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# Intramuscular dimethyl trisulfide: Efficacy in a large swine model of acute severe cyanide toxicity

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## Abstract

**Background—**Cyanide is a deadly compound used as a terrorist agent. Current FDA approved antidotes require intravenous administration, limiting their utility in a mass casualty scenario. Dimethyl trisulfide (DMTS), a sulfur-based molecule, binds cyanide converting it to the less toxic by-product thiocyanate. Studies evaluating efficacy in rodents have been performed, but a large, clinically relevant animal model has not been reported.

**Objective—**This study evaluates the efficacy of intramuscular DMTS on survival and clinical outcomes in a swine model of acute, severe cyanide toxicity.

**Methods—**Anesthetized swine were instrumented for continuous monitoring of hemodynamics. Prior to potassium cyanide infusion animals were acclimated and breathing spontaneously. At 5-minutes post apnea animals were treated with DMTS or saline. Vital signs, hemodynamics, and laboratory values were evaluated at various time points.

**Results—**Baseline values and time to apnea were similar in both groups. Survival in the DMTS treated group was 83.3% and 0% in saline controls (p=0.005). The DMTS group returned to breathing at a mean time of 19.3±10 min after antidote, control animals did not return to breathing (CI difference 8.8, 29.8). At the end of the experiment or time of death, mean lactate was 9.41 mmol/L vs. 4.35 mmol/L (CI difference −10.94,0.82) in the saline and DMTS groups, respectively and pH was 7.20 vs. 7.37 (CI difference −0.04, 0.38). No adverse effects were observed at the injection site.

**Conclusion—**Intramuscular administration of DMTS improves survival and clinical outcomes in our large animal swine model of acute cyanide toxicity.

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### Keywords

cyanide poisoning; DMTS; Dimethyl trisulfide; terrorism; KCN; swine; intramuscular

## Introduction

Cyanide poisoning remains a major threat to civilians and military personnel worldwide from accidental, as well as intentional exposures [1]. The mechanism of cyanide toxicity is primarily by binding cytochrome c oxidase and inhibiting cellular respiration, causing lactic acidosis, altered mentation, apnea, hypotension, and finally cardiac arrest [2, 3]. The threat of cyanide use by terrorists is a major concern of the US chemical defense program, which makes finding a non-intravenous, safe antidote for acute cyanide toxicity a high priority [1]. While effective antidotes are available for treating individual victims, current antidotes must be given intravenously and often in large volumes [2]. Currently an antidote that could be administered in a mass casualty cyanide poisoning event does not exist, representing a major gap in treating patients in this type of scenario.

Nithiodote®, an FDA approved therapy for cyanide poisoning, contains sodium nitrite and sodium thiosulfate [3]. Sodium thiosulfate acts as a sulfur donor, converting cyanide to the less toxic, renally excreted compound thiocyante [4, 5, 6]. Thiosulfate relies on the sulfur transferase rhodanese, which is primarily found in the mitochondria of the liver and kidneys. Furthermore, thiosulfate is minimally lipophilic, limiting its ability to penetrate the cell and blood brain barrier, a target organ of cyanide toxicity [4, 7]. Dimethyl trisulfide (DMTS), like the FDA approved drug sodium thiosulfate, has been found to be therapeutic following cyanide poisoning [8]. Similar to sodium thiosulfate, DMTS, a sulfur based molecule found in garlic, onion and other plants, acts as a sulfur donor making it an antagonist for cyanide, converting cyanide to the less toxic compound thiocyanate [9,10]. However, compared to thiosulfate, DMTS has been shown to clear cyanide with greater efficiency, making it a potentially ideal candidate drug for cyanide toxicity [8, 9].

Due to its minimally toxic effects of DMTS, its long shelf-life, and the potential to be delivered intramuscularly (IM) by minimally trained individuals or via self-administration, DMTS could be an ideal antidote to cyanide poisoning. The goal of this study is to examine the efficacy of IM DMTS compared to saline control on survival and clinical outcomes in swine following acute cyanide toxicity.

