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14. ABSTRACT

Under Task Order 0001, the MREF's laboratories and facilities were maintained and operated in compliance with government regulations. The NREF successfully passed all inspections and certifications. Major contract activities performed include: conducting inventories of CA and maintaining usage reports, preparing seven Test Execution Plans (TEP) for task orders, and scientific meetings with DoD Team representatives to develop current and projected tasks. A TEP was prepared for Task Order 0002 to support government testing of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA). Task Order 0003 is for performance of toxicity testing of MMB-4 dimethanesulfonate (MMB-4 DMS). Task Order 0004 is to compare cutaneous sulfur mustard injuries with thermal burns in a weanling pig models, and Task Order 0005 is to use this model to determine efficacy of Epitram-B in improving healing of superficial dermal sulfur mustard injuries. Task Order 0006 is an in vitro study to investigate skin penetration of a non-traditional agent (NTA). Task Order 0007 is for toxicity testing of 4-pyridinealdoxime.

15. SUBJECT TERMS
Chemical Defense, medical countermeasures, animal models, protection, genotoxicity, treatment, HD, SERPACWA, penetration cell, NTA, MMB-4, toxicology, wound healing, GLP, microarray, thermal injury, HD injury

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ANNUAL REPORT ON CONTRACT
W81XWH-05-D-0001

Operate a Chemical Surety Program and Studies Supporting the Medical Chemical Defense Research Program

INTRODUCTION

The Department of Defense (DoD) Team consisting of the Defense Threat Reduction Agency (DTRA), Chemical Biological Medical Systems (CBMS), a division of the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD), and the U.S. Army Medical Research and Materiel Command (USAMRMC) has a mission-critical goal to improve military effectiveness and survivability of the service member in a chemically contaminated environment. Battelle's Medical Research and Evaluation Facility (MREF) supports this goal.

Battelle's MREF operates to answer and validate basic, applied, and developmental biomedical questions critical to providing improved medical countermeasures against existing chemical agents (CA), toxins and emerging threats. This research, development, testing, and evaluation (RDT&E) facility uses animal models, alternatives to animal models, and complex laboratory procedures utilizing CA and other hazardous chemicals. The facility provides ample space for studies in multiple animal species, *in vitro* models, including isolated organ systems and cell cultures, and analytical, medicinal, and synthetic chemistry procedures. Studies involve parenteral, oral, or topical administration of candidate prophylaxes, pretreatment compounds, protective and/or decontamination materials in conjunction with percutaneous, parenteral, or inhalation challenge with threat agents utilizing *in vivo* model systems. The facility meets all safety and surety requirements for storage, handling, use, and disposal of CA, RDT&E dilute solutions of CA and other hazardous materials. The MREF has the capability of conducting research and testing to satisfy the requirements of Good Laboratory Practices (GLP) regulations and ISO 9001 standards.

BODY

Task Order 0001 – Operate a Chemical Surety Program and Provide Management Support

The MREF's laboratories and facilities were maintained and operated in compliance with government regulations. Major contract activities performed include: conducting inventories of CA and maintaining usage reports, preparing seven Test Execution Plans (TEP) for task orders, and numerous scientific discussions with DoD Team representatives for the development of current and projected tasks. The MREF successfully passed all inspections or certifications by the U.S. Department of Agriculture, Ohio Environmental Protection Agency, Madison County (OH) Health Department, Battelle's Institutional Animal Care and Use Committee (IACUC), ISO 9001 Registrar, Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International), the USAMRICD Safety and Chemical Operations

Branch, and the U.S. Army Inspector General. The MREF provides administrative support for an on-site Contracting Officer's Representative (COR).

Task Order 0002 - Support of Government Testing of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA)

A TEP for this task order was requested on June 16, 2005, and the task order was awarded on September 15, 2005. There were three objectives with this task order. The first is to test if application of SERPACWA affected efficacy of decontamination of sulfur mustard (HD) with the M291 skin decontamination kit (SDK). The second objective is to test if use of the M291SDK following HD exposure interferes with the effectiveness of SERPACWA against that exposure. The third objective is to test the impact of use of the M291 SDK on SERPACWA efficacy against subsequent HD exposure prior to SERPACWA re-application. These objectives are to provide answers to questions raised by the U.S. Food and Drug Administration (FDA) following their clinical review.

