CASE REPORT

Toshiko Tanaka,[1] *Ph.D.; Kentaro Kasai,*[1] *M.P.Sc.; Toshiro Kita,*[1] *Ph.D.; and Noriyuki Tanaka,*[1] Ph.D.

Distribution of Phenol in a Fatal Poisoning Case Determined by Gas Chromatography/Mass Spectrometry

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ABSTRACT: A victim who was presumed to have ingested waste fluid containing phenol of DNA extraction was found dead in his laboratory. The skin was partially chemically burned, with blisters as maps. No mechanical injuries were observed. The pathological findings of the liver and kidney were typical of those of acute substantial poisoning. Phenol concentrations in the blood, urine, stomach contents and organs were determined by gas chromatography/mass spectrometry. Phenol was distributed throughout the body. The concentration of free phenol in the blood was found to be 60 μg/mL, and in the urine it was 208 μg/mL. The phenol concentrations in the organs were found as follows: 106 μg/g in the brain; 116 μg/g in the lungs; 166 μg/g in the liver, and 874 μg/g in the kidney, respectively. Significantly high concentrations were observed in the kidney, urine, and liver. To the best of our knowledge, such an intoxication through this kind of ingestion has never been reported before. Distributions of phenol in fatal poisonings have been reported, but colorimetry was used as the analytical method and it cannot exclude the interference of other phenolic compounds.

KEYWORDS: forensic sciences, forensic toxicology, phenol, acute poisoning, gas chromatography/mass spectrometry, death

Phenol is used commercially as a disinfectant, chemical intermediary, extractive solvent and wood preservative, and is also used to extract DNA from biological specimens. Phenol causes severe irritation and corrosion on contact with skin or other tissue. Absorption of phenol may produce cyanosis, shock, weakness, collapse, convulsions, liver and kidney damage, coma, and death (1–5). Fatal concentrations of phenol were previously reported in the oral ingestion of Lysol™ (3), Castellani's paint (4), and dermal exposure to phenol solution (4,5). The phenol concentrations in these fatal cases were determined by colorimetry. Phenol determination by colorimetry cannot exclude the interference of other phenolic compounds, such as cresol, resorcinol, xylenol and catechol (6). The analysis of phenol in blood or urine was previously performed by the use of gas chromatographic (GC) methods (7–9), in which phenol can be distinguished from the other phenolic compounds. However, the fatal distribution of phenol determined by GC methods has not previously been reported. In this case report, the fatal distribution of phenol was determined by the GC method, and the data are discussed in comparison with previously reported cases.

[1] Assistant professor, instructor, associate professor, and professor, respectively, Department of Forensic Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu 807, Japan.

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Case History

A 27-year-old graduate student had been drinking in a bar with his colleagues at night. They returned to their laboratory, where they intended to drink the pure ethanol in a 3-L glass bottle prepared for experimental use. The colleagues left him for home after a while. The next morning, the first clerk to come to the laboratory found him lying dead on the floor. Beside the ethanol bottle on the lab table, there was a waste fluid bottle of the same shape as the ethanol bottle. The bottle had been prepared for phenol-chloroform waste fluid because of their involvement in DNA extraction. His soaked trousers and a glass were on the same table, and had an aromatic odor. The waste fluid in the bottle was separated into two layers. The waste was analyzed by GC in the Forensic Science Laboratory, Fukuoka Prefectural Police Headquarters. The upper layer contained mostly phenol—water and a slight quantity of chloroform. The lower layer contained mostly chloroform and a small quantity of phenol. There were ethanol and phenol remaining in the glass.

An autopsy was begun at 2:00 p.m. on that day. The body surface was grayish in color, and had a sweet aromatic odor. The skin in the large area extending from the right arm to both legs had changed color to dark brown, and some parts of its surroundings were chemically burned. There were also blisters in the skin across the burned area. The lips and oral mucous membranes had changed color to dark brown. No mechanical injuries were observed. The walls of the orsopharynx, larynx, bronchus, esophagus and stomach were also dark brown and were inflamed, and the mucous membranes were partially separated from the walls. There were approximately 20 g of dark brown mucous and a colorless clear liquid in the stomach. There were approximately 150 mL of urine of light reddish yellow color in the bladder. All organs appeared congestive only macroscopically. The following histological observations were made: inflammatory changes in the lungs; extension of sinusoid lumens and centrilobular increase of cytoplasmic eosinophility in the liver; interstitial edema and renal tubular hemorrhage in the kidneys; and interstitial hemorrhage in the pancreas and adrenal

glands. All of these abnormal findings were slight. The other organs, such as the brain, heart and spleen, were congestive, but no other abnormalities were noticed.

Method and Results of Analysis

Two kinds of drug screening analyses were performed on the heart blood, urine and the supernatant of the stomach contents. Nicotine and cotinine were the only drugs that were detected by the thin layer chromatography (TOXI-LAB®, TOXI-LAB Inc., CA). No drugs were detected by the high-performance liquid chromatography (HPLC) (REMEDi™ HS, Bio-Rad, CA). Blood ethanol was 0.70 mg/mL, and blood chloroform was 0.12 μg/mL, as detected by gas chromatography using a flame ionization detector with headspace sampling (GC-9A, Shimadzu, Kyoto, Japan).

Analysis of free phenol was performed by gas chromatography/mass spectrometry (GC/MS) (Automass 150, JEOL, Tokyo, Japan) on extracts obtained from modified ethyl acetate extractions (10). The whole blood and organs were each added to more than five times their quantity of cold distilled water. The sample analyses were carried out in duplicate. The organs were homogenized in an ice-cold bath. p-Ethyl phenol was used as an internal standard in 100 μL of distilled water. After the addition of the internal standard to 1 mL of the homogenates or liquid samples such as blood, urine and supernatant of stomach contents, the samples were acidified to pH1 with 5 MHCl, saturated with sodium chloride (NaCl), and extracted three times with 3 mL of ethyl acetate. The organic layers were collected and condensed by a dry stream of nitrogen. The extracts of the samples were subjected to GC/MS. GC/MS was performed using capillary column (DB-WAX, 0.25 mm i.d. × 30 m length, 0.25 μm film thickness) (J&W Scientific, CA). The flow rate of the carrier gas was 1 mL.min-1, and the split ratio was 50. The column temperature was programmed from 30°C to 220°C at 10°C/min. The other temperatures were 200°C for injection and 250°C for the ion source. Scan mode was used for the qualitative analysis, and SIM mode for the quantitative analysis. The monitoring ions in SIM mode were m/z 66, 94, 95 for phenol, and 107, 122 for p-ethyl phenol, respectively.

Recovery for determining the concentrations of the phenol was prepared in each of five samples by adding a known amount of phenol to drug-free blood (for blood analysis), urine (for urine and stomach content analysis) and organs. Recoveries with this procedure were greater than 90% in all samples, and were used for the estimation of phenol concentrations. The data are presented in Table 1. The concentration of phenol in heart blood was found to be 60 μg/mL, together with higher concentrations in the other samples. Phenol was distributed throughout the body, with significantly high concentrations in the kidney, urine and liver.

Discussion

The pathological findings in this case, such as mucous changes in the digestive and respiratory organs, dermal burning and substantial toxic changes of the liver and kidney, were consistent with phenol poisoning (1,2). The concentrations of ethanol and chloroform in this case were far below the toxic or anesthetic range (1,11). Therefore, it was conjectured that the victim in our case ingested the upper layer of waste fluid containing phenol, vomited and spilled it over his body, thus burning the skin chemically. The phenol absorbed through the skin could also have added to the phenol distribution absorbed by ingestion, and contributed to his death.

Fatal concentrations of phenol were previously reported in oral ingestion of Lysol (3), Castellani's paint (4), and in dermal exposures to phenol solution (4,5). The data are summarized in Table 1. All of these fatal cases were male and died rapidly, and phenol concentrations were determined by colorimetry, except for this present case. The interference of other phenolic compounds, such as cresol, resorcinol, xylenol and catechol, cannot be excluded from the phenol concentrations determined by colorimetry (6). Lysol consists of a large amount of cresol, potassium hydroxide, plant oil and a small amount of phenol. Castellani's paint is a mixture containing phenol, basic fuchsin, resorcinol, acetone and ethanol. It has not been clarified in poisoning cases by Briglia (3) and Soares et al. (4) whether or not the toxicity of phenol is completely identical to other phenolic compounds. The contribution of the other ingredients of these products to the death was not discussed in these cases (3,4).

The concentrations of phenol in the blood in the cases of fatal ingestion were reported as 46 μg/mL (3) and 56 μg/mL (4). In fatalities due to accidental percutaneous absorption of phenol, postmortem blood concentrations were reported as 27 μg/mL (4), 4.7 μg/mL (5) and 90 μg/mL (R. Consden, personal communication, 1967). Independent of analytical method or route of absorption, the fatal concentration in the blood of phenol or phenolic compounds was estimated at between 27 μg/mL and 90 μg/mL in the previously reported cases, except in the case of Lewin et al. (5). The concentration of phenol in the blood in the case reported here was in the fatal range. Phenol has a very short half-life (30.3 ± 2.8 min) and is quickly excreted into the urine, conjugated with sulfate and glucuronide (10). Only in Lewin's case (5) was resuscitation and cardiac massage tried, so that the phenol concentration might have become lower than in the case of no treatment.

TABLE 1—*Fatal concentrations of phenol reported in the literature (μg/mL or μg/g).*

References	Present Case	(3)	(4) Case 1	(4) Case 3	(5)	**
Blood	60	46	56	27	4.7	90
Urine	208	...	...	...	n.d.	...
Stomach contents	111	...	...	...	n.d.	...
Brain	106	...	...	...	...	...
Lung	116	471	...	...	...	...
Liver	166	269	74	...	3.3(7.1*)	...
Kidney	874	259	...	...	...	...
Age (years)	29	unknown	43	17	24	unknown
Material	phenol	Lysol	Castellani's paint	phenol	phenol	phenol
Route of absorption	oral + dermal	oral	oral	dermal	dermal	dermal
Analytical method	GC/MS	c	c	c	c	c

NOTES:—n.d. = not detected; * = hydrolyzed phenol; c = colorimetry; ** = R, Consden, personal communication, 1967.

Free phenol concentration in the blood correlated with the dosage administered, while the conjugated phenol did not correlate with the dosage (10). The toxicity of conjugated phenol is less than that of free phenol. Therefore, the free phenol concentration in the blood can be regarded as an index of poisoning. The concentration of free phenol in the blood was found to be 60 μg/mL in this case, together with even higher concentrations in the other samples. The lipophilic quality of phenol may be related to its high distribution in the other organs. The concentrations of phenol in the liver and kidney varied among the cases reviewed. The reason for the variation is not clear. The individual variation of metabolic activity, time course, route of absorption, and the ingredients of the absorbed materials may affect the phenol distribution. The method of analysis also can affect the concentration determined, because, contrary to GC, the colorimetry cannot exclude other phenolic compounds. In order to estimate the fatal concentration and fatal distribution of phenol appropriately, a GC method should be used.

This is the first reported case of which we are aware in which the distribution of phenol in tissues was determined using a gas chromatographic method, although we realize that GC has been used for some time to determine phenol in forensic toxicology laboratories.

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Additional information and reprint requests:
Toshiko Tanaka
Department of Forensic Medicine
School of Medicine, University of Occupational and Environmental Health, Japan
Kitakyushu 807, Japan