## Materials and Methods

DMTS (126.25 g/mol), Span 80 and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO). A 40% (440.5 mg/ml) DMTS solution was prepared by adding 2 g DMTS to a surfactant mixture containing 0.75 g Span 80 and 2.25 g Tween 80. The mixture was votexed and stored in sealed glass vials prior to use [11].

### Study Design

We conducted a randomized control trial comparing an IM DMTS treatment group to an IM saline control group in cyanide exposed swine, a species commonly used to evaluate efficacy

of medical countermeasures to chemical toxins that cannot be evaluated in humans. All experiments were approved by the University of Colorado's Institutional Animal Care and Use Committee (IACUC) 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, and experimentation took place in the animal care facility at our institution.

## Animal Subjects

Adolescent female Yorkshire swine (*Sus scrofa*) (Oak Hill Genetics, Ewing, IL) weighing 45–55 kg were used for this study. Anesthesia was induced with IM administration of 10–20 mg/kg ketamine (MWI, Boise, ID) and isoflurane (MWI, Boise, ID) via nosecone. Animals were intubated with a cuffed 8.0 mm endotracheal tube (Teleflex, Morrisville, NC), and peripheral venous access obtained. Sedation was maintained using the Drager Fabius GS anesthesia machine (Drager, Houston, TX) with 1–3% isoflurane and 0.4 $FIO_2$. Tidal volume was set at 8 ml/kg and a respiratory rate of 16–20 breaths per minute, adjusting the minute volume to maintain an end tidal $CO_2$ of 45–55 mmHg. A 7.5 ml/kg bolus of 0.9% saline (B. Braun, Bethlehem, PA) was given prior to central line placement. The external jugular and femoral artery were visualized using the M9 ultrasound system (Mindray, Mahwah, NJ) and central venous and arterial access were obtained. The Drager Infinity Delta monitor (Drager, Houston, Tx) was used to monitor and record respiratory parameters, pulse oximetry, body temperature, invasive blood pressure, and electrocardiogram (ECG) throughout the experiment. Invasive hemodynamic variables were measured via pulmonary artery catheterization using an eight-French Swan Ganz CCOmbo catheter and the Edwards Vigilance II monitor (Edwards Lifesciences, Irvine, CA). Once vascular access was obtained a one-time bolus of heparin (100 units/kg) was given and isoflurane was weaned to 0.8–1% and 0.21 $FiO_2$ until the animal was breathing spontaneously, without mechanical ventilation. Sedation was maintained with isoflurane throughout the experimental procedures to minimize pain and discomfort.

## Experimental Protocol

Following a 10-minute acclimation period, animals were randomized into one of two treatment groups; IM DMTS (6 animals) (provided in kind by Dr. Rockwood, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD) or IM saline control (6 animals). Potassium cyanide (Sigma Aldrich, St. Louis, MO) was diluted in saline (B.Braun, Bethlehem, PA) and delivered via continuous infusion into the right jugular vein until five minutes after apnea occurred [2,3,12,13]. At five min after apnea, a mean time of 11.2 minutes after cyanide infusion was started, animals were treated with either DMTS or saline control and the cyanide infusion was terminated. The DMTS treatment arm received 82.5 mg/kg DMTS injected in equal volumes into the right and left gluteal muscle (total volume injected was approximately 8.8 ml). Control animals received an equivalent volume of saline injected into the right and left gluteal muscle. Animals were observed for 90 minutes post treatment or until death occurred, which was defined as a mean arterial pressure (MAP) of less than 30 for 10 continuous minutes.

### Outcome Measures

The primary outcome measure was time of survival between groups following treatment. We also compared physiologic variables including: return to spontaneous breathing following apnea, pulse rate, respiratory rate, pulse oximetry, invasive blood pressure, and systemic vascular resistance. All physiological parameters were monitored continuously and recorded every 5 minutes. Laboratory studies including chemistry, arterial blood gases and lactate concentration were obtained every 10 minutes.

