Efficacy of Oral Administration of Sodium Thiosulfate and Glycine in a Large, Swine Model of Oral Cyanide Toxicity

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Study objective: Cyanide is a deadly poison, particularly with oral exposure, in which larger doses can occur before any symptoms develop. Multiple governmental agencies highlight oral cyanide as an agent that can be used in a terrorist attack because it can be easily weaponized and is readily available. Currently, there are no Food and Drug Administration–approved antidotes specifically for oral cyanide. An oral countermeasure that can neutralize and prevent absorption of cyanide from the gastrointestinal tract after oral exposure is needed. The objective of this study is to determine if the combination of glycine and sodium thiosulfate administered orally is effective in reducing mortality in a large, swine model of oral cyanide toxicity.

Methods: Nine swine (45 to 55 kg) were instrumented, sedated, and stabilized. Potassium cyanide (at 8 mg/kg) in saline solution was delivered as a onetime bolus through an orogastric tube. Three minutes after cyanide administration, animals that were randomized to the treatment group received sodium thiosulfate (508.2 mg/kg, 3.25-M solution) and glycine (30 mg/kg, 3.5-M solution) through an orogastric tube. Survival at 60 minutes was the primary outcome. We compared survival between groups by log-rank Mantel-Cox analysis and trended laboratory results and vital signs.

Results: At baseline and treatment, all animals were similar. Survival at 60 minutes was 100% in treated animals compared with 0% in the control group (P=.003). By the study end, defined as death or 60 minutes after cyanide administration, there was a significant difference in the lactate concentration between the treatment and control groups (control 9.43 mmol/L [SD 4.08]; treatment 1.66 mmol/L [SD 0.82]; difference between means 7.69 mmol/L [SD 2.07]; 95% confidence interval difference −14.05 to −1.32). Mean arterial pressure was significantly different between the treatment and control groups at study end (control 26 mm Hg [SD 6.7]; treatment 81 mm Hg [SD 14]; difference between means 55.2 mm Hg [SD 7.1]; 95% confidence interval difference 37.8 to 72.6). pH and oxygen saturation were also significantly different between the treatment and control groups at study end.

Conclusion: The combination of oral sodium thiosulfate and glycine significantly improved survival and physiologic parameters in a large-animal model of oral cyanide toxicity. [Ann Emerg Med. 2019;■:1-7.]

Please see page XX for the Editor's Capsule Summary of this article.

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INTRODUCTION

Cyanide is a deadly poison.[1,2] Its ingestion can lead to headache, confusion, nausea, vomiting, hypotension, metabolic acidosis, hyperlactatemia, coma, seizure, and death, similar to the signs and symptoms of inhalational exposure. A primary mechanism of toxicity is through inhibition of cytochrome a3/complex IV of the electron transport chain.[3-5]

Oral cyanide exposure can occur through ingestion of salts such as potassium cyanide.[1-6] In 1978, greater than 900 people died as a result of ingestion of a beverage laced with cyanide in the Jonestown massacre in Guyana, and cyanide use was well documented in World Wars I and II.[2,3] Multiple governmental agencies highlight oral cyanide as a potential agent that can be used in a terrorist attack because it can be easily weaponized through contamination of food and water supplies.[1-7] In January 2019, envelopes containing potassium cyanide were sent to multiple Japanese companies, with letters threatening to lace medications and food with the poison if a large monetary payment to the senders was not made.[8]

Available antidotes for cyanide intoxication (ie, hydroxocobalamin) are not Food and Drug Administration approved specifically for oral exposure.[7,9] Furthermore, they are not ideal for use in mass casualty settings because they require intravenous access or have to be administered in large volumes. In a mass casualty, patients could present with various degrees of toxicity. An oral antidote may have

Editor's Capsule Summary

What is already known about this topic

No Food and Drug Administration–approved antidote exists for oral cyanide exposure.

What question this study addressed

A porcine model of lethal oral cyanide exposure evaluated the effect of postexposure oral sodium thiosulfate and glycine on survival at 60 minutes in 9 pigs.

What this study adds to our knowledge

Combination thiosulfate-glycine administered 3 minutes after oral cyanide ingestion significantly improved survival, acidemia, and hemodynamic parameters compared with that of controls.

