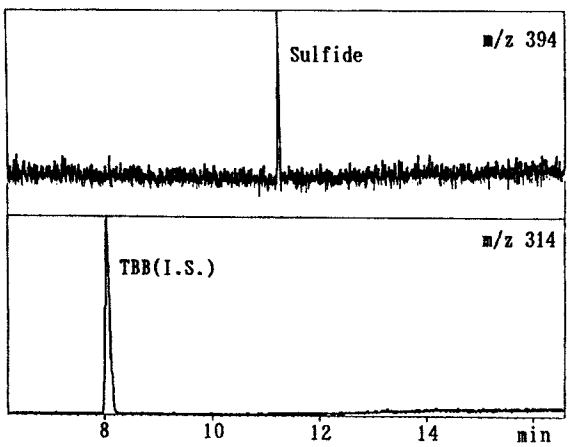

FIG. 3—*Mass chromatograms of the derivatized extract for sulfide obtained from a blood sample of Victim A.*

Since plaster-boards containing calcium sulfate had been dumped in the industry waste works as stable industrial waste until the year 1998, the sulfide detected in the seepage may have derived from sulfate which was reduced to sulfide by microorganisms.

Analysis of Sulfide and Thiosulfate in Blood and Urine

Figure 3 shows mass chromatograms of the derivatized extract for sulfide obtained from a blood sample of the victim (A). Figure 4 shows mass chromatograms of the derivatized extract for thiosulfate obtained from a urine sample of the victim (D). The peaks with retention times of 8.00, 11.20, 13.45 min were identified as I.S., the derivative of sulfide and that of thiosulfate, respectively. Interfering peaks were nonexistent on the chromatograms.

Concentrations of Sulfide in Blood of Victims

The concentrations of sulfide in blood samples from A and B were 0.13 and 0.11 mg/L, respectively (Table 1). The concentrations were somewhat higher than the level in healthy persons (2,3), and were lower than levels previously reported in fatal cases of humans (7,8) and animal experiments (12). Since the victims were given oxygen inhalation in the hospitals, the sulfide had presumably decomposed.

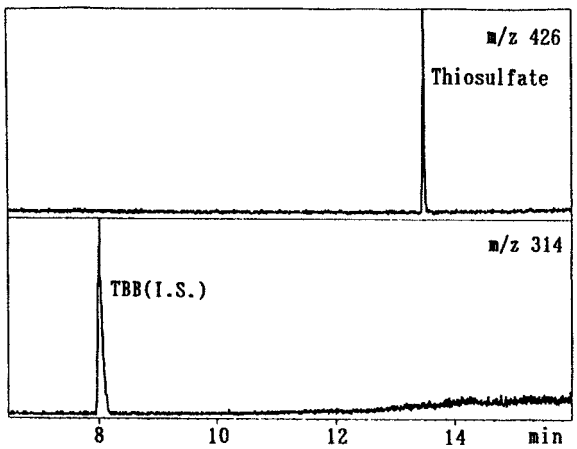

FIG. 4—*Mass chromatograms of the derivatized extract for thiosulfate obtained from a urine sample of Victim D.*

TABLE 1—*Sulfide and thiosulfate concentrations in blood samples from four victims (mg/L).*

	Victims			
Compound	A, Whole Blood	B, Whole Blood	C, Plasma	D, Plasma
Sulfide	0.13	0.11	N.D.*[1]	N.D.*[1]
Thiosulfate	10.53	4.59	4.14	N.D.*[2]
Time of sample collection*[3]	Time of autopsy	Time of autopsy	2	6

N.D. = not detected (*[1]; below 0.03 mg/L, *[2]; 0.3 mg/L). *[3] = hours after the accident.

Sulfide was not detected in plasma samples from C and D. The sulfide in plasma samples was considered to be less stable than that in whole blood samples. Therefore, we considered that sulfide was not a sufficient indicator of hydrogen sulfide poisoning.

Concentration of Thiosulfate in Blood and Urine of the Victims

Concentrations of thiosulfate in the blood of A and B were 10.53 and 4.59 mg/L, respectively (Table 1). These concentrations are 35 and 15 times higher than the level in healthy persons (17), and were similar to those previously reported in fatal cases of humans (7,8). In contrast to this high level in the blood, thiosulfate in urine samples from A and B were 0.90 mg/L and not detected, respectively (Table 2). Therefore, the cause of death in these two victims was attributed to hydrogen sulfide gas poisoning, hence it was proved that they died soon after the inhalation of hydrogen sulfide gas.

The concentration of thiosulfate in the plasma of C was 4.14 mg/L (Table 1), that is 14 times higher than the level in healthy persons (17), and similar to those noted in fatal cases (7,8). The concentration of thiosulfate in the urine of C was 137.20 mg/L (Table 2), that is 43 times higher than the level in healthy persons (17). Based on these findings, the cause of death of C was attributed to