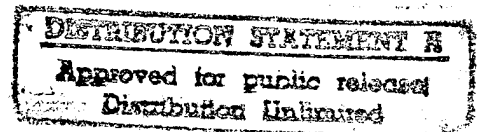

QUARTERLY REPORT

**RESEARCH ON NAVY-RELATED COMBAT
CASUALTY CARE ISSUES, NAVY OPERATIONAL-
RELATED INJURIES AND ILLNESSES AND
APPROACHES TO ENHANCE NAVY/MARINE
CORPS PERSONNEL COMBAT PERFORMANCE**

Prepared for

Naval Medical Research Institute
Bethesda, Maryland 20814



As Required By
Contract Number N00014-95-D-0048
(GC-2728)

Prepared by
GEO-CENTERS, INC.
7 Wells Avenue
Newton Centre, MA 02159

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GEO-CENTERS, INC.

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QUARTERLY PROGRESS REPORT
1ST QUARTER OF OPTION YEAR ONE
GC-PR-2728-00

CONTRACT NUMBER: N00014-95-D-0048

REPORTING PERIOD: December 1, 1995 - February 29, 1996

REPORT DATE: March 25, 1996

**RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES,
NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND
APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL
COMBAT PERFORMANCE**

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the first quarter of the contractual option year one for the Naval Medical Research Institute (NMRI) under Contract N00014-95-D-0048, Delivery Orders 002 and 003. The delivery orders encompass a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; and the National Naval Medical Center-Bethesda, MD.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Summary of work performed during current reporting period, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles are being provided as part of this annual technical progress report. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

- A. Infectious disease threat assessment and enterics programs.
- B. Immune cell biology, wound repair and artificial blood studies.
- C. Biomedical diving programs.
- D. Personnel performance enhancement programs.
- E. Breast Care Center.
- F. Dental related diseases.
- G. Toxicological studies.



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II. NMRI, Bethesda, MD

A. INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

DESCRIPTION OF WORK TO BE PERFORMED

Fernando

- Performs research in order to develop efficient molecular assays for the detection and identification of gene sequences of orthopox viruses, which can be a warfare as well as an epidemiological threat. The research involves the study of background literature on the genomic structures and characteristics of these viruses and their current DNA detection methodologies, design of gene probes and tests that will identify and characterize orthopox genomic profiles, and evaluation and optimization of these tests for sensitivity, specificity and efficiency. These tests fall into three levels, namely, primary screening, secondary confirmatory and tertiary characterization.

Jendrek

- Conducts fermentations in a BL-3 suite and depending upon the organism being fermented may also perform some or all of the purification associated with the project. He also has to create all documentation associated with any aspect of his position, including Standard Operating Procedure, Batch Records, and any documentation required for newly installed equipment. He also either installs all new equipment related to his projects or oversees their installation by the technicians sent by the supplier. He must also assist in the Molecular Biology aspects of his position, DNA purification, Plasmid isolation, Electroporation, and other techniques which are performed regularly.

Weeks

- Serve as an associate of the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses. Conducts surveys of the scientific literature to develop background data on techniques



and formulates approaches for the investigations, develops experimental protocols, defines the objectives and priorities of subsidiary problems and arranges the details of cooperative investigations with other organizations when necessary. Is responsible for the general administration of the laboratory reagents, solutions, enzymes, and other materials and equipment used in conducting the studies described. Is responsible for the cleanliness and orderliness of working areas, freezers, and refrigerators. Is responsible for the training and orientation of all new laboratory technicians. Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation of the histories of each. Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory. Performs other duties as assigned.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Fernando

- Will collect and study the literature on orthopox viral molecular biology and DNA detection methodologies for the design of gene probes and PCR tests for orthopox genes. Begin to evaluate the probes by PCR analysis of orthopox DNA.

Jendrek

- Next quarter, perform more fermentations of Delta Sterne pPA102(CR4) to stockpile the protein for future research and trials. The 20 liter fermentations will also be repeated to show repeatability and to test the batch record for that process. He will also grow various strains of WB600 that produce PA and various mutants of the protein. He will continue with the molecular biology to attempt to get a plasmid shuttle vector between E.coli and B. Subtillus.

Weeks

- Finish the mapping of the pFra plasmid of Yersinia pestis. The other objective for this quarter is to start and finish the characterization of the PLA gene of the pPst plasmid of Y. pestis.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Fernando

- Studied a vast collection of literature on the genomic structures and detection tests for orthopox viruses. He has been evaluating several sets of gene amplification primers by PCR analysis. The primers were designed to target the conserved genes, namely, (a) hemagglutinin, and (b) the contiguous cluster comprised of DNA polymerase, glutaredoxin, intermediate and ribonucleotide reductase operons of the orthopox genomic family. These primers are expected to generate 455-460 bp and 10,000 bp DNA amplicons for (a) and (b), respectively, from the four orthopox viral DNA samples, namely, Vaccinia, Cowpox, Monkeypox and Camelpox. Vero cell DNA served as the negative gene control. The tests were successfully conducted yielding the expected positive results.

Jendrek

- Conducting various molecular biological tasks to create a plasmid shuttle vector for the PA gene between *E. coli* and *B. subtilis*. Experiments range from plasmid isolation and digestion to ligating fragments of PCR DNA into plasmids. Completed fermentations of the WB600 for some of the mutant forms of the PA protein, continued fermentations of WB600 for some of the mutant forms of the PA gene into various bacterial species. He has also continued his fermentations of the wild type PA for researchers in the BAC-T division.

Weeks

- Finished mapping the pFra plasmid of *Yersinia pestis*. The plasmid map was generated and requires fine tuning. More map unit points must be verified, therefore more sequence and digest information is currently being generated. Initiated work on the deletion/addition mutations in the pPst plasmid of *Y. pestis*. This will verify the virulence capabilities of the plasminogen activator properties of the PLA gene of the pPst plasmid in order to obtain a viable vaccine candidate.



GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Fernando

- Will run several experiments using modified parameters in order to optimise these PCR tests for greater efficiency. He will perform the optimization assays with and without d-UTP incorporation, which will be required for the secondary tests. The latter tests will involve automated fluorescent RFLP analysis, using the ABI Prism Automatic DNA Sequencer. He will perform restriction enzyme site map analysis on the genomes for selecting best RE candidates for the RFLP DNA fingerprinting.

Jendrek

- Will continue with molecular biological aspects of the position. He will also begin to do sequencing gels and work with the actual mutation aspect of the Protective Antigen gene. The shuttle vector, will then be employed for moving the mutant PA gene into various bacterial species. Continue to do fermentations of WB600 and B. anthracis to stockpile PA for other researchers.

Weeks

- Fine tune the mapping of the pFra plasmid and finish characterization of the PLA gene of the pPst plasmid.



II. NMRI, Bethesda, MD

**B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND
ARTIFICIAL BLOOD PROGRAM**

DESCRIPTION OF WORK TO BE PERFORMED

Li

- Lipopolysaccharide (LPS) is considered to be the responsible agent for the induction of endotoxic shock, affecting the liver and intestine as a target organs. Intestinal cell lines and endothelial cell line have been selected for this work. For these experiments, cells will be stimulated with various concentration of LPS (endotoxin) and incubated in low oxygen (hypoxia) environment to mimic hemorrhagic shock (HS) *in vitro*.

