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TITLE: Optimization of Repeat Dosing with Intramuscular Dimethyltrisulfide Following Acute Cyanide Poisoning

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CONTRACTING ORGANIZATION: University of Colorado-Denver

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The US chemical defense program has identified finding a non-intravenous, safe antidote for acute cyanide toxicity a high priority. Current antidotes require intravenous infusion making their utility in a mass casualty poisoning limited. Dimethyl trisulfide has been shown to be an effective antidote when administered via intramuscular injection in rodent and swine models of cyanide poisoning. However, efficacy following repeat dosing based on clinical indicators has not been evaluated. We have demonstrated that repeat dosing of DMTS rescues from highly lethal cyanide poisoning. These studies are important since certain cyanide exposures may result in ongoing toxicity over extended periods of time, requiring additional antidote administration.

15. SUBJECT TERMS
Cyanide, DMTS, Intramuscular, Swine

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The Department of Homeland Security and the US chemical defense program in the DoD Combat Casualty Care Research Program have identified finding a non-intravenous, safe antidote for acute cyanide toxicity a high priority. Current antidotes require intravenous infusion making their utility in a mass casualty poisoning limited. We have shown short-term and long-term efficacy of intramuscular DMTS in a large, swine model of acute cyanide poisoning. However, we have not previously evaluated efficacy of repeat administration of DMTS following acute cyanide poisoning. Based on evaluation of mean arterial blood pressure following treatment with DMTS, we believe repeat dosing with IM DMTS may further improve efficacy.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Cyanide, Dimethyl Trisulfide, Swine, Apnea, Intramuscular

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goals of this project are outlined in the specific aims below.

Specific Aim 1: Determine the optimal dosing regimen of intramuscular DMTS to rescue from a lethal cyanide poisoning and improve survival.

- Local IACUC approval
- ACURO approval
- Determine the optimal dosing regimen of intramuscular DMTS

Specific Aim 2: Determine the therapeutic dose range and plasma drug levels that rescue from lethal cyanide poisoning.

- Administration of intramuscular DMTS and blood sample collection for analysis of DMTS blood levels
- Blood sample collection and processing for analysis of DMTS blood levels

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities

Optimization of repeat dosing with IM DMTS – We have conducted studies aimed to optimize a repeat dosing regimen of IM DMTS. Swine between 45-50 kg were used for these studies. Anesthesia was induced with intramuscular ketamine and sedation maintained with isoflurane via nosecone (see detailed procedures below). Following induction endotracheal intubation was performed and sedation maintained with a mixture of oxygen (FiO2 0.21-0.40) and isoflurane (1-3%). Animals were instrumented with central venous and arterial catheters for blood collection. Once stable and breathing spontaneously, baseline blood samples were collected and potassium cyanide (KCN) administered via intravenous infusion. At 5 minutes after apnea the cyanide infusion was stopped, and IM DMTS administered. A second dose of DMTS was administered if the animal's mean arterial blood pressure dropped below 15% of baseline. Dose ranging studies were conducted to determine the optimal doses of DMTS required for rescue and maintenance of mean arterial blood pressure. Swine were monitored continuously under sedation for 90 minutes after treatment.

Therapeutic dose range and plasma drug concentrations that rescue from cyanide poisoning

To evaluate the therapeutic dose range and plasma drug concentration of repeat dosing of IM DMTS blood samples will be collected at baseline and at 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes after the first IM DMTS administration.

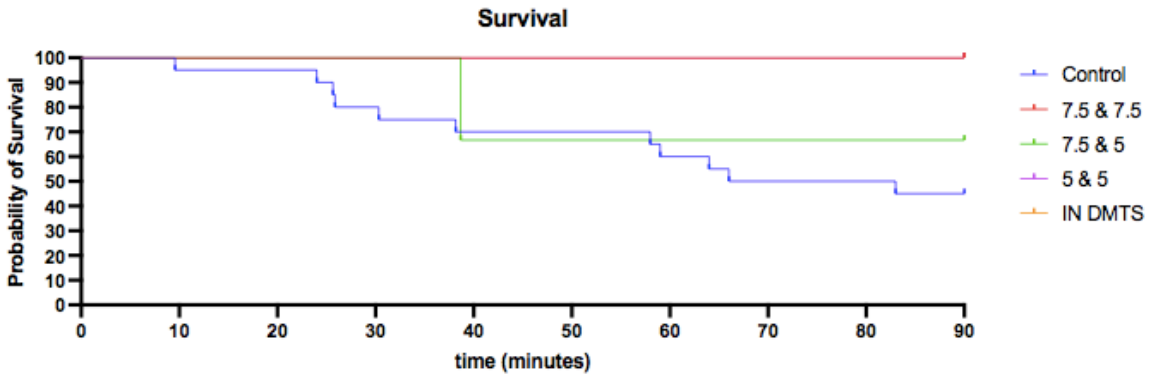
Specific Objectives

- Optimization of repeat dosing of intramuscular DMTS that rescues from cyanide poisoning
- Identify the therapeutic concentration of DMTS that rescues from cyanide poisoning
- Survive swine for 3-7 days following DMTS rescue to evaluate neurocognitive outcomes

Significant Results

The studies conducted demonstrate that repeat dosing with intramuscular DMTS rescues from cyanide poisoning. Dose finding studies identified repeat dosing with 7.5 mg/kg provided the most efficacy (figure 1). Studies aimed at identifying the therapeutic dose of DMTS required to rescue suggest that a blood concentration of 0.1 micromolar is required for rescue since animals that survived cyanide poisoning had blood concentrations 0.1 micromolar. However, comparison with animals that did not survive is still needed to conclusively determine the therapeutic blood concentration.

Figure 1 Survival Following Repeat Treatment with DMTS After Cyanide Poisoning



Swine received 2 doses of DMTS. The first dose of DMTS was administered at 5 minutes post apnea, the second dose was administered when the mean arterial blood pressure reached 15% of the baseline value.

Future Directions

At the time of this report, we have scheduled studies to evaluate the long-term outcomes of cyanide poisoned swine after rescue with DMTS. In addition, we have developed a scoring system for the assessment of neurobehavioral outcomes using spatial object recognition and open field evaluations.

Conclusion

The major goals of the specific aims were accomplished during this period. The completed studies have demonstrated that repeat dosing with DMTS is efficacious and improves survival following acute cyanide poisoning. We are on schedule to complete the studies aimed at evaluating long-term survival and neurobehavioral outcomes.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Goals and objectives have been accomplished for year 1. Studies are scheduled to being in January 2023 to assess the long-term outcomes following repeat dosing.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Determining the most effective treatment regimen of intramuscular DMTS administration, as well as therapeutic blood concentrations is an important step toward FDA approval. Future studies will evaluate long-term efficacy. If repeat dosing of DMTS is shown to be efficacious it could fulfill the need for a rapid acting, easy to administer, safe cyanide antidote applicable to mass casualty incidents.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Our swine vendors experienced an outbreak of porcine reproductive and respiratory syndrome (PRRS) resulting in a minor delay in study execution. We have identified a third source of swine for our studies in an effort to avoid future delays.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animal

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: *Vikhyat Bebarta, MD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*
Contribution to Project: *Dr. Bebarta has contributed to the design and execution of experiments.*

Name: *Tara Hendry-Hofer, BSN, RN*
Project Role: *PM*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*

Contribution to Project: *Ms. Hendry-Hofer has contributed to the design and execution of experiments.*

Name: *Carter Severance, BS*
Project Role: *PRA*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*
Contribution to Project: *Carter Severance has contributed to the execution of experiments.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*

- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.