How this is relevant to clinical practice

Although this study suggests efficacy, oral antidote would be most beneficial in patients with mild to moderate symptomatology rather than the lethal doses studied. Moreover, it would likely be administered more than 3 minutes after exposure. The optimal population to benefit from this antidotal approach has yet to be determined.

the greatest effect on people who are unsure whether they were exposed, or who have early toxic exposure and present with mild to moderate symptoms before developing severe illness. Intravenous and intramuscular antidotes can be reserved for individuals who are already severely ill and cannot ingest an oral countermeasure.[10] An oral antidote that can be self-administered would allow health care providers to direct other resources to patients who are critically ill. With ingestion, a large amount of cyanide can accumulate in the stomach before absorption and the development of severe illness, which may necessitate large doses of antidote that cannot be tolerated through intravenous or intramuscular administration.[9,10] In these situations, a safe, readily available oral countermeasure that neutralizes cyanide in the stomach and prevents its absorption would be valuable.

Cyanide is likely systemically absorbed as hydrogen cyanide gas, which is rapidly formed after cyanide ions are exposed to the acidic pH of the stomach. Preventing the formation of hydrogen cyanide by alkalinizing the gastric pH may mitigate toxicity. Glycine has been shown to be an effective buffer in a rabbit model of ingested cyanide.[10]

Systemically, the enzyme rhodanese transfers sulfur to cyanide to form thiocyanate, which is relatively nontoxic. In toxic exposures to cyanide, this mechanism of metabolism becomes overwhelmed, leading to cyanide toxicity. Thiosulfate can nonenzymatically react with cyanide to form thiosulfate and also serves as a sulfur donor to rhodanese.[10] Furthermore, it has been safely used in human beings. We hypothesized that the combination of oral glycine and oral thiosulfate would increase survival in swine poisoned by oral cyanide by mitigating hydrogen cyanide formation and promoting formation of thiocyanate. Because human trials are not feasible to study cyanide intoxication, antidote approval will require data obtained from well-characterized small- and large-animal models.[10] In this study, we used a recently developed large, swine model of oral cyanide poisoning that mirrors human toxicity to evaluate the efficacy of oral sodium thiosulfate and oral glycine versus no-treatment controls.[4]

MATERIALS AND METHODS

Study Design

We conducted a randomized controlled trial comparing animals treated with oral sodium thiosulfate/oral glycine with no-treatment controls after oral cyanide exposure. All experiments were approved by the University of Colorado's Institutional Animal Care and Use Committee and complied with the regulations and guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care. Animals were housed in an animal care facility, where experimentation took place.

Selection of Participants

Animals (female Yorkshire swine [*Sus scrofa*]) weighing 45 to 55 kg were randomized into treatment groups (oral sodium thiosulfate/oral glycine) and control groups. Anesthesia was induced with ketamine at 10 to 20 mg/kg (MWI, Boise, ID) and isoflurane (MWI) by nose cone. Animals were intubated, an orogastric tube (B. Braun, Boise, ID) was placed, and peripheral venous access was obtained. Sedation was maintained with a Drager Apollo anesthesia machine (Drager, Houston, TX) with 1% to 3% isoflurane and 0.4 Fio_2. Tidal volume was set at 8 mL/kg and a respiratory rate of 16 to 20 breaths/min was used, with adjustment of the minute volume to maintain an end tidal carbon dioxide level of 38 to 42 mm Hg. A 0.9% saline solution bolus of 7.5 mL/kg (B. Braun, Bethlehem, PA) was given before central line placement. With the M9 ultrasonographic system (Mindray, Mahwah, NJ), central venous (external jugular) and

Table 1. Physiologic parameters at baseline between the swine treated with glycine/sodium thiosulfate and no-treatment controls.

	Control, n=4	Glycine Thiosulfate, n=5	Difference Between Means	95% CI Difference
Weight, kg*	50.8 (5.4)	50.3 (3.7)	−0.5 (3.2)	−8.6 to 7.6
Lactate, mmol/L*	0.60 (0.10)	0.94 (0.23)	0.35 (0.14)	−0.15 to 0.84
pH*	7.42 (0.02)	7.41 (0.05)	−0.01 (0.02)	−0.07 to 0.05
SBP, mm Hg*	117 (15.5)	100 (18.8)	−17.3 (11.4)	−44.3 to 9.8
MAP, mm Hg*	94 (10.5)	80 (18.8)	−14.5 (9.9)	−38.4 to 9.4
Pulse rate, beats/min*	84 (32.6)	91 (13.4)	7.6 (17.4)	−41.5 to 56.8
Pulse oximetry, percentage oxygen*	92 (3.1)	92 (2.7)	−0.3 (2.0)	−5.1 to 2.0
SBP, mm Hg†	108 (9.5)	93 (22.5)	−15.0 (11.1)	−42.7 to 12.7
MAP, mm Hg†	78 (15.7)	71 (18.7)	−6.8 (12.3)	−38.5 to 24.9
Pulse rate, beats/min†	104 (38.4)	90 (13.8)	−14.5 (20.2)	−72.9 to 44.0

SBP, Systolic blood pressure; *MAP*, mean arterial pressure.
Data are presented as mean (SD).
*There was no significant difference in animal weight, laboratory values, hemodynamics, or respiratory rate at baseline.
†There was no significant difference in hemodynamics at treatment.

arterial (femoral artery) access was obtained. The Drager Infinity Delta Monitor (Drager) was used to monitor physiologic parameters. After instrumentation, isoflurane was weaned to 0.8% to 1% and 0.21 Fio_2 until the animal was breathing spontaneously. The ventilator was then set to pressure support.

Potassium cyanide at 8 mg/kg (Sigma Aldrich, St. Louis, MO) was diluted in saline solution (<10 mL) and delivered as a onetime bolus dose through the orogastric tube. Three minutes after cyanide administration, swine in the treatment group received sodium thiosulfate (508.2 mg/kg, 3.25-M solution) and glycine (30 mg/kg, 3.5-M solution) (Sigma Aldrich) by orogastric tube in 2 sequential boluses (total volume <100 mL).

Doses were calculated with guidance from previous studies.[4,10] From a rabbit study, the human equivalent dose

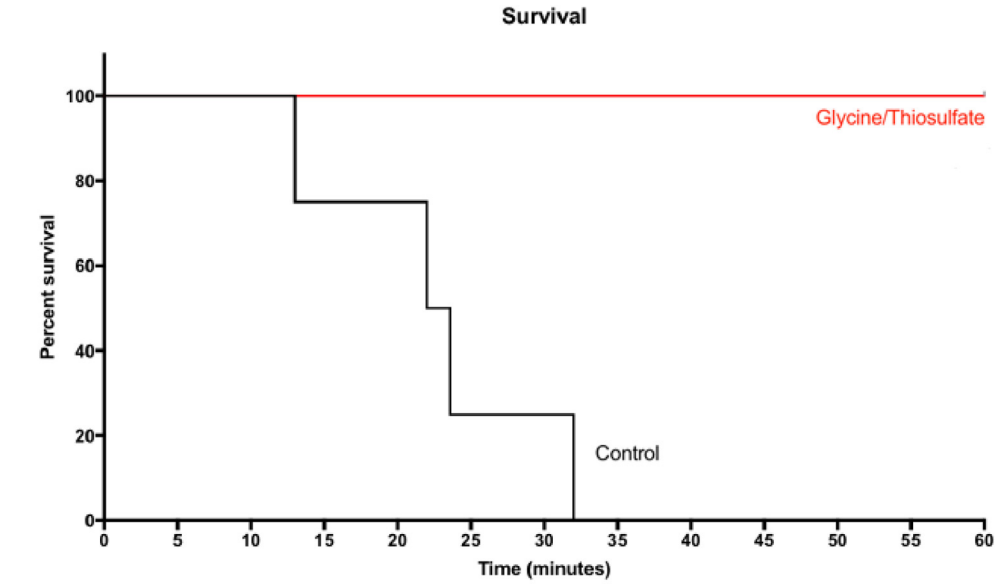

Figure 1. Survival analysis using a Kaplan-Meier plot comparing treatment and control groups of cyanide-poisoned animals.

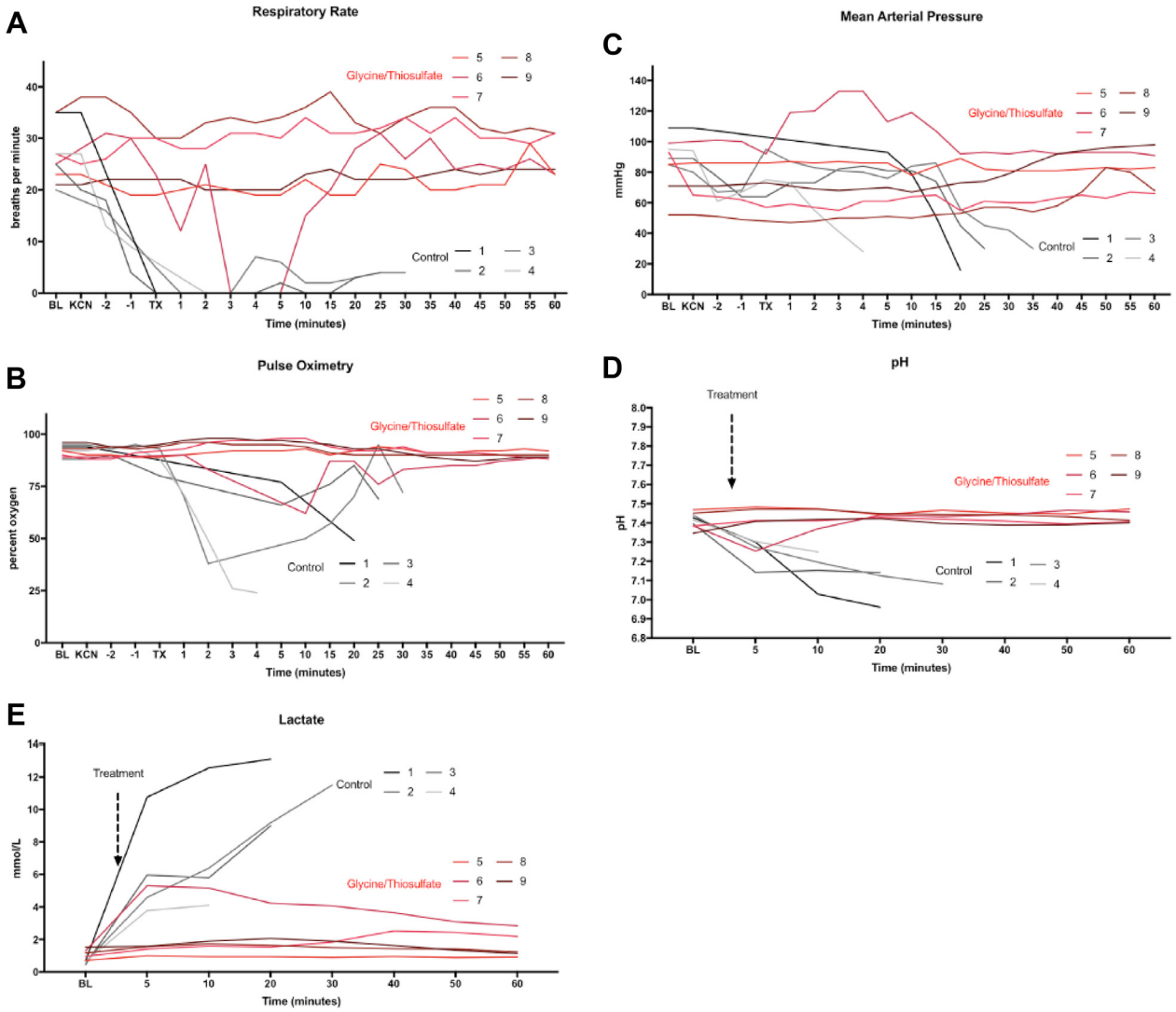

Figure 2. Physiologic parameters of animals over time. *A*, Respiratory rate over time. *B*, Pulse oximetry over time. *C*, Mean arterial pressure over time. *D*, pH over time. *E*, Lactate concentration over time.

for glycine and thiosulfate was calculated.[10] Using the human equivalent dose and the dose-by-factor approach, we calculated the no observed adverse effect levels of glycine and sodium thiosulfate in swine and maximally concentrated the solutions. Cyanide dosing was based on previous studies that demonstrated that potassium cyanide at 8 mg/kg is lethal in swine.[4] The timing of countermeasure administration was guided by a previous study using rabbits.[10]

Animals were monitored for 60 minutes or until death, defined as a mean arterial pressure of less than 30 mm Hg for 10 minutes. This pressure has been used as a clinically relevant endpoint for cyanide toxicity in previous large animal studies.[4,5] Animals that may have survived to the 60-minute mark but had not yet sustained a mean arterial pressure of less than 30 mm Hg for 10 minutes would have been categorized as nonsurvivors. Variables including pulse rate and mean arterial pressure were continuously monitored. Laboratory study results were obtained every 10 minutes.

Outcome Measures

The primary outcome was survival at 60 minutes. Other variables assessed included pulse rate, oxygen saturation, respiratory rate, blood pressure, arterial blood gas level, serum lactic acid concentration, and blood chemistry data.

Death or 60 minutes after cyanide exposure marked the end of the study, after which all animals were euthanized (sodium pentobarbital intravenously at 100 mg/kg) in compliance with the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care.

Primary Data Analysis

Prism (version 7.0; GraphPad, La Jolla, CA) was used for statistical analysis. An anticipated sample size of 8

Table 2. Significant improvement of lactate and pH in the treatment group compared with controls.*

	Control, n=4	Glycine Thiosulfate, n=5	Difference Between Means	95% CI Difference
Lactate, mmol/L	9.43 (4.08)	1.66 (0.82)	7.69 (2.07)	−14.05 to −1.32
pH	7.12 (0.14)	7.43 (0.03)	−0.31 (0.07)	0.09 to 0.53
SBP, mm Hg	61 (10.5)	104 (14.3)	43.1 (8.3)	8.3 to 23.5
MAP, mm Hg	26 (6.7)	81 (14.0)	55.2 (7.1)	37.8 to 72.6
Pulse rate, beats/min	64 (13.1)	98 (17.2)	33.8 (10.1)	9.9 to 57.6
Pulse oximetry, percentage oxygen	54 (22.2)	90 (1.5)	36.1 (11.1)	0.9 to 71.3

Data are presented as mean (SD).
*Physiologic parameters were also significantly improved in treatment animals compared with controls. Values presented are at study end, defined as death or at 60 minutes after treatment. All control animals died before 60 minutes. All treatment animals survived until 60 minutes.

animals per group was determined, with α=.05 and a power of 0.80, estimating an 80% difference in survival between groups. We stopped after 9 experiments (4 control animals, 5 treatment animals) because analysis of the data at that time revealed a statistically significant difference in the primary outcome, survival at 60 minutes.

Values are expressed as mean (SD). An unpaired *t* test with Welch's correction was used to calculate 95% confidence intervals (CIs), means, and SDs. A 2-tailed *t* test was used for comparison between groups. $P<.05$ was considered significant. Survival between groups was analyzed by generation of a Kaplan-Meier survival curve and comparison of percentage survival between groups by log-rank Mantel-Cox analysis.

RESULTS

At baseline and treatment, physiologic parameters, including weight, pH, lactate, and blood pressure, were similar in both groups (Table 1 and Table E1 [available online at http://www.annemermed.com]). Survival at 60 minutes, the primary outcome, was 100% in the treatment group compared with 0% in the control group (P=.003) (Figure 1). Control animals died at 12.55, 22.01, 23.35, and 32.75 minutes after potassium cyanide exposure. All animals in the control group developed apnea (Figure 2*A*), hypoxia (Figure 2*B*) (P=.047), hypotension (Figure 2*C*), acidemia (Figure 2*D*), and elevated serum lactate concentrations (Figure 2*E*). By the study end, defined as death or 60 minutes after cyanide administration, there was a significant difference in the lactate concentration between the treatment and control groups (control 9.43 mmol/L [SD 4.08]; treatment 1.66 mmol/L [SD 0.82]; difference between means 7.69 mmol/L [SD 2.07]; 95% CI difference –14.05 to –1.32). Mean arterial pressure was significantly different between the treatment and control groups (control 26 mm Hg [SD 6.7]; treatment 81 mm Hg [SD 14]; difference between means 55.2 mm Hg [SD 7.1]; 95% CI difference 37.8 to 72.6). pH and oxygen saturation were also significantly different between the treatment and control groups (Table 2).

LIMITATIONS

All animals have differences compared with humans, but the 50-kg pigs used in our study have an anatomy, size, and cardiovascular system similar to that of humans, making swine an excellent choice for an animal model of human cyanide toxicity.[4] Anesthesia was used; however, both treatment and control animals received similar doses, and use of anesthesia was required by our Institutional Animal Care and Use Committee. Animals were observed for a short period, but all the control animals died, and for our primary outcome of survival, the observation time was adequate. Control animals did not receive a volume of oral saline solution equivalent to that which treatment animals received; however, we maximally concentrated the countermeasure solutions to minimize the volume of saline solution delivered. Some physiologic parameters, such as the respiratory rate and oxygen saturation, may have been different between the 2 study groups at treatment. Two animals in the control group rapidly developed apnea (4 minutes and 30 seconds, and 3 minutes and 56 seconds). The earliest time to apnea in the treatment group was 6 minutes and 30 seconds. The differences in these parameters at treatment may have been a result of the small sample size. Further studies are needed to confirm that animals in each group were similar.

The oral treatments were administered 3 minutes after cyanide exposure, which may have prevented severe toxicity from developing in the treatment group. In severe toxicity, victims may rapidly lose consciousness and develop apnea, rendering them incapable of ingesting an oral antidote within that time frame. Future studies will be directed at understanding the effects of later timing of antidote administration.

An oral antidote would be most valuable for patients who are unsure whether they were exposed or those who present with mild or moderate signs of toxicity before developing severe illness. This study was conducted in a model using lethal doses of cyanide. We anticipate that an oral antidote that is efficacious for lethal doses of cyanide will also be effective for individuals with mild to moderate toxicity and may prevent the development of severe illness in these patients.

This study evaluated treatment with the combination of sodium thiosulfate/glycine. Currently, glycine is not commonly available in the out-of-hospital setting. We plan to study the efficacy of oral sodium thiosulfate as a single agent for treatment of cyanide ingestion in swine, explore the effects of changing the timing of oral antidote administration, and consider expanding observation time beyond 60 minutes.

DISCUSSION

We found that early intervention with oral thiosulfate and oral glycine yielded 100% survival compared with no treatment (0%) in a swine model of cyanide poisoning. The model parallels that of human exposure to cyanide and is dose dependent, which complements data from a murine model of oral cyanide poisoning.[4,6] Limitations with experiments involving mice include difficulty in hemodynamic monitoring and challenges with dose scaling of cyanide and antidote. Furthermore, there are major differences in gastric pH and anatomy between mice and humans.[6]

Alkalization of the gastric pH with bicarbonate prolonged survival but did not prevent death in rabbits poisoned with oral cyanide.[7] In the same study, the addition of cobinamide improved survival. Also in the rabbit model, Brenner et al[10] reported 100% survival in rabbits treated with oral glycine and oral sodium thiosulfate versus 0% in the controls. There are size, anatomic, and physiologic differences between rabbits and humans that pose a challenge in scaling doses of medications for potential human application.

According to the Food and Drug Administration Animal Rule (2015), countermeasures for exposures that cannot be studied in humans, such as cyanide, can be approved according to animal data from at least 2 species expected to mimic human toxicity. The data presented from this large-animal study build on those reported from previous experiments.[4,5-7,10]

The combination of oral sodium thiosulfate and glycine significantly improved survival and physiologic parameters in a large-animal model of oral cyanide toxicity.

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All authors attest to meeting the four ICMJE.org authorship criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND (2) Drafting the work or revising it critically for important intellectual content; AND (3) Final approval of the version to be published; AND (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REFERENCES

1. Agency for Toxic Substances and Disease Registry. Public health statement for cyanide. Available at: https://www.atsdr.cdc.gov/phs/phs.asp?id=70&tid=19. Accessed April 15, 2019.
2. Morocco AP. Cyanides. *Crit Care Clin*. 2005;21:691-705, vi.
3. Way JL. Cyanide intoxication and its mechanism of antagonism. *Annu Rev Pharmacol Toxicol*. 1984;24:451-481.

4. Ng PC, Hendry-Hofer TB, Witeof AE, et al. Model of oral potassium cyanide intoxication. *Comp Med*. 2018;68:375-379.
5. Hendry-Hofer TB, Witeof AE, Lippner DS, et al. Intramuscular dimethyl trisulfide: efficacy in a large swine model of acute severe cyanide toxicity. *Clin Toxicol (Phila)*. 2019;57:265-270.
6. Sabourin PJ, Kobs CL, Gibbs ST, et al. Characterization of a mouse model of oral potassium cyanide intoxication. *Int J Toxicol*. 2016;35:584-603.
7. Lee J, Mahon SB, Mukai D, et al. The vitamin B12 analog cobinamide is an effective antidote for oral cyanide poisoning. *J Med Toxicol*. 2016;12:370-379.
8. Barnes T. Potassium cyanide sent to Japanese newspapers, food and drug companies under names of "doomsday cult" leaders. Independent. Available at: https://www.independent.co.uk/news/world/asia/japan-potassium-cyanide-letter-threats-aum-shinrikyo-shoko-asahara-newspapers-drug-food-companies-a8752591.html. Accessed April 15, 2019.
9. Hendry-Hofer TB, Ng PC, Witeof AE, et al. A review on ingested cyanide: risks, clinical presentation, diagnostics and treatment challenges. *J Med Toxicol*. 2019;15:128-133.
10. Brenner M, Azer SM, Oh JK, et al. Oral glycine and sodium thiosulfate for lethal cyanide ingestion. *J Clin Toxicol*. 2017;7:355.