### Euthanasia

At the end of the study all (surviving) animals were euthanized with an intravenous administration of 100 mg/kg sodium pentobarbital. All experiments were approved by the University of Colorado's Institutional Animal Care and Use Committee (IACUC) and complied with the regulations and guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care.

### Data Analysis

Prism 7.0 software (GraphPad, La Jolla, California) was used for statistical analysis. Using power analysis with an alpha of 0.05 and a power of 0.80 the anticipated sample size was 6 per group, estimating a 70% difference in survival between groups. We defined death as a MAP of less than 30 mmHg. Values are expressed as mean ± standard deviation. An unpaired *t* test with Welch's correction was used to calculate 95% confidence intervals, means, and standard deviations. A two-tailed *t* test was used for comparison between groups. A *P* value of less than 0.05 was considered significant. Survival between groups was analyzed by generating a Kaplan-Meier survival curve and comparing percent survival between groups by log-rank, Mantel-Cox analysis.

## Results

All physiological and laboratory parameters were similar between treatment and control animals at baseline (Table 1). Additionally, the dose of cyanide to achieve apnea was similar across both groups (Table 1). Similarly, at the time of apnea physiological and laboratory parameters were also comparable, as was the time to achieve apnea and the total amount of cyanide infused across both groups (Table 2).

Animals receiving IM DMTS demonstrated a significant increase in survival (p=0.005) compared to saline controls by 90 minutes post treatment (Figure 1). Animals in the DMTS treatment group (5/6) returned to spontaneous breathing after receiving the antidote, whereas animals in the saline group did not (0/6), and subsequently died (Table 3). Additionally, animals receiving DMTS showed improvement in clinical and laboratory parameters by the end of study (Table 3). Animals receiving DMTS showed improvement in heart rate, 115.5±41.2 versus control 50.8±19.1 beats per minutes (CI difference −44.5, 74.2), respiratory rate, 22.2±12.1 versus control 0±0 breaths per minute (CI difference 9.5, 34.9), pulse oximetry 81.2%±12.2% versus control 44.5%±3.8% (CI difference 23.9, 49.4) mean arterial pressure 72±21 versus control 20±5mmHg (CI difference 30.3, 74.3), and systemic vascular resistance 1069±398.9 versus control 604.7±40.4 dynes·sec·cm$^{-5}$ (CI difference

46.7, 882.7) (Table 3 and Figure 2). Though not statistically significant, blood pH and lactate improved over time in the DMTS treatment group (Figure 3). We did not observe any gross adverse effects at the saline or DMTS IM injection sites.

## Discussion

In our study of severe cyanide poisoning in swine, we found IM DMTS improves survival as well as clinical and laboratory parameters following acute cyanide poisoning as compared to our saline treated animals. Unlike saline controls, animals treated with DMTS (5/6) returned to breathing following treatment and survived for the duration of the experiment. DMTS also improved hemodynamics and blood gases demonstrating resolution of acidosis. With regards to blood pH and lactate, the lack of statistical significance with DMTS treatment compared to saline control is likely due to early drop out of control animals since the majority of control animals died within 60 minutes following treatment with saline.

Our experimental model mimics a prehospital and emergency department setting. For monitoring purposes, it utilizes human-grade medical equipment to assess physiological outcomes in our study. Additionally, since the swine used in this study are similar in size to humans, scaling the dose of DMTS required to treat human victims is simplified [14]. Our swine model of cyanide poisoning results in outcomes similar to what is seen in human cyanide exposures, characterized by hypotension, apnea, acidosis, and myocardial depression [2,3].