A TEP was drafted and submitted to CBMS on October 6, 2005. After discussions among MREF and CBMS personnel and the COR, Dr. David Lenz, an updated study design was prepared and submitted to CBMS on October 20, 2005 for review. On November 1, 2005, Battelle was notified to provide CBMS an official copy of the revised protocol, and this was submitted on November 3, 2005. CBMS requested a review by the FDA prior to implementing the test protocol submitted by Battelle. Execution of laboratory studies are on hold pending FDA's response to CBMS on review and approval of the protocol.

Task Order 0003 - Toxicology Testing of MMB-4 Dimethanesulfonate (MMB-4 DMS)

A TEP for this task order was requested on January 27, 2006, and the task order was awarded on March 31, 2006. Two *in vitro* studies and one *in vivo* genotoxicity study conducted in accordance with GLP regulations under approved protocols that comply with 21 CFR 58 were requested. The studies include Ames testing to evaluate the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in several strains of *Salmonella typhimurium*, and at the tryptophan locus of *Escherichia coli* strain WP2*uvr*A, in the presence or absence of an exogenous mammalian metabolic activation system (S9) containing microsomal enzymes. The assay design is based on the Organisation for Economic Co-operation and Development (OECD) Guideline 471, updated and adopted 21 July 1997. The second *in vitro* study is to evaluate the ability of the test article to induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells with and without an exogenous metabolic activation system. The assay design meets the OECD Guideline 473, updated and adopted July 21, 1997.

In vivo genotoxicity testing is to evaluate the test article for clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in mouse bone marrow. The assay design is based on OECD Guideline 474, updated and adopted July 21, 1997.

The data from this study are in support of a future Investigational New Drug (IND) application to the FDA by the Medical Identification and Treatment Systems Joint Product Management Office (MITS-JPMO). Execution of laboratory studies are expected to begin after June 20, 2006 when MITS-JPMO receives the MMB-4 DMS produced under current Good Manufacturing Practices (cGMP).

Task Order 0004 - Comparison of Cutaneous Sulfur Mustard Injuries and Thermal Burns in a Weanling Pig Model

A TEP for this task order was requested February 8, 2006, and following the granting of a time extension, a TEP was submitted on March 8, 2006. Following discussions with Dr. John Graham, U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), a revised TEP was submitted March 24, 2006. Procedures to produce similar superficial dermal cutaneous injuries by heat and by HD on the ventral abdominal surface of weanling pigs are to be determined in this task order. Following development of the model, progression and healing of these injuries are compared using contemporary genomic techniques.

The study is performed in three phases. Phase I is a dose ranging study to determine the temperature and application time of a metal rod necessary to produce a 3-cm diameter, superficial dermal burn. Phase II is to verify that the chosen parameters will generate a burn of similar depth and severity as that caused by an 8-min exposure to 400 µl of HD applied to a 3-cm diameter area. Phase III compares thermal and HD injuries using the validated model. Tissues are collected at 1, 3, 6, 12, 24, and 48 hr and at 7 days to evaluate wound progression and early healing. Microarray analysis of selected skin biopsies is performed, and a temporal gene expression profile of wound progression obtained. Molecular pathways of healing and potential therapeutic targets are identified when possible.

Task Order 0005 - Efficacy of Epitram-B in Improving Healing of Superficial Dermal Sulfur Mustard Injuries in a Weanling Pig Model

The request for a TEP was received February 8, 2006. Following discussions with Dr. John Graham, USAMRICD, a TEP for this task order was submitted March 3, 2006. This task order is a study to compare the efficacy of a cream, Epitram-B, to silver sulfadiazine in improving the healing of superficial dermal sulfur mustard injuries in the weanling pig. Epitram-B is reported to help activate the proliferative phase of wound healing, decrease swelling and edema, control bleeding, suppress scar tissue, decrease

gram negative bacteria, and stimulate circulation. The HD superficial dermal injury weanling pig model developed at the MREF is used to compare two different doses of Epitram-B to one dose of silver sulfadiazine, with a fourth site receiving no treatment.

Task Order 0006 - An *In Vitro* Study of Skin Penetration Rate of a NTA

A TEP for this task order was requested on April 18, 2006 and the proposal is due May 3, 2006. This task includes *in vitro* experiments and mathematical approaches to provide dermal penetration kinetics of a nontraditional agent (NTA) that poses a skin hazard. The task is completed in three phases. In phase 1, prior to conducting static diffusion cell/skin penetration experiments, analytical chemistry procedures are performed. Specifically, the solubility, stability, compatibility and calibration range for the test compound in receptor fluid, the fluid that is in contact with the dermal side of the skin sample, must be determined. A liquid chromatography - electrospray ionization- tandem mass spectrometry (LC-ESI-MS/MS) method of analysis of the NTA has been developed, but may need to be adapted for analysis of the compound within receptor fluid samples. Phase 2 determines the NTA flux through skin. The dermal penetration of the NTA compound is evaluated using static diffusion cells and dermatomed weanling pig skin. Skin samples with a defined exposure area are placed onto static diffusion cells and a known amounts of the NTA (~ 10 g/m^2) is applied to the surface of the skin. At specific time intervals, the receptor solution is analyzed, using the method developed in phase 1, to determine the concentration of the NTA. Phase 3 determines the NTA flux through skin following contact transfer of the NTA from a concrete matrix. Previous work demonstrated environmental persistence of the test NTA, and this phase supplies information regarding the rate of NTA penetration of skin following contact with a contaminated surface.

Task Order 0007 - Toxicology Testing of 4-Pyridinealdoxime

A TEP for this task order was requested on May 1, 2006 and the proposal is due May 15, 2006. Four studies, including two *in vitro* genotoxicity studies, one *in vivo* genotoxicity study, and one *in vivo* 7-day repeat dose study, were requested to be conducted in accordance with GLP regulations under approved protocols that comply with 21 CFR 58. The studies include Ames testing to evaluate the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in several strains of *Salmonella typhimurium*, and at the tryptophan locus of *Escherichia coli* strain WP2*uvr*A, in the presence or absence of an exogenous mammalian metabolic activation system (S9) containing microsomal enzymes. The assay design is based on the OECD Guideline 471, updated and adopted 21 July 1997. The second *in vitro* study evaluates the ability of the test article to induce chromosomal aberrations in cultured CHO cells with and without an exogenous metabolic activation system. The assay design meets OECD Guideline 473, updated and adopted July 21, 1997.

In vivo genotoxicity testing evaluates the test article for clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in mouse bone marrow. The assay design is based on OECD Guideline 474, updated and adopted July 21, 1997.

For the 7-day repeat dose study, two lots of 4-pyridinealdoxime, with certificates of analysis, are procured from separate sources, and an analytical method is established and validated to compare the lots. Solutions of 4-pyridinealdoxime in normal saline for injection are prepared fresh on each dosing day, and dose confirmation samples collected and assayed. Initially, a dose range-finding study is performed to estimate a no-observable-adverse-effect level (NOAEL) for 4-pyridinealdoxime administered by intramuscular injection in rabbits. A highest dose level less than the NOAEL is selected for the subsequent 7-day repeated dose study.

KEY RESEARCH ACCOMPLISHMENTS

Task 0001

- Provided program management support and prepared seven TEPs for task orders.
- Conducted inventories of CA and maintained usage reports.
- Passed all regulatory inspections.
- Provided program administrative support for an on-site Contracting Officer's Representative (COR).

Task 0002

- Provided CBMS with a test protocol to submit to FDA for review and approval.

REPORTABLE OUTCOMES

Not applicable.

CONCLUSION

Not applicable.

REFERENCES

Not applicable.

APPENDICES

Not applicable.

SUPPORTING DATA

Not applicable.