Fan

- Currently conducting molecular biological research at Septic Shock Research Program, Naval Medical Research Institute. Projects include "Detection of inducible nitric oxide mRNA in cardiac myocytes and cardiovascular smooth muscle cells of septic rats" and "Regulation of protein kinase C mRNA isotypes expression in rat cardiovascular smooth muscle cells".

Chavez

- Perform basic research on the physical properties of hemoglobin and hemoglobin-based blood substitutes. Hemoglobin is the protein responsible for oxygen transport. Hemoglobin oxidation, heme stability within the hemoglobin, and nitric oxide binding are the major focus areas at this time.
- Provide scientific consulting on a variety of pertinent operations including analytical assay development, hemoglobin biochemistry, and protein purification and stabilization.



TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Li

- To investigate the mechanisms that oral interleukin-6 (IL-6) improves gut barrier function partially restores immune function after HS.
- An endotoxic and hypoxic cell model which mimic the HS *in vitro* has been established.
- Investigate cellular cytokine secretion and gene expression in those cells.
- Investigate cytoprotection of endotoxic and hypoxic intestinal cells using IL-6.

Fan

- Continue the investigation on gene regulation of inducible nitric oxide synthase (iNOS) and protein kinase c (PKC) isoforms in rat cardiac myocytes and vascular smooth muscle cells during LPs-induced sepsis using RT-PCR technique.
- Optimize the "mimic" competitive PCR technique in order to quantitatively analyze changes of iNOS and PKC mRNA levels during LPs-induced sepsis.

Chavez

- Completion of heme affinity experiments using rapid scanning spectroscopy has been completed.
- Heme exchange under anaerobic conditions has been initiated.
- Completion of rough draft of the pilot plant paper.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Li

- Using neutral red assay, a variety of cellular deteriorations have been found in these intestinal cell lines induced by LPS 0.5-1.0 mg/ml and 1%O₂ hypoxia incubation at different time points.
- Mice fed with IL-6, had their spleen cell proliferation evaluated by labeling *in vivo* with bromodeoxyuridine (BrdU).



- IL-6 restores intestinal blood flow in mice which were bled using the HS model. Doppler flow measurement were made at the intestinal mucosa surface. After resuscitation the intestine remains ischemic until exposed IL-6.
- Publication: F.M. Rollwagen, Y-Y Li, N.D. Pacheco, T.B. Nielsen. Systemic bacteraemia following haemorrhagic shock in mice: alleviation with oral interleukin 6. *Cytokine*, 8(2):121-129, 1996.

Fan

- Generated satisfactory results of gene regulations of iNOS and PKC isotypes in LPS-treated rat cardiac myocytes and aortic smooth muscle cells.
- Li, S., S.X. Fan, and T.M. McKenna. Role of nitric oxide in sepsis-induced hyporeactivity in isolated rat lungs. *Shock*. 1996; 5(1):1-8.
- Li, S., S.X. Fan, and T.M. McKenna. In Vitro lipopolysacchride increases PKC mRNA and PKC isotype synthesis in rat aortic tissue. Nineteenth annual conference on shock. 1996. Submitted.

Chavez

- The following abstract was presented: "Heme Stability of Partially Oxidized Hemoglobins," M.D. Chavez, B.E. Shrader, H.S. Zahwa, and V.W. Macdonald, 1996 Biophysical Meeting, February 17-21, Baltimore, MD. The most enlightening result of the work was that an increased population of liganded ferrous (reduced) heme within a partially oxidized alpha-alpha cross linked hemoglobin tetramer increases the rate of loss of oxidized heme. This is further evidence that the liganded ferrous alpha-alpha cross linked homoglobin exists in an intermediate destabilized configuration.
- Heme affinity experiments have been completed. In performing the heme extraction procedure, it was observed that heat treated, flash photolyzed hemoglobin A0 was less stable than hemoglobin A0 not subjected to this treatment. Experiments to verify this observation are listed in next quarters objective.
- To our surprise, anaerobic conditions increased rate of heme exchange in partially oxidized hemoglobin. Oxidation of the ferrous deoxy-heme is accelerated under experimental conditions, leading to the increased heme exchange. Completion of this set of experiments is expected in April.
- The draft of the pilot plant paper is complete. The format is being modified for the publication "Biologicals". Presentation of the results will be published here and also in the 1996 Current Issues in Blood Substitute Research and Development.



GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Li

- Repeat the neutral red assay using 1%O₂, 5%CO₂, 94%N₂ long term incubated intestinal cells.
- To investigate the mechanisms that oral IL-6 improves gut barrier function.

Fan

- Continue the ongoing investigation of gene regulation of the cellular mediators of cardiovascular hypocontractility under various experimental conditions and treatments.

Chavez

- Experiments to test the effect of heat treatment and flash photolysis on hemoglobin A0 will be done. Circular dichroism can be done to check both the overall protein folding and the heme pocket environment. NMR and Raman differential spectroscopy will be performed if more detailed structural analysis is required.
- Development of a large scale preparation of hemoglobin A0 will be started. A two stage ion exchange column chromatographic procedure will be utilized to save time and material cost.
- Invitation to participate in the 1996 Current Issues in Blood Substitute Research and Development in San Diego, CA, March 17-20. The following abstract has been submitted:

“An Improved Process for the Production of Sterile Modified Hemoglobin Solutions”

M.D. Chavez(1), F.A. Highsmith(2), C.M Driscoll(2), B.C. Chung(2), V.W.

Macdonald(1), J.M. Manning(3), L.E. Lippert(2), R.L. Berger(2), and J.R. Hess(2).

(1) Blood Research Detachment, Walter Reed Army Institute of Research;

(2) Bionetics Corporation, Rockville, MD;

(3) The Rockefeller University, New York, NY.



II. NMRI, Bethesda, MD

C. BIOMEDICAL DIVING RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Cortes

- To conduct surgeries for the Alzheimer's project.
- To perform other surgeries such as cannulations and probe implants.
- To collect and analyze data from animal models.
- To assist in experiments using a hyperbaric oxygen chamber.
- To care and maintain laboratory animals in excellent condition for experiments.

Shea

Alzheimer Project:

- To perform microdialysis experiments in the CNS of rats which have previously been lesioned at the nucleus basalis Mynert (NBM) via the drug NMDA.
- Analyze the neurotransmitters acetylcholine (Ach), norepinephrine (NE), and serotonin (5-HT), in microdialysis perfusate obtained from the above experiments.

Oxygen Toxicity Project:

- Run trial microdialysis experiments in the newly designed hyperbaric chambers prepared for 100% oxygen environment under deep dive conditions.

Genetic Seizure Project:

- A collaborative project within Diving Medicine where a genetic strain of mice prone to spontaneous seizures will be analyzed for baseline neurotransmitter amounts in various brain regions and compared to normal litter mates.

Free Radical Project:

- A HPLC method will be set up in order to measure free radical formation in various brain regions of awake animals. Microdialysis samples will contain a free radical trapping agent which will then be assayed via HPLC electrochemical detection.



Dityrosine Project:

- This is a new and collaborative project with the Department of Neurology at the Uniform Services University of Health Sciences (USUHS).

Obowa

- Provide technical assistance in the Diving Medicine research laboratory investigating exposure to hyperbaric oxygen (HBO) and its effects on the CNS. Prepare brain tissues for staining, section tissues using the cryostat, perform immunohistochemical staining methods on tissue sections, care for animals, perform surgical procedures on rats, and order laboratory equipment and supplies.