Rockwood and colleagues reported DMTS to be 43–79 times more efficient at clearing cyanide in an in-vitro comparison with thiosulfate [8]. In the mouse model of cyanide toxicity DMTS was shown to be efficacious against nearly 4 times the LD50 of KCN [11]. Based on the chemistry of DMTS (molecular weight 126.26 g/mol), it is highly likely that the fast therapeutic reaction is 1:1 with cyanide. However, this reaction can only occur when DMTS molecules find CN molecules in the body. The fraction of administered DMTS that actually finds CN will likely vary depending on the route of administration, and the level of cyanide intoxication. It is diffuclut to know how much cyanide is converted by DMTS without knowing how much DMTS is circulating. However, in their mouse model of cyanide poisoning DMTS offered more protection against cyanide compared to thiosulfate [8]. In previous studies, we have shown the sulfur donor sodium thiosulfate by itself does not reverse the effects of cyanide toxicity in our model when given intravenously alone [3]. Unlike sodium thiosulfate, DMTS is lipophilic, effective in the absence of rhodanese, and a more efficient sulfur donor, allowing for administration of smaller volumes into the muscle [8,11]. Additionally, DMTS is a single agent antidote, whereas sodium thiosulfate must be given with sodium nitrite to be efficacious. Evalutating DMTS in conjunction with other antidotes, as well as comparing it to other antidotes is warranted.

While Rockwood and colleagues had similar findings to ours, the rodent model of cyanide poisoning is limited. It does not allow invasive monitoring of clinical parameters, and physiologic comparisions to human can be challenging. Swine are commonly used for evaluating countermeasures to chemical toxic agents that cannot be evaluated in humans, and the species has been accepted by the FDA for chemical countermeasure development

[14]. The data presented here provide evidence that DMTS could fill the treatment gap and provide first responders with an antidote that can be administered quickly to a large number of victims in a mass casualty scenario. These studies focused on short term survival. In order to thoroughly evaluate efficacy, pharmacokinetics, and adverse effects of DMTS treatment, long term survival and clinical outcomes should be evaluated.

### Limitations

Our study does have limitations. Although improvement in blood pH and lactate were not statistically significant, we did however, show a trend towards improvement with DMTS treatment. It is likely that small sample size and the number of control animals which died prior to developing severe lactic acidosis contributed to the lack of statistical significance.

An additional limitation of this study is it does not replicate human toxicity exactly. Our model uses an intravenous infusion model of cyanide poisoning, inhalation or ingestion of cyanide is more realistic. However, our model does allow for invasive assessment of clinically relevant parameters. While most cases of cyanide toxicity result from ingestion or inhalation, we chose intravenous administration of potassium cyanide in an effort to induce reproducible, predictable, controlled, and rapid toxicity [2, 3, 12, 17, 18, 20]. Intravenous administration minimizes the risk to research staff compared to the inhaled route. Another limitation is the use of potassium cyanide to induce toxicity. The potassium dose was small, approximately 2 mEq in 30 minutes, which does not cause adverse cardiac effects [2, 15, 16]. Furthermore, potassium cyanide has been used in chemical attacks and is listed specifically on the Public Health Emergency Medical Countermeasures Enterprise (PHEMCE) list of threat agents [15].

These studies focused on one dose of DMTS (82.5 mg/kg) based on initial preliminary data. Rockwood and colleagues published in-vitro studies indicating much lower doses of DMTS may prove to be efficacious [8,11]. Based on this data, additional studies examining lower doses in swine are warranted. Furthermore, knowing the amount of DMTS in the systemic circulation following intramuscular administration would help guide dose optimization studies. We are currently working to optimize methods to analyze DMTS levels in swine blood and plasma. Future studies will be aimed at examining long term efficacy and evaluating neuro outcomes. Additionally, we used saline in the control arm, whereas DMTS was prepared in Span80/Tween80. However, efficacy studies in a mouse model of cyanide poisoning did not show improvement with intramuscular injection with Span80/Tween80. Finally, we performed short term survival studies, however cyanide's effects occur quickly, and we propose using DMTS soon after exposure in the field or mass casualty setting, in an effort to minimize the long term effects. Nonetheless, additional studies on long term survival should be performed.

## Conclusion

Intramuscular administration of DMTS improves survival and clinical outcomes in our large animal swine model of acute cyanide toxicity.

