CASE REPORT

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An Acetonitrile-Related Death

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ABSTRACT: A 39-year-old female who lived alone was discovered dead in her home. An autopsy produced no cause of death. The blood sedative screen was negative and only diphenhydramine was found by the urine organic base analysis. Examination of the blood and urine for volatiles produced an unexpected peak by GC analysis, which was then identified as acetonitrile by GC/MS. Acetonitrile concentrations were 31 and 56 mg/dL in two separate blood samples and 44 mg/dL in the urine. The blood cyanide concentration was 4.4 μg/mL. The cause of death was determined to be acetonitrile poisoning although the source of the acetonitrile was not discovered.

KEYWORDS: toxicology, acetonitrile

Acetonitrile exerts toxicity by being converted to cyanide. Feierman has good evidence from rat liver microsome studies that this metabolism is a two-step process in which acetonitrile is first converted to a hydroxy intermediate by liver cytochrome P-450 [*1*]. Cyanide is then liberated by the action of catalase. Unlike cyanide, acetonitrile poisoning typically takes several hours before toxic symptoms develop. One reason for this is the slow conversion to cyanide [*2*]. Another possible explanation for delayed toxicity is the time required to deplete the substrates for the cyanide detoxification reaction in the liver catalyzed by rhodanese that converts cyanide to thiocyanate.

Specific treatment for acetonitrile poisoning is to treat the cyanide poisoning. The amyl nitrate and sodium nitrite in the cyanide kit that is used in the U.S. converts hemoglobin to methemoglobin, which is capable of trapping cyanide in cyanmethemoglobin to prevent the poisoning of the electron transport chain. Sodium thiosulfate, the other component of the cyanide kit, serves as a substrate for the rhodanese-catalyzed conversion of cyanide to the much less toxic thiocyanate.

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Case Report

The deceased was a 39-year-old divorced woman who lived alone. She was a registered nurse employed at a nursing home. She had been admitted to an alcohol treatment center eight years previously and there was some indication that she had been fired from a previous job for alcoholism. There had been a suicide attempt with an unknown drug four years previously. Her lupus was well controlled and was being treated with prednisone. There were no known heart or renal problems. Her sister and her physician both felt there were no indications of depression or suicidal tendencies at the time shortly before her death.

The subject was found dead and unclothed on her bathroom floor about 30 h after last being seen alive. There was no evidence of foul play and there were no notes or other indications of suicide.

Laboratory Methods

Blood Sedative Screen

This assay is a modification of the method of Jain [*3*]. Two mL of blood were mixed with 0.5 mL of pH 4.5 buffer and extracted with 10 mL $CHCl_3$. Eight mL of the organic layer were transferred to another tube and the solvent was evaporated. The residue was taken up in 0.5 mL of heptane and 50 μL of methanol were added, followed by vortex mixing. Three μL of the methanol were gas chromatographed on an OV-17 packed column. The temperature program was 160–280°C at 16°C/min. The final temperature was held for 8 min at the end of the run.

Urine Organic Bases

The procedure is a modification of a method reported by Jain [*4*]. Five mL of urine were mixed with 1 mL of pH 11 buffer and extracted with 10 mL of $CHCl_3$:isobutanol, 20:1. The organic phase was isolated and mixed with 0.6 mL of 0.1 N sulfuric acid. The aqueous phase was separated and neutralized with 0.2 mL of 0.75 N KOH and three drops of chloroform were added, followed by vortex mixing. Three μL of the chloroform were chromatographed on an OV-1 coated, packed column. The temperature program was 100–280°C at 16°C/min with a 4 min hold at the maximum temperature.

Volatiles

Volatiles were analyzed by a modification of a published method [*5*]. One half mL of blood or urine was mixed with 0.5 mL internal standard (3.5 mL 1-propanol diluted to 1 L with water). Seven tenths of a μL of the mixture was chromatographed at 160°C on a 4 ft × $^1/_8$ in stainless steel column packed with Porapak S. Nitrogen was used as carrier gas with a flow rate of 30 mL/min and detection was by FID. A standard mixture of methanol, ethanol, acetone and isopropanol was used to identify unknowns by retention time. A 0.079 weight % acetonitrile standard was prepared by diluting 100 μL of acetonitrile to 100 mL with Type I water.

Cyanide

The cyanide method reported by Guilbault was used [*6*]. Fifty μL of cyanide reagent were placed in the center well of a Conway microdiffusion dish. This reagent consisted of 1 mL of 2 mg/mL *p*-benzoquinone in dimethylsulfoxide and 0.1 mL of 0.05 M phos-

phate buffer pH 7.5. Two mL of blood or standard were placed in the outer chamber. The sample/standard was acidified with 6 drops of 1.9 M H_2SO_4 and the chamber was immediately sealed. Diffusion was allowed to take place for 4 h. Three mL of phosphate buffer pH 2.75 were added to the center well, mixed, and the entire contents transferred to a cuvette. Fluorescence of standards and unknowns was measured with excitation at 350 nm and emission at 450 nm.

Results

The blood sedative screen was negative and only diphenhydramine was identified in the urine by the organic base procedure. In the volatile procedure methanol, ethanol, acetone, isopropanol and 1-propanol eluted at 0.90, 1.84, 2.33, 3.13 and 4.61 min, respectively. Blood and urine from the subject produced a peak at 2.09 min in addition to the 1-propanol internal standard peak. This peak was identified as acetonitrile by GC/MS. Acetonitrile concentrations of 31 and 56 mg/dL were determined on two blood samples. There were no records as to collection site for the two samples. The urine acetonitrile concentration was 44 mg/dL. A blood cyanide concentration of 4.4 μg/mL was obtained for the blood sample that contained 56 mg/dL of acetonitrile. No source of acetonitrile was ever found in the subject's home although acetonitrile-containing artificial nail remover preparations were not specifically looked for.

Previous Cases

Table 1 gives a summary of the six, previously reported acetonitrile-caused deaths. The first is an industrial exposure [*7*] that lead to the death of one worker, hospitalization of two, and the development of symptoms in others. An acetonitrile-containing solution was used to apply a coating to the inside of a large tank. The application took two days and some of the workers inhaled the vapors for several hours. The 23-year-old worker who later died, was well until about 4 h after leaving work and then experienced nausea, vomiting and blood spitting. His symptoms progressed to seizures and coma by the time of hospital admission and he died about an hour after admission which was about 14 h after his last exposure. The second, fatal inhalation exposure occurred in an industrial photographic laboratory when two men used acetonitrile mixed with boiling water to clean a floor [*8*]. One of the men became ill enough to be hospitalized but no more information is given about him. The other man first complained of stomach pains and nausea in the evening about four hours after leaving work. During the night he experienced massive vomiting and next morning was admitted to hospital where he became comatose and experienced seizures. He was in coma for the remainder of the hospitalization. He was hypotensive, was resuscitated from a cardiac arrest, and was maintained on a ventilator. The nature of his poisoning was not realized until day three when he was treated with cobalt tetracemate and dihydroxycobalamin for cyanide toxicity. He died of cardiovascular collapse on day five after having been brain dead since day three.

While the previous two deaths were from inhalation, Caravati and Litovitz reported the first death from ingestion of acetonitrile [*9*]. A 16-month-old boy drank 15 to 30 mL of an acetonitrile-containing artificial-fingernail remover. Although the poison center was contacted, the product was assumed to be primarily acetone and little toxicity was expected. The child was put to bed and left to sleep and was found dead next morning, about 12 h after ingestion. Another fatal ingestion, presumably of acetonitrile, occurred in a 22-year-old female with history of depression and suicide attempts [*10*]. She was found semiconscious at work and friends reported she had probably taken an unknown poison about 8 h before admission to hospital. She was drowsy but rousable for the next 18 h and then became agitated, experienced convulsions and cardiac arrest. After resus-

TABLE 1—*Acetonitrile death cases.*

Report	Dose/route	Manner	Agent	Age	Acetonitrile mg/dL	Cyanide μg/mL
Amdur, 1959	Inhalation	Accident	Coating, Solvent	Adult		Blood, 8.0
Dequidt, 1974	Inhalation	Accident	Solvent	Adult	Blood, 1.2 Urine, 3.1	Blood, 1.1
Caravati, 1988	15–30 mL, oral	Accident	Nail Remover	16 Months		Blood, 3.1
Boggild, 1990	Oral	Suicide	Solvent	Adult		
Jones, 1992	Oral	Accident	Lab Reagent	2 Adults	M. Blood, 80 Urine, 102 F. Blood, 77 Urine, 101	M. Blood, 2.4 Urine, 0.7 F. Blood, 4.5 Urine, 0.7
This Report	Unknown	Unknown	Unknown	Adult	Blood, 56 & 31 Urine, 44	Blood, 4.4

citation she was hypotensive with severe metabolic acidosis. Despite adequate ventilation, treatment with pressor agents and bicarbonate the subject died about 30 h after the supposed ingestion. A bottle containing acetonitrile was found in her possessions. Although neither acetonitrile nor cyanide was measured in blood or urine in this subject and there is no report of a witnessing of the subject's ingestion, the convulsions, hypotension and metabolic acidosis several hours after exposure are consistent with what is seen in the reported cases of acetonitrile poisoning.

The most recently reported acetonitrile fatalities involved a middle-aged couple who where found dead in bed in the morning after an evening of drinking at home [*11*]. The couple were both ill during the night. A son had returned home in the early morning and found the father vomiting. He was told the mother also was ill. It appears that the couple were mixing drinks from reagent bottles labeled as ethanol that the man had brought home from work. The solutions in these bottles were found to be not ethanol but acetonitrile.

In addition to the fatalities there have been several cases of toxicity from acetonitrile exposure. Some information from these cases is summarized in Table 2. Several workers were exposed during the tank painting incident that caused the death that was mentioned previously [*7*]. Case 2 from that report painted for about three hours in late afternoon. He slept well that night and walked to work before becoming nauseous with vomiting about 8 AM. About 2 h later he was hospitalized for observation and became hypotensive with shallow respirations and a decreased respiratory rate. His deep tendon reflexes were barely obtainable. He was treated with oxygen, blood transfusion, ascorbic acid and sodium thiosulfate. Within a few hours his condition was much improved. Case 3, after painting in the afternoon left the job and felt well until about 11 p.m. when he had chilly sensations. He had diarrhea during the night and felt nauseous and listless in the morning. He didn't feel well enough to go to work and recalls nothing up through the time he was located by his sister and then hospitalized at midday. On admission he was slate gray and semiconscious with a low pulse rate and body temperature. Respirations were slow and shallow and his motor power was impaired. Deep tendon reflexes were not found. He was treated the same as Case 2 and was noticeably improved within half an hour. Several other workers were less seriously affected. Their symptoms included headache, nausea, and weakness. Three of them had measurable blood cyanide concentrations that peaked at 0.7, 0.6 and 0.3 μg/mL respectively. Cyanide was not detectable in the other workers.

Jaeger reported the case of a 26-year-old man who attempted suicide by drinking 40 mL of acetonitrile [*12*]. After three hours the patient was nauseous and vomiting. Later his condition was characterized by coma, respiratory insufficiency, metabolic acidosis, shock and two cardiac arrests. At 10 h after ingestion he was treated with cyanide antidotes but stayed in coma for six days. The patient had severe complications including muscle and liver degeneration but eventually recovered. The authors reported that thiocyanate, metabolite of cyanide was detectable in the patient's urine for 20 days after the poisoning.

There are several reports of toddlers being hospitalized after accidental ingestion of acetonitrile-containing artificial-fingernail remover. A two-year-old boy appeared to have spilled sculptured nail remover on himself [*9*]. Eight hours later he was found poorly responsive and having vomited. On admission to hospital he was hypotensive and in metabolic acidosis. Supportive treatment with fluids, oxygen and bicarbonate brought a good response and cyanide antidotes were not used. Kurt reported a case of accidental ingestion of this material by a two year old [*13*]. She was without symptoms for 12 h after ingestion before experiencing vomiting, seizures, coma, and metabolic acidosis. She was treated with oxygen and the cyanide antidote kit and was fully recovered after two days. A mildly affected three-year-old subject who drank 15 to 30 mL of artificial-nail

TABLE 2—*Acetonitrile toxicity cases that recovered.*

Report	Dose/route	Manner	Agent	Age	Acetonitrile mg/dL	Peak cyanide, μg/mL
Amdur, 1959	Inhalation	Accident	Coating, Solvent	Adult 1		3.1
				Adult 2		10
Jaeger, 1977	40 g, oral	Suicide Attempt	Solvent	Adult		
Caravati, 1988	30 mL, oral, skin	Accident	Nail Remover	2 Years		6.0
Kurt, 1991	5–10 mL, oral	Accident	Nail Remover	2 Years		1.8
Geller, 1991	15–30 mL, oral	Accident	Nail Remover	3 Years		1.2
Turchen, 1991	59 mL, oral	Suicide Attempt	Nail Remover	Adult		13
Michaelis, 1991	5 mL, oral	Suicide Attempt	Lab Reagent	Adult	8	17.3
Losek, 1991	60 mL, oral	Accident	Nail Remover	2 Years		3.8

remover was reported by Geller [*14*]. The child had symptoms of vomiting and confusion about 13 h after the ingestion, but was never acidotic. He was treated with sodium thiosulfate and recovered. Another accidental ingestion in a child involved a 23-month-old who drank about 60 mL of artificial-nail remover [*15*]. He had vomited at 6 h postingestion and was seen in the hospital at 12 h and did not appear ill. At 24 h postingestion the patient had staring episodes and was unresponsive. Although the blood pH was never low, lactic acid was elevated. Sodium thiosulfate was given every four hours for five doses. The child's consciousness returned to normal and he was discharged on the third hospital day.

A 39-year-old woman drank 59 mL of artificial-nail remover in a suicide attempt [*16*]. She was asymptomatic for seven hours, then vomited. She was treated with gastric lavage and charcoal and had a seizure at eleven hours after ingestion. A difficult course of hospitalization followed in which the metabolic acidosis was treated with bicarbonate, and sodium nitrite and sodium thiosulfate were given several times to treat the cyanide toxicity. She was also hemodialyzed. She ultimately recovered and was discharged on the sixth hospital day. Michaelis reported a 30-year-old man who drank about 5 mL of acetonitrile in a suicide attempt [*17*]. At five hours after ingestion he was brought to the hospital with malaise. He was given 1 g of sodium thiosulfate at 4.5 h, on the way to the hospital, and 31 g starting at 5.5 h after ingestion. The patient never developed seizures or metabolic acidosis and was transferred to psychiatric care at 30 h after ingestion. Acetonitrile and cyanide concentrations were measured from several blood samples taken over the course of the hospitalization and elimination half lives of 32.4 and 15.1 h respectively were calculated.

Discussion and Conclusions

Acetonitrile concentrations have been reported in only a few of the acetonitrile death and toxicity cases. The concentrations found in this case, 31 and 56 mg/dL for two blood samples and 44 mg/dL for the urine, are a bit lower than those found in the two oral ingestion deaths reported by Jones: 77 and 80 mg/dL in blood and 101 and 102 mg/dL in urine [*11*]. Peak serum concentrations of 8 mg/dL were measured in the surviving suicide attempt case reported by Michaelis [*17*]. This subject was only mildly affected, perhaps as a result of treatment with the antidote sodium thiosulfate at 5.5 h after ingestion. The paper by Dequidt [*8*] is the only other one to report quantitation of acetonitrile. Blood and urine concentrations of 1.2 and 3.1 mg/dL were reported. These are much lower than those mentioned. These samples were apparently collected 3 to 4 days after the inhalation exposure. This time would equal about three excretion half lives of 32 h as calculated by Michaelis [*17*]. If this assumption is correct then peak acetonitrile concentrations would have been about 5 mg/dL, which is still much lower than the concentrations found in the other three deaths. This paper gives little detail about the methods used to quantitate acetonitrile.

The difference between the acetonitrile concentrations of the two blood samples, 31 and 56 mg/dL, requires some comment. The lower blood result was measured in the sample that was collected in a tube without anticoagulant or preservative. The other blood tube contained oxalate and fluoride. We would expect that acetonitrile would be stable in blood and we do not suspect that the difference in results is because of the different tubes. We had no information about collection site for the two samples and it seems possible that different collection sites and postmortem redistribution explain the difference. Acetonitrile is very soluble in water and we expect that it should be distributed uniformly in body water. The greatest postmortem redistribution is found with drugs that have high V_D values. However since acetonitrile is very water soluble it should have a low V_D. It may be that the stomach concentration was higher than blood at the time of

death and some diffusion from the stomach produced a gradient of acetonitrile concentration along the large blood vessels.

Review of the reported cases shows that in the instances where acetonitrile ingestion was recognized and where treatment with cyanide antidote was started within a few hours the subjects have survived. Exposures that have not been treated promptly with the cyanide antidote have resulted in death.

There is a complete overlap of blood cyanide concentrations in the subjects that died and those that survived. Inspection of the two tables shows that the postmortem blood cyanide concentrations range from 1.1–8 μg/mL while the peak concentrations in the patients that recovered range from 1.2–17.3 μg/mL. However, two research studies on the mechanism of nitrile toxicity in mice draw the conclusion that cyanide concentrations in brain, not blood, are the best indicator of lethality [*2,18*].

In the two acetonitrile deaths reported by Jones [*11*] the volatile in the blood samples was initially misidentified as ethanol because of similar retention times even when the assays were done with two different columns. Our Porapak column method did not have this problem as the retention time of acetonitrile was sufficiently different from the commonly expected volatiles methanol, ethanol, acetone, and isopropanol.

Although acetonitrile quantitation is not complex, few of the reports of acetonitrile toxicity and death have included acetonitrile concentrations. The main value of acetonitrile measurement is probably to confirm absorption of acetonitrile. Concentrations of cyanide and acetonitrile together in a death investigation should be adequate to confirm that death is due to cyanide poisoning resulting from acetonitrile exposure.

The lethal concentrations found in our case and the one by Michaelis were readily detectable with a gas chromatographic methods for measuring blood ethanol and other volatiles, techniques that are readily available in toxicology laboratories. However a major problem in detecting an unexpected acetonitrile poisoning is that the acetonitrile retention time may be close enough to one of the common volatiles as to be misidentified and reported incorrectly as ethanol, acetone, or isopropanol.

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