Porter

- To support in the selection of a hyperbaric CO₂ analyzer for fleet submarine dry deck shelter use.
- To support analysis of fleet soda lime for possible contamination, and to analyze the samples for specific dye concentrations.
- To assist with other laboratory duties as needed.

Ruby

- Providing gas analysis support for Navy diving studies at NMRI.
- Development of new gas analysis methods in support of Navy Fleet requirements.
- Specification, procurement and installation of laboratory chemical analytical instrumentation to support NMRI/DBTFA gas analysis capabilities.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Cortes

- To continue and finish the Alzheimer's project by including more data points to the curve.
- To continue experiments involving the oxygen chamber.
- To start a microdialysis study on the effect of NMDA in the formation of free radicals in the brain.
- To train military staff in surgical procedures and sterile techniques.



Shea

Alzheimer Project:

- To continue the experiments in the NMDA lesioned rats by increasing the number of observations at various time points post lesion.

Oxygen Toxicity Project:

- A number of rats implanted with microdialysis probes will be run in the new hyperbaric chamber under various depths in order to test the microdialysis equipment under 100% oxygen levels.

Genetic Seizure Project:

- Eight mice with specific genetic alterations will be sacrificed and their brains dissected into various regions. After extraction a number of neurotransmitters will be measured and comparisons made between seizure and non-seizure prone animals.

Free Radical Project:

- A HPLC proceed will be set up to measure and separate the free radical formation of 2,3 dihydroxybenzoic acid and 3,5 dihydroxybenzoic acid, products of free radicals and salicylic acid. The salicylic acid is the trapping agent used in the microdialysis perfusion medium.

Dityrosine Project:

- The compound dityrosine, presumed to be formed from free radicals is not commercially available. Therefore we are attempting to purify this compound. When this is accomplished we will then set up a method for quantitative analysis of dityrosine in microdialysis, CSF, and samples of brain tissue.

Obowa

- Perform immunohistochemical staining of tissue sections for detection of c-fos and heat shock proteins (HSP).
- Refine technique and isolate brain region involved in performing olfactory bulbectomies in rats.



Porter

- To continue with fleet soda lime analysis as samples come in from the manufacture.
- To continue the testing program for the candidate CO₂ analyzers.

Ruby

- Purchase aerosol particle size analyzers for evaluating their potential for monitoring airborne particles on submarines.
- Provide input to Naval Sea Systems Command (NAVSEA), PMS-395, in formulating a test plan for analyzing the divers air banks on 688 class submarines.
- Assist NAVSEA in the development of a portable carbon dioxide analyzer capable of operation in hyperbaric atmospheres for use in Dry Deck Shelters (DDS) and host ships.
- Provide technical support to the cleanva, the MRCC and the gas farm operations.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Cortes

- Coordinated and supervise the use of the stereotaxic apparatus as well as surgical procedures and protocols.
- Performed cannulations, probe implants as well as other surgical procedures.
- Conducted microdialysis trials on implanted animals to create a base line.
- Collected and processed cerebro-spinal fluid and brains from animals after experiments.
- Train military staff in surgical procedures such as probe implants, cannulations and cerebro-spinal fluid collection in animals as well as techniques in anesthetics and sterile procedures.

Shea

Alzheimer Project:

- The number of animals at various time points after NMDA lesioning has been increased.
- The analysis of neurotransmitters is on schedule and has been included in the results.



Oxygen Toxicity Project:

- Equipment for the diving chamber microdialysis experiments has been delayed due to technical problems with the diving chamber. It is hope that these problems will be remedied this quarter and experiments will commence.

Genetic Seizure Project:

- HPLC procedures for various neurotransmitters have been established. Normal mice brains have been dissected and tested in these chromatography methods.

Free Radical Project:

- HPLC conditions have been worked on for the separation of salicylic acid-free radicals products. A post-doctoral fellow has taken over these experiments with my consultation.

Dityrosine Project:

- We are receiving a crude preparation of synthesized dityrosine from Dr. A.J. Verma, Department of Neurology at USUHS and it is being purified by various HPLC methods.

Obowa

- Performed animal surgeries, including olfactory bulbectomies on rats to refine technique and determine optimal procedure. Cut brain sections for HSP and c-fos studies using cryostat. Stained tissue sections for c-fos and HSP detection using immunocytochemistry. Continued studies implanting EEG electrodes in rats for c-fos studies. Performed Western blot gel electrophoresis on brain tissue samples for determination of heat shock protein. Assisted investigators with dive chamber operation while diving rats for different projects. Ordered laboratory equipment and supplies.

Porter

- A chamber gas delivery system was designed and constructed to meet testing requirements for the hyperbaric CO₂ analyzers.
- Hyperbaric testing of four production CO₂ analyzers for dry deck shelter use is underway. Modifications are being made by the manufacture as problems are identified.



- A test program is currently being formulated for the commercial units prior to final recommendation for fleet hyperbaric use.
- 16 buckets of military grade soda lime were tested and approved for fleet use.
- Performed other laboratory tests as requested.
- Co-author on MNRI Technical Report #95-76 Evaluation Of Portable Carbon Dioxide Analyzers For Use AT 1-ATA On U.S. Navy Submarines.

Ruby

- Several candidate portable particle size analyzers were purchased. The MIE Co. MINIRAM unit was selected as the best candidate for field use and the GRIMM Model 1.105 Portable Dust Monitor was selected as a primary standard for calibrating the field units. Both units have been received and initial tests are promising.
- A preliminary test plan has been submitted to PMS-395 related to air bank sampling on 688 class submarines.
- The prototype units for the NAVSEA portable carbon dioxide analyzers have completed all of the initial screening tests. The two best units are being subjected to an extensive test program designed to measure their performance under conditions that mimic actual field conditions.
- Support has been provided, as required, to other functional areas of the diving medicine department.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Cortes

- To continue the microdialysis trials with perfusion of NMDA to assess the effect of a lesion on the production of free radicals in the brain.
- To gather enough data to write an abstract to be presented at the 1996 Society for Neuroscience convention in Washington D.C.
- To assist in the experimental procedures using the implanted animals.
- To continue in the training of military staff of more advanced surgical procedures.



Shea

Alzheimer Project:

- Finish the time course study for post-lesioning NMDA animal studies.
- Set up halthane anesthesia apparatus for rats in a stereotaxic frame, whereby they can be lesioned and dialyzed immediately after injection of drug.

Oxygen Toxicity Project:

- Set up microdialysis procedures in a hyperbaric chambers, especially under high oxygen conditions.

Genetic Seizure Project:

- Determine the neurotransmitter contents of various brain parts in genetically altered mice and compare these with control litter mates.

Free Radical Project:

- This project will now be turned over to a new post-doc Dr. Ekram Elayan.

Dityrosine Project:

- To purify dityrosine and confirm the purity by GCMS. Also to set up an HPLC method to be able to measure this compound in biological fluids.

Obowa

- Perform olfactory bulbectomies in rats to observe any changes in HBO seizure activity and c-fos distribution in the brain following bulbectomies. Perform surgeries implanting EEG electrodes after bulbectomies. Continue immunohistochemical staining procedures for both c-fos and HSP identification.

Porter

- To continue analysis of fleet soda lime for contaminants and dye concentration as needed.
- To continue testing program for dry deck shelter hyperbaric CO₂ analyzers.