## Acknowledgments

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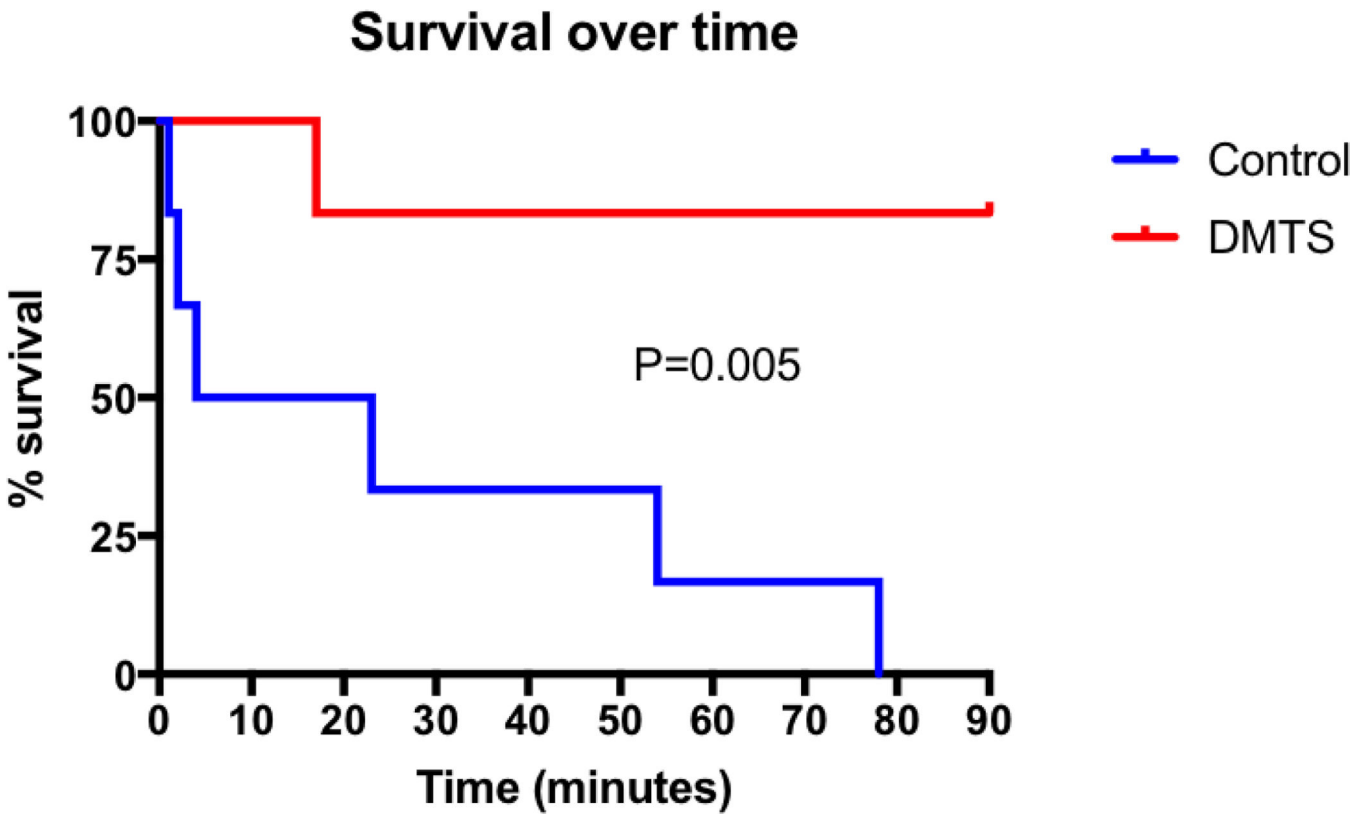


**Figure 1.**

Percent survival in swine treated with intramuscular DMTS as compared to saline control Survival is improved with IM DMTS administration following acute cyanide toxicity compared to saline controls. P value determined by log rank (Mantel-Cox) test, for comparison, P value less than or equal to 0.05 considered significant.

DMTS: dimethyl trisulfide

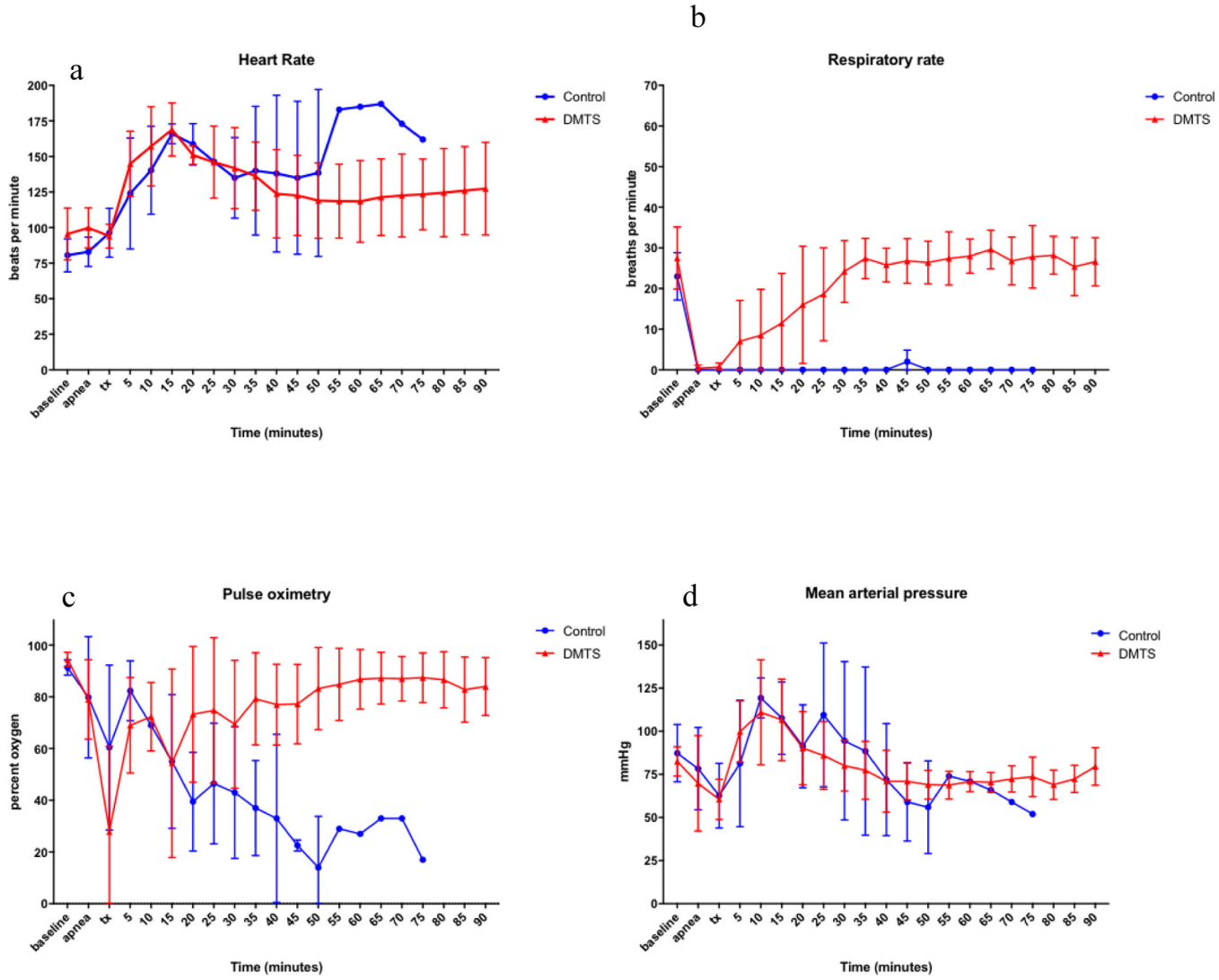


**Figure 2.**

Clinical outcomes over time between the swine treated with DMTS and saline control

Heart rate, respiratory rate, pulse oximetry, and mean arterial pressure are significantly improved in DMTS treated animals compared to saline control animals at the time of death/end of the study. P value determined using a two-tailed, unpaired *t* test for comparison, P value less than or equal to 0.05 considered significant, data is presented as means ± standard deviation. Comparisons made at death/end of study due to control animals dying prior to the end of study.

DMTS: dimethyl trisulfide, mmHg: millimeters of mercury

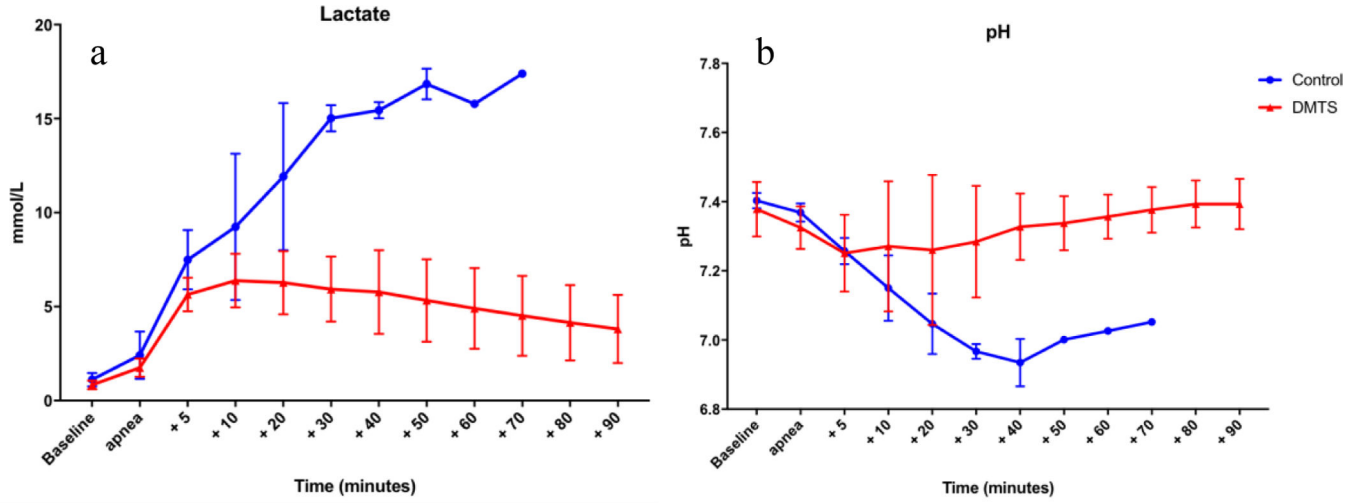


**Figure 3.**

Arterial blood gas values over time between the swine treated with DMTS and saline control

Lactate and pH improved in animals treated with DMTS compared to saline control over time, however was not statistically significant (p=0.080 and 0.096, respectively).

P value determined using a two-tailed, unpaired *t* test for comparison, P value less than or equal to 0.05 considered significant, data is presented as means ± standard deviation.

Comparisons made at death/end of study due to control animals dying prior to the end of study.

DMTS: dimethyl trisulfide, mmol/L: millimoles/liter; mEq/L: milliequivalents/liter

**Table 1.**

Physiological parameters at baseline between the swine treated with DMTS and saline control
There is no significant difference in animal weight, blood gases, hemodynamics, or respiratory rate at baseline.

| | Control n=6 | DMTS n=6 | Difference between means | 95% CI difference |
|---|---|---|---|---|
| **Weight (kg)** | 49.2±2.6 | 47.0±2.5 | −2.18±1.47 | −5.5, 1.1 |
| **Lactate (mmol/L)** | 1.11±0.35 | 0.83±0.23 | −0.28±0.17 | −0.68, 0.11 |
| **pH** | 7.40±0.02 | 7.38±0.08 | −0.025±0.03 | −0.11, 0.06 |
| **SBP (mmHg)** | 103±10.9 | 101±10.9 | −2±6.68 | −16.2, 11.8 |
| **MAP (mmHg)** | 83±12 | 83±9 | −1.00±5.86 | −13.7, 12.7 |
| **Pulse rate (beats per minute)** | 81±12 | 96±18 | 15.00±8.80 | −5.1, 35.1 |
| **Respiratory rate (breaths per minute)** | 23.0±5.8 | 27.5±57.6 | 4.50±3.92 | −4.3, 13.3 |

Data is presented as means ± standard deviation.

kg: kilogram; mg/kg: milligram/kilogram; KCN: potassium cyanide; mmol: millimole; L: liter; mmHg: millimeters of mercury; CI: confidence interval; DMTS: dimethyl trisulfide

**Table 2.**

Physiological parameters at apnea between the swine treated with DMTS and saline contro
There is no significant difference in total dose of KCN, time to apnea, blood gases, or hemodynamics at apnea.

| | Control n=6 | DMTS n=6 | Difference between means | 95% CI difference |
|---|---|---|---|---|
| **KCN mg/kg at apnea** | 1.08±0.15 | 1.02±0.09 | −0.06±0.18 | −0.47, 0.35 |
| **KCN mg/kg at treatment** | 1.95±0.31 | 1.90±0.19 | −0.05±0.15 | −0.38, 0.30 |
| **Time to apnea (minutes)** | 6.37±2.22 | 5.99±1.32 | −0.38±1.053 | −2.8, 2.04 |
| **Lactate (mmol/L)** | 2.41±1.26 | 1.78± 0.53 | −0.63±0.60 | −2.18, 0.91 |
| **pH** | 7.37±0.03 | 7.35±0.03 | −0.02±0.03 | −0.07, 0.02 |
| **SBP (mmHg)** | 112±28.7 | 94±32.5 | −17.5±17.7 | −57.1, 22.1 |
| **MAP (mmHg)** | 78±23.9 | 70±27.7 | −8.5±14.9 | −41.9, 24.9 |
| **Pulse rate (beats per minute)** | 83±10.4 | 100±14 | 16.8±7.1 | −0.8, 32.9 |

Data is presented as means ± standard deviation.

KCN: potassium cyanide; mg/kg: milligram/kilogram; mmol: millimole; L: liter; mmHg: millimeters of mercury; CI: confidence interval; DMTS: dimethyl trisulfide

**Table 3.**

Animal characteristics at death or end of study between the swine treated with DMTS and saline control Animals in the DMTS treatment arm return to breathing following apnea, whereas control animals do not. DMTS treatment results in increased survival time, improved blood lactate and pH, improved hemodynamics, pulse oximetry, and respiratory rate. Comparisons made at death/end of study due to control animals dying prior to the end of study.

| | Control n=6 | DMTS n=6 | Difference between means | 95% CI difference |
|---|---|---|---|---|
| **Time to rebreathing post treatment (minutes)** | 0 | 19.3±10.0 | 19.3±4.1 | 8.8, 29.8 |
| **Time to death (minutes)** | 27±32.2 | 77.8±29.8 | 50.8±17.9 | 10.9, 90.8 |
| **Lactate (mmol/L)** | 9.41±5.69 | 4.35±2.10 | −5.06±2.44 | −10.94, 0.82 |
| **pH** | 7.20±0.20 | 7.37±0.09 | 0.17±0.09 | −0.04, 0.38 |
| **Systolic blood pressure (mmHg)** | 27±10 | 86±27 | 59±11.7 | 30.6, 87.4 |
| **Mean arterial pressure (mmHg)** | 20±5 | 72±21 | 52±8.9 | 30.3, 74.3 |
| **Heart rate (beats per minute)** | 50.8±19.1 | 115.5±41.2 | 64.7±18.79 | −44.5, 74.2 |
| **Respiratory rate (breaths per minute)** | 0±0 | 22.2±12.1 | 22.2±4.9 | 9.5, 34.9 |
| **Pulse oximetry (% oxygen)** | 44.5±3.8 | 81.2±12.2 | 36.7±5.2 | 23.9, 49.4 |
| **Systemic vascular resistance (dynes·sec·cm$^{-5}$)** | 604.7±40.4 | 1069±398.9 | 464.3±164.5 | 46.7, 882.7 |

Data is presented as means ± standard deviation.

mmol: millimole; L: liter; mmHg: millimeters of mercury; CI: confidence interval; DMTS: dimethyl trisulfide