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Ruby

- Assist the Naval Sea Systems Command (NAVSEA) in a study to characterize the volatile organic compounds that may be present in the divers air banks of 688 class submarines when the banks are charged from the sub air after prolonged time at depth without ventilation.
- Continue the development of the NAVSEA candidate carbon dioxide analyzers and the divers air bank sampling procedures.
- Continue the screening and selection process to obtain portable analyzers for field testing of compressors used to produce divers breathing air. Parameters will be oil mist/particulate, oxygen and carbon monoxide.
- Support the needs of the diving medicine functional areas as required.



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II. NMRI, BETHESDA, MD

D. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Wolf

- Provide management support to the Combat Casualty Care Program at Naval Medical Research and Development Command. Duties include reviewing medical research plans and progress reports, recommending laboratory guidance, evaluating research proposals, drafting periodic and ad hoc management reports and developing presentation materials.

McCowin

- Provide management support to the Special Operations Forces Medical Technology Development Program at the Naval Medical Research and Development command. Duties include reviewing and evaluating medical research proposals, reviewing progress reports and comparing them with the approved research plans, recommending guidance, and drafting periodic and ad hoc management reports and developing draft presentation materials. The scope of research includes all topics within the Special Operations Forces Medical Technology Development Project. This includes investigations relevant to the treatment of disease, trauma, effects of environmental extremes and treatment for medical support of Special Operations Forces. In addition, from time to time, collect, process and report findings on critical issues which are directly related to other urgent military medical research issues within the purview of the Special Operations Forces Medical Technology Development Program.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Wolf

- Become versatile in the various data management programs provided by the Naval Medical Research & Development Command as well as those provided by the Office of Naval Research.



- Allow my colleagues to be more comfortable in the use of those same data management programs. Facilitate the production of reports for the Director of Research and Development.

McCowin

- Collect monthly obligation and expenditure reports from principal investigators.
- Collect 1st incremental progress reports.
- Review 1st incremental progress reports for proposal relevance.
- Literature search to locate expertise in the area of SOF research. Send request for pre-proposal letters to military commands and universities to address the FY97 Tasking Statements.
- Collect and review pre-proposals to ensure the tasking was met.
- submit FY96 obligations and expenditure report to special Operation Defense Acquisitions Center (SORDAC) for Execution Review Conference.
- Submit FY95 unobligated funds report to SORDAC
- Prepare project reference book of SOF Medical Technology R&D
- Attend Biomedical Initiative Steering Committee (BISC) meeting.
- Prepare 1st incremental and pre-proposal package and submit to BISC.
- Evaluate work unit file of principal investigators for funding and deliverable status.
- Prepare draft baseline agreements for all SOCOM funded projects.
- Prepare FY96 funds for distribution in conjunction with BISC chairman.
- Prepare and submit FY97 funding requirements for Med-Tech program to the President's budget committee to OSD/OMB.

Support Species Differences in Skin Penetration Project:

- Dermal image analysis provided for research animal specimens to quantitate epidermal and dermal endpoints following four in-vitro exposures; methods to quantitate human cutaneous endpoints in a manner similar to previous animal studies is under development.
- Continued communication with the Center for In-Vivo Microscopy regarding collaborative efforts to develop Magnetic Resonance Microscopy as a research tool to study the cutaneous penetration of model chemicals.



Support Combustion Toxicology of Advanced Materials (ACM) Project:

- Support efforts to complete final reports for phase I: due to the loss of staff involved with key portions of the draft technical report, portions of previous research are being recompiled.
- Support efforts to plan and implement phase II: Meeting and planning sessions are ongoing to support in-life exposure, the role of image analysis is being evaluated and determined.

Support Ammonium Perchlorate Study (A10):

- Thyroid morphometric analysis was completed and involved 120 images with over 8,000 follicles from 24 animals at 5 dosage groups.

Assist Tri-Service Marketing/Program Development Team:

- Co-authored a report detailing past, present and future directions of the Program Development Team titled "Program Development Strategic Plan".
- Co-authored a poster presentation titled "Risk Assessment Of Hazardous Materials In The Department of Defense" for the Navy Occupational Health and Preventative Medicine Workshop (Virginia Beach, VA, March 1996).
- Attended a symposium on an advanced INTERNET programming language (Java) to determine its potential role in the Tri-Service Toxicology INTERNET site.
- Initiated joint effort to redesign and reorganize the Tri-Service Toxicology Web site.

Other duties as requested or required:

- A research proposal titled "Research Proposal: Developmental Toxicology Methods Development" was submitted on 22 February in response to a verbal request from the Chair, Research Management Council.
- A DOS-based image analysis system was successfully ported to the Windows 95 Operating System (OS) completing a 6 month effort to correct technical issues.
- Installation of a Unix-based image analysis system was completed.

PERIOD SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING

Wolf

- Participate, with my counterparts in the Army Medical Research and Materiel Command, in the creation of the Defense Technical Area Plan. This plan is to be



used by Director, Defense Research & Engineering, as the overall goal and objective for all defense funded biomedical research and development. In addition to the creation of this document, I was tasked with checking content for accuracy and completeness after the contract organization completed its work.

- Completed the NMRDC spread sheets for use by the Director of Research and Development. It was necessary to confirm and cross check each figure against three different sources.
- Initiated a data base for investigator accomplishments. It is the goal of this data base to allow the Director of Research and Development to readily pull together all the significant actions which have been accomplished by our funded investigators. It is intended, also, that this data base will feed into the major work-unit-tracking data base the Command has commissioned from a separate contract.
- Participated with my local colleagues in the creation of the command brief, a new presentation book for the Commanding Officer of NMRDC to use in his promotional efforts. This entailed the collection and redisplay of a significant amount of information.
- Using the Office of Naval Research newly created information management system, INRIS, eight Procurement Requests were initiated. It is necessary to periodically track the status of those requests to see that our contract funded investigators will be paid in a timely fashion. The conversion from the older information management system was difficult for all users, but more so for NMRDC as we have to travel to Ballston to use the new secure system.
- Developed a new Memorandum of Understanding for the Hemoglobin Production Facility. This memorandum will be signed by Flag Officers from the Army and Navy and coordinates the responsibilities and activities of both headquarters commands as well as two field laboratories.

McCowin

- Work from the Technical Objectives for this Quarter section was performed during this reporting period.



GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Wolf

- Continue to identify those areas where GEO-CENTERS, INC. May assist NMRDC in the transition to whatever its future will be. Develop capabilities with the new Local Area Network and Windows Programs at NMRDC.

McCowin

- Attend BISC in March 1996.
- Collect and evaluate 2nd incremental progress reports.
- Collect monthly obligation and expenditure reports from principal investigators.
- Submit monthly obligation and expenditure reports to SORDAC.
- Solicit and evaluate proposals for FY97.
- Plan to attend June 96 BISC meeting.
- Prepare final baseline agreements for SORDAC, BISC Chairman and Program Manager to sign.
- Submit baseline agreements to Mr. Smith Director of SORDAC.



E. BREAST CARE CENTER, Bethesda, MD

DESCRIPTION OF WORK TO BE PERFORMED:

Patient Service Representatives

Grimes, Jenkins, Lozoya, Williams

- Develop a system for processing and interviewing patients. Establish uniform policy for check-in/check-out procedures.
- Devise a system for completion and collection of third party insurance forms on each patient.
- Use standard patient registration procedures.
- Perfect receiving patients and incoming telephone calls/inquiries, determine priorities and refer to proper person/department.
- Develop a schedule that would ensure all mammogram films would be pulled 1-2 days ahead of current clinic schedule.
- Ensure that all incomplete patient records and third party forms are corrected or returned to proper staff for completion/correction.
- Set up records and filing system for paperwork associated with each patient record. Ensure that all documents processed are in accordance with department standards and that all forms are in designated order in the patient records. Label files for permanent shadow files.
- Assist with establishment of standard operating procedures.
- Orient new support team members and clinical team staff to office routine.
- Devise a system of notifying all no-shows, record information in shadow file and then initial.
- Call/notify all physicians the day before they are scheduled for clinic; let them know approximately how many patients they will see.
- Ensure that all mail is picked up/delivered daily.
- Print Composite Health Care System (CHCS) daily schedule and end of day reports.

Balintona

- Responsibilities include addressing the psychosocial status, mental status, patient concerns, and the impact of diagnosis on family relationships of breast cancer patients.
- Facilitation of the Stage I & Stage II Breast Cancer Survivors' Group
- Co-Facilitate the Advanced Breast Cancer Support Group



- Facilitate the Spouse's of Breast Cancer Patient's Support Group
- Collect and analyze research data on the adjustment and social support in male spouses based on the support group.
- Liaison with the National Naval Medical Center Social Work Staff.
- Coordinate individual, family, group and marital psychotherapy based on Social Work assessment and clinical intervention needed.

Durand

- Liaison between the patient and the family and all other health care providers, intervening at key points (and or when significant problems occur) for individual patient. Addresses and resolves issues that have a negative impact, creating opportunities and systems to enhance positive outcomes.
- Performs on site visits with patients in various clinical areas. (i.e. Radiology Oncology (Rad. Onc.) Medical Oncology (Med. Onc.)
- Initiates and contributes via multidisciplinary team approach modifications or changes in caregiver practice patterns to maximize quality patient care and resource utilization.
- Assists in the development and implementation of the Task Manager program with Digital Equipment Corporation.
- Checks daily for outstanding biopsy results.
- Page and inform physician of biopsy results if positive.
- Make follow-up phone calls to postoperative patients to check on their well-being.
- Schedule follow-up appointments to Rad. Onc., Med. Onc., Physical Therapy, Nuclear Medicine, and C.T. Scan.
- Verify consults to clinical areas for breast cancer patients with follow-up phone call.
- Prepare information data form for Tumor Board presentation of positive cancer patients.
- Follow-up visit to clinical areas with patients after surgery.
- Verify surgical dates via surgical clinic and OR schedules.
- Perform pre-op teaching specific for nurse case manager with patient and family members.
- Acts as support system for patient and family, in conjunction with the Social Worker and Nurse Educator for newly diagnosed cancer patients.
- Acts as supervisor for 4 Registered Nurses solely to prepare evaluations and prepare time sheets.
- Instructs post-op mastectomy patients on breast prosthesis, and issues dealing with the appointments for fitting.



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- Initiate order forms with prescription for the patient's acquisition of breast prosthesis.
- Acts as liaison between prosthetic company and patient/clinic.
- Deliver consults to various clinical areas.

Fields

- Perform technical services including mammograms.
- Assisting in biopsies and ultrasounds.
- Perform quality control.
- Record keeping.

Higgins

- Responsible for all patient flow activities.
- Acts as Relief Clinical Nurse Manager, in the absence of the Nurse Manager.
- Responsible for opening and closing all clinical areas and preparing exam rooms for patient use.
- Check crash cart in APU on designated day.
- Triage telephone calls and walk-ins.
- Reviewing and sorting mammogram reports, ordering new labs/radiographs in CHCS as per doctor's orders.
- Gather all results for patient visits.
- Processing linen and hazardous material within the Center
- Check and order supplies for clinical exam rooms and needle/syringe cart.
- Coordinate all FNAs and procedures and notify Nurse Case Manager of all such cases.
- Attending seminars/conferences for staff and professional development.

Lopez

- Develop and integrate a breast care educational program for female Department of Defense beneficiaries and their support persons.
- Educational program to include all breast care issues with an emphasis on early detection of breast cancer.
- Provide pre-operative teaching and educate patients regarding breast cancer and treatment options.



GEO-CENTERS, INC.

- Be available as an information resource person for the patient and their support person.
- Plan staff development programs and maintain BCC staff development records.
- Act as relief Ambulatory Care Nurse under the direction of the nurse manager.
- BCC designated safety representative, responsible for safety manuals, monthly safety meetings and BCC safety issues.

McIntyre

- Support a research program which focuses on breast cancer.
- Liaison between the Radiology Department-Mammography Section, the Breast Care Center, (BCC), and other hospital departments.
- Performs nursing duties.
- Performs managerial duties.

Mitchell

- Provide care for patients with both malignant and benign diseases of the breast, including: initial evaluation, definitive medical or surgical therapy, and long term follow-up.
- Develop a data collection system which will allow patient data to be collected in a manner which will permit clinical research to be performed at a later date.
- Educate medical students, residents, nurses, and other physicians in the diagnosis and treatment of malignant and benign diseases of the breast.
- Develop research protocols for the Breast Care Center.

Prindle, Snee

- Coordinates patient flow activities.
- Acts as relief clinical nurse manager in the absence of nurse manager.
- Collaborates with physicians concerning unscheduled patient appointments.
- Performs professional nursing assessments.
- Teaches breast self examination and pre and post-biopsy education.
- Triage patient phone calls and consults with physicians as needed.
- Prepares patient charts with appropriate medical, lab, and x-ray reports.
- Responsible for entering physician orders into computer.
- Assists physicians with all procedures such as Fine Needle Aspirations or cyst aspirations.



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- Provides physical and emotional support to patients during their appointment.
- Collaborates with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management.
- Responsible for preparing all clinical areas for patients and securing clinical areas at the end of the day.
- Processes linens and hazardous wastes within the BCC.
- Maintains supplies at par level and reorders supplies as needed.

Vaughn

- Medical filing for the Radiology Department and The Breast Care Center.
- Tracking films.
- Handle mail and telephone correspondence regarding radiology films.
- Pull and file mammograms.
- Make copies of mammograms..

Wallace

- Coordinate administrative activities of the Breast Care Center (BCC).
- Manage patient/physician schedule templates in the Composite Health Care System (CHCS).
- Collect and report monthly workload statistics.
- Collect and report monthly manhours reports.
- Become proficient in use/manipulation of CHCS.
- Financial analysis of past expenditures for creation of budget, including Center Operations (Facilities, Outfitting, Travel, Research, Education) and Information systems.
- Train Technical Assistant.
- Research patient level accounting system.
- Research alternative method for patient charting to reduce amount of paper files.



GEO-CENTERS, INC.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Grimes, Jenkins, Lozoya, Williams

- Streamline and organize front desk procedures.
- Retrieve and ensure completion of third party insurance forms.
- Improve routing and response to incoming telephone calls/inquires.
- Use standard registration procedures requiring plastic green card for imprinting all forms pertinent to each patient.
- Provide roster for pulling mammogram films 1-2 days prior to the scheduled visit.
- Coordinate policies for scheduling appointment/procedures for patients calling/walk-ins/consults/cards.
- Streamline physician schedule notification process.
- Refine CHCS daily schedule and end of day reporting.
- Ensure pick-up delivery of mail in a timely manner.

Balintóna

- Liaison with Dr. Miller, Nursing Researcher at USUHS, for feedback on the research proposal.
- Addressed the psychosocial status and individual concerns of patients in the Breast Care Center.
- Developed information packets to be given to newly diagnosed patients. The packet address the psychosocial aspects of treatment.

Durand

- Continue to improve the Task Manager through ongoing collaboration with Digital Equipment Corporation.
- To continue to develop through multidisciplinary collaboration, Standard Operating Procedures (SOP) for the nurse Case Manager's position.
- Continue to update "Care Manager" data base with retrospective cases of breast cancer patients originally seen in the surgical clinic 1 year ago.
- Continue to enter in "Care Manager" program all newly diagnosed Breast Care Patients.
- Refine techniques for capturing statistical data that would impact future studies of the Breast Care Center.



Fields

- Continues efforts to fine tune skills using various procedures and modalities within the department.
- Anticipates interaction with the Breast Cancer Center (BCC) to expand and strengthen.
- Continue to broaden patient understanding as well as influence a team approach with co-workers to establish a happier healthier work environment.
- Continue to broaden knowledge of mammography and breast diseases.

Higgins

- Preparing clinical areas for patient care.
- Investigative tool to assess variables for nursing research.
- Developing protocols and procedures for telephone triage.
- Stock all clinical areas with par level supplies.
- Identify nursing roles for the Ambulatory Care Nurse.
- Further develop computer skills utilizing Windows '95 software, especially CHCS.
- Nursing documentation and care plans tool development.
- Organization of triage area and triage files.
- Continue to further develop breast self exam teaching in a timely and effective manner.
- Utilizing past radiology reports to improve clinical impression reporting for mammograms/cxrs/ultrasounds.
- Creating a patient package for cancer follow-up patients, to include cxr, mammogram and blood work prior to visit.

Lopez

- Continue to setup educational resource room.
- Continue to identify educational materials needed and obtain approved materials.
- Participate in round table discussions to streamline patient flow while meeting patient needs in a timely and effective manner.
- Participate in NNMC health fair providing information on Breast Care Center, breast cancer prevention and early detection.
- Screen video tapes and begin setting up resource library for patient use.
- Provide pre-operative, breast cancer, treatment options, and breast self exam teaching to patients and their support persons.
- Initiate teaching documentation forms when approved.



McIntyre

- Assist the Radiologists/staff with stereotactic and ultrasound guided breast biopsy procedures.
- Perform assessments on all stereotactic/ultrasound biopsy patients and provide these patients with post breast biopsy teaching instructions.
- Assist in developing the critical pathways in nursing.

Mitchell

- Maintain the high standard of care that has been established at the Breast Care Center and provide comprehensive care to patients with diseases of the breast.

Prindle, Snee

- Continues development in the role of the ambulatory care nurse.
- Continues development of computer skills, especially the use of the hospital systed call CHCS.
- Ongoing evaluation and revision of nursing assessment tool.
- Prepares presentation for BCC conference; role of ambulatory care nurse to be presented to an audience of approximately 40.
- Continues to gain further knowledge and education in breast cancer and it's treatment.
- Ongoing development of nursing protocols for telephone triage.
- Continue working with Patients Service Representatives to achieve a fluid transition between PSR/Patient/Nurse (Developing Algorithm format)

Vaughn

- Alphabetize the main file system.
- Systematic checking for quality improvment.
- Improve report filing to allow a more efficient operation.
- Readily available for assistance to co-workers, The Breast Care Clinic staff, physicians, patients, and staff requiring help with radiology films.



Wallace

- Maintain schedule templates for BCC attending physicians in five subclinics.
- Coordinate with Radiation Oncology and Medical Oncology Clinics to aid in the establishment of working relationship with the BCC.
- Collect and Report monthly Manhours and workload.
- Coordinate administrative activities of the BCC.
- Further proficiency in use/manipulation of CHCS.
- Create budget.
- Train Technical Assistant.
- Research patient level accounting system.
- Research alternative method for patient charting to reduce amount of paper files.
- Develop Strategic Plan and Marketing Plan.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Grimes, Jenkins, Lozoya, Williams

- Began organization of front-desk procedures.
- Processed and interviewed patients through CHCS and designated forms, obtained and updated all patient demographic information and ensured completion of forms.
- Obtained and verified pertinent insurance information utilizing available forms. Obtained third party insurance forms from physicians at end of each visit.
- Required identification card from each patient and imprinted all clinic forms pertinent to that patient.
- Received patients and incoming telephone calls/inquiries, determined priorities and referred to the proper source.
- Explained clinic procedures to patients.
- Retrieved/returned Mammogram films daily.
- Ensured completion of incomplete patient records and third party insurance forms.
- Set up records and maintain filing system for paperwork associated with each patient record. Ensured that all documents processed are in accordance with department standards. Filed all forms in designated order in patient record. Labeled files for permanent shadow files.
- Scheduled and coordinated front desk procedures in accordance with department policy.



- Oriented new support team members and clinical team staff to office routine.
- Participated in team planning to assure team members meet team quality standards. Maintain department standards of productivity.
- Notified physicians the day before they are scheduled for clinic; let them know approximately how many patients they will have.
- Picked-up and delivered mail daily.

Balintona

- Completed the Partner of the Breast Cancer Support Group research proposal.
- Obtained approval from the Clinical Investigation Department at the National Naval Medical Center to run the research.
- Completed a pilot study composed of spouses of breast cancer.
- Started two new support groups in the Breast Care Center which included a Spouse's Support Group and a Advanced Breast Cancer Support Group.
- Addressed the psychosocial status, mental status and patient/family concerns in the Breast Care Center.
- Co-facilitation of the Stage I & Stage II Breast Cancer Survivors' Group.
- Development of the Social Work Assessment tool and critical pathways.
- Worked closely with the BCC Nurse Case Manager to provide seamless care to patients.
- Liaison with the National Naval Medical Center Social Work Department.

Durand

- Assisted Digital Equipment Corporation in the development of the Task Manager.
- Worked collaboratively with staff members on other units, and have successfully established a system of making appointments in advance of receipt of consults.
- Successfully entered approximately 30 new patients in the Task Manager system.
- Resolved critical issues between patient and providers concerning their methods of treatment.
- Been influential in the tracking of patients with positive mammography's that would be otherwise lost within the system.
- Initiated the documentation of specific information (i.e. Breast prosthesis dispensation, monthly positive patients) that can be looked at retrospectively for study/work load.
- Initiated 4 SOP's for the referrals of patients to: Physical Therapy, Radiation Oncology, Hematology Oncology and Plastic Surgery.



- Attended 3 educational seminars to enhance the Nurse Case manager's position.
- Prepared and presented the role of the Nurse Case Manager at the Opening of Breast Care Center.
- Demonstrated on an ongoing basis the functions of the "Care Manager" to various visitors and dignitaries.

Fields

- Performed a variety of mammograms, stereostatic biopsies, needle localizations and ultrasound procedures.
- Patient load increased due to the BCC becoming fully operational.
- Developed a stronger working relationship with co-workers.
- Interfaced with mammography doctors to increase knowledge in the areas of mammography and breast disease.

Higgins

- Coordinated patient flow activities in the clinical areas with patients, nurses and doctors.
- Stocked all exam rooms and clinical areas, appropriately.
- Further identified the nursing assignments as an Ambulatory Care Nurse.
- Coordinated all FNAs and procedures and notified the Nurse Case Manager of such procedures.
- Stayed later than normal business hours due to patient care requirements.
- Triageed telephone calls and walk-ins. Also organized triage area and files.
- Decreased time of breast exam teaching, while remaining effective.
- Improved written clinical impression on radiology order request.
- Processed linen and hazardous material.
- Developed results folder and cancer follow-up patient package.
- Participated in multidisciplinary meetings, to further enhance the relationship between BCC and Radiology.
- Attendance at various seminars/conferences within and outside of facility.
- Participation and case study presentation at BCC conference.



Lopez

- Assumed responsibility of the designated safety representative of the BCC.
- Instituted safety, disaster, and fire manuals for the BCC.
- Leads monthly safety meetings.
- Started staff development files on BCC employees.
- Plans and institutes staff education calendar and events.
- Functions as ambulatory care nurse providing breast self exam teaching, assisting the physicians with physical exams, procedures, and scheduling of diagnostic test when needed.
- Developed BSE brochure for BCC and initiated use.
- Continues to provide patient education.
- Developed multidisciplinary teaching documentation form for the APU patient.

McIntyre

- The above technical objectives were met during the current reporting period.
- Coordinated mammography scheduling.
- Supervised other mammography personnel.
- Obtained mammography statistical data for FDA purposes.
- Obtained credentialing information on radiologists.
- Tracked 6 month follow-up patients via BCC Task management tool and outcome analysis.
- Correlated mammography and pathology findings via Composite Health Care System (CHCS).

Mitchell

- The Breast Care Center averaged approximately 100 patient visits per week during the last quarter. The BCC surgeon saw approximately 35% of the total patients, and performed 31 operative procedures during the last quarter.
- Medical students and residents have been integrated into the Breast Care Clinic and are actively involved in the care of patients with diseases of the breast.
- A system to collect patient data has been developed, and data is being recorded in a manner which will allow for retrospective clinical research to be conducted at a later date.
- Several retrospective clinical studies have been initiated, including: an evaluation of the occurrence of genetic markers for breast cancer in patients with bilateral breast



cancer, the evaluation of estrogen receptor status using new immunohistochemical techniques in patients with breast cancer, and a retrospective review of the treatment of breast cancer at NNMC.

Prindle, Snee

- Coordinated patient flow activities.
- Acted as relief nurse manager for the BCC on several occasions.
- Collaborated with many physicians concerning unscheduled patient visits.
- Performed professional nursing assessments.
- Provided BSE and biopsy teaching.
- Triageed patient phone calls and made telephone consults to physicians.
- Prepared patient charts appropriately with medical, lab, and x-ray reports.
- Entered physician orders into the computer.
- Assisted physicians with many procedures done in the BCC.
- Provided physical and emotional support to patients.
- Collaborated with social service, nurse case manager, clinical nurse educator, physical therapist and many physicians to ensure exceptional patient care.
- Prepared patient areas and secured them at the end of the day.
- Disposed of linens and hazardous wastes appropriately.
- Presented the role of the ambulatory care nurse at the BCC conference.
- Wrote Algorithm for Patient Flow for visiting physicians.
- Attended telephone triage conference.
- Read many journal articles about breast cancer and it's treatment.

Vaughn

- Provided assistance to staff requesting help with radiology films.
- Organized a log book to improve film tracking as well as devised a new way to disseminate films to patients by using the CHCS computer system.

Wallace

- Developed a successful working relationship with the Medical and Radiation Oncology Clinics.
- Managed schedule templates for all subclinics of the BCC.
- Coordinated administrative activities of the BCC.
- Became proficient in use/manipulation of CHCS



- Began financial analysis of past expenditures for creation of budget, including Center Operations (Facilities, Outfitting, Travel, Research, Education) and Information Systems.
- Developed training schedule for Technical Assistant.
- Began research on patient level accounting system.
- Researched alternative methods for patient charting to reduce amount of paper.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Grimes, Jenkins, Lozoya, Williams

- Streamline and organize front-desk procedures.
- Coordinate scheduling with other clinics for smoother follow-up visit for the patient.
- Maintain department standards.
- Attend classes involving CHCS training as well as computer training.
- Develop User Defined Keys for greater efficiency in completing tasks which require routine keystrokes.
- Assist in development of a personal development program to assure continuing professional growth.
- Assist in development of SOP's.

Balintona

- Continue with the development of the STRETCH program in conjunction with the Breast Care Center physical therapist.
- Liaison with Dr. Ken Miller, Nursing Researcher, for further feedback and input on the research proposal.
- Provide social work services to patients by addressing psychosocial status, mental status, patient and family concerns.
- Coordinate individual, family and group psychotherapy for patients.



Durand

- Continue to work in collaboration with Digital to improve the "Care Path Manager"
- Continue to develop the Standards of Procedures for the position of the Nurse Case Manager.
- Provide Prop teaching in conjunction with the physician, for the patients and their families regarding alternative treatments for breast cancer. (Required by the Department of Health and Mental Hygiene)
- Distribute educational material for treatment options to patients and their families.

Fields

- Perform various studies within the department thereby increasing knowledge and experience.
- Broaden understanding of the BCC's procedures and personnel.
- Will take full advantage of any educational opportunities which may arise as time/schedule permits.

Higgins

- Refine breast self exam teaching and monitor patient's teaching needs.
- Continue to enhance nursing knowledge base on breast cancer issues.
- Continue to further develop personal computer skills.
- Continue to attend a seminars/conferences on breast cancer issues and professional nursing issues.
- Function as team member to develop nursing care plans and documentation tools.
- Improve patient flow processes in the clinical area.
- Completely stock all exam rooms with par level supplies.
- Become more involved with the nursing research aspect of the Center.
- Continue to orient new staff members to patient flow processes and forms within the Center.
- Continue to participate in meetings to further enhance the relationship between Radiology and BCC.



Lopez

- Will continue to provide patient education.
- Initial set-up of patient centered reference library.
- Continue to act as relief ambulatory care nurse.
- continue to develop array of patient educational materials.
- Continue staff development and safety representative responsibilities.

McIntyre

- Continue to perform nursing and managerial duties, as described above.
- Become more proficient in utilizing the BCC Task Management computer system.

Mitchell

- Continue to provide care for patients with both benign and malignant diseases of the breast at the Breast Care Center. Increase the number of patients seen in the Breast Care Center to approximately 500 patient visits each month.
- Expand the educational opportunities available at the Breast Care Center by developing a curriculum for medical students and physicians to receive intensive training in the management of breast diseases over a 2 to 4 week period at the Breast Care Center.
- Continue to develop protocols for both retrospective clinical research involving patients with breast cancer.

Prindle, Snee

- Continue to enhance education in breast cancer and it's treatment.
- Continue development of nursing protocols and quality assurance documents especially in the area of telephone triage.
- Continue to improve patient flow management.
- Attend a seminar/conference related to breast cancer.
- Continue to participate in multidisciplinary meetings.
- Improve upon a fine tun presentation on ambulatory care nursing for future presentations.
- Improve expertise in patient education for APU patients having breast surgery.



Vaughn

- Reduce turn around time for mammogram film return to the department from the Breast Care Center, General Surgery, and patient.
- Implement a new system with release forms for dissemination of patient films.
- Assist with mammogram screening program.

Wallace

- Continue to nurture relationship with the Radiation Oncology Clinic and the Medical Oncology Clinic.
- Develop SOP Manual for the BCC.
- Research possibility of ad hoc reporting in CHCS.
- Continue to coordinate administrative activities of the BCC.
- Complete financial analysis of past expenditures for creation of budget, including Center Operations (Facilities, Outfitting, Travel, Research, Education) and Information systems. Develop Long-term tracking mechanism.
- Complete research on patient level accounting system, begin implementation.
- complete research on alternative methods for patient charting to reduce amount of paper and select a system.
- Develop Strategic Plan and Marketing Plan.



III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD

A. DENTAL DISEASES-RELATED RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Turner

- To continue to procure peripheral blood from Naval recruit volunteers and to isolate polymorphonuclear neutrophils (PMNs) from these blood samples.
- To extract the granule contents from the PMNs.
- To continue with a new series of experiments focused on the bactericidal activities of factors in the PMN granule fractions.

Lamberts

- To assist as an editorial consultant in the preparation or review of manuscripts to be submitted for publication.
- To aid in the preparation of research presentations (such as posters) for scientific meetings, and in the review of research proposals, research communications (abstracts, letters, rebuttals), etc.

Ovsey

- Identify, order and sustain the required equipment necessary to perform mercury analysis by Perkin-Elmer method (PE) 245.1A using a PE5100 PC Atomic Absorption Spectrophotometer, FIAS400 - flow injection system, and AS-90 - auto-sampler.
- Optimize the instrument, attain the Initial Demonstration of Competence (IDC), perform the Method Detection Limit (MDL) estimate (40 CFR App. B part 136), and approximate the Linear Dynamic Range to define the upper limit of mercury analysis. Analyze Quality control Samples (QCS) quarterly. Enroll and participate in all available Water Pollution (WP) and Water Supply (WS) studies. Passing the WS study is required to be eligible for the IEPA certification samples.



Miller

- Responsible for all aspects of Immunological and Microbiological activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, serving as a link between NIH sponsored research and naval dental research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

Beck

- Provide technical assistant with ongoing Immunology research projects. Participate in NIH projects of Genetic Epidemiology. Maintain the upgrade the laboratory such that the research experiments are carried out smoothly. Maintain and record proper technical procedures and data produced for each experiment.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Turner

- To test purified PMN factors, obtained from commercial sources, for bactericidal activity. Granule extracts will be related to this effort.
- To achieve competence with various chromatographic media on our high performance liquid chromatography (HPLC) ["SMART"] system.

Lamberts

- To continue (complete, if possible) the preparation of a manuscript for E. Pederson et al. on a new bacterial medium.
- To assist Naval Dental Research Institute (NDRI) investigators editorially whenever requested.



Ovsey

- Develop a draft of the laboratory Quality Assurance Plan (QAP). The final QAP will be submitted to the IEPA with the Laboratory Questioner and WS results.
- Prepare the laboratory towards eventual IEPA laboratory inspection.
- Aid NDRI in the facilitation of the identification of this organization's role in a program to aid the Naval dental facilities with the improvement of quality of dental waste water.

Miller

- Work Unit : 061152N.MR00001.001-0063. Evaluation of the influence of superantigens and polyclonal B-cell activators for periodonatal disease. To complete work on one additional manuscript. This will bring the project ot completion.
- To continue work on a new program entitled "Evaluation of disproportionate expression of T-Cell receptor V β regions in lymphocytes from patients with advanced periodontitis".
- Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. Complete writing of the secod of two final manuscripts.
- Work Unit: 63706N.M0095.006-3014. Influence of growth factors on gingival and periodontal ligament fibroblasts. Complete preparations of manuscripts.
- To begin to expand the procedures used in 2-Delectrophoresis to more fully study the varius proteins found in gingival crevicular fluid. In addition, preliminary data generated will be used to write an Individual Research (IR) proposal to be submitted in March, 1996.
- Work Unit: 0601152N.MR00001.001-0063. Cytokine production by polymorphonuclear leukocytes resident in peradicular and periodontal lesions. To continue evaluation of cytokine expression by neutrophils. Attempts will also be made to develop an *in situ* hybridization procedures for identification of IL-1 and IL-6 in clinical tissue samples.

Beck

- Continue gathering samples of stimulated lymphocytes for RNA analysis study.
- Learn the techniques and concepts of in situ hybridization and immunohistochemical staining. Carryout these experiments using various prepared tissues slides.
- Prepare slides for *in situ* hybridization and immunohistochemical staining experiments from cultured cells.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Turner

- Diagnosed a malfunction in the HPLC system. Parts are on order.
- Further refined electrophoresis (PHAST) system for silver staining micro samples to detect proteins that may be present in ultra-low concentrations.
- Began activation of fast protein liquid chromatography (FPLC) equipment to supplement the capabilities of HPLC.
- Co-authored one paper published in Archives of Oral Biology (1995), Volume 40, 1151-1155, by E. D. Pederson et al.
- Co-authored one presentation for the March 1996 meeting of the International Association for Dental Research. [Pederson, E.D., Turner, D.W., Schade, S.Z. and Simonson, L.G. Bactericidal Activity of PMN Factors on Treponema denticola. J Dent Res 76:490 (Abst. 3231),1996].

Lamberts

- Reviewed text of two posters (Pederson, Kuehne) that are being prepared for presentation at the March, 1996 meeting of the International Association for Dental Research.
- Reviewed a manuscript that was prepared by M. Cohen.
- Reviewed a protocol of V. Ovsey for measurements of heavy metals in dental-operatory waste-water.
- Reviewed an Independent Research (IR) proposal that was prepared by D. Turner and E. Pederson.
- Began work on the revision of a manuscript for S. Schade et al. The manuscript is to be updated and resubmitted for publication.
- Co-authored a paper entitled "Salivary Levels of Alpha-2 Macroglobulin, Alpha-1 antitrypsin, C-Reactive Protein, Cathepsin-G and Elastase in Human Subjects with or without Destructive Periodontal Disease" by E.D. Pederson et al., published in Archives of Oral Biology (1995), Vol. 40, 1151-1155.



Ovsey

- Ordered and received all equipment necessary for the performance of mercury analysis by the Perkin-Elmer method (PE) 245.1A. Satisfied the additional equipment requirements of the IEPA Certification Program. Continuing to purchase laboratory supplies and services based on maintenance and experimental needs. Completed the method validated in accordance with IEPA and EPA requirements.
- The IDC established the laboratories capability to analyze mercury in water and waste water samples. The IDC analyte concentration has to be 5 to 50 times the MDL, and must be as a minimum within 80 to 120% recovery. The IDC was conducted before the MDL estimation.
- The MDL was estimated at approximately 0.2 micrograms per liter. This means that, if a drop of mercury weighing 1 gram was dissolved in a pool of water with the volume of 5 million liters or 1.32 million gallons of water, this laboratory would be able to detect such small mercury concentrations. The Illinois mercury discharge requirements are 2.5 times higher than this laboratories MDL (i.e. 0.5 micrograms per liter).
- The LDR estimate was performed to identify the upper bound of linearity of this analysis method.
- The QCS confirms this laboratories analyses with an externally prepared and NIST Certified mercury standard.
- Participated in the EPA's WPO35 study. Completed the analysis in January, 1996. Expected to receive the WSO37 samples. However the Federal Government shutdown delayed the WPO35 study completion date and the start of WSO37 study. Approximately six weeks after February 29, 1996 the EPA will release the official WPO35 results. However, received unofficial comparison results from the regional EPA office, projecting a likely successful results. WSO37 samples will be sent out in the beginning of April, 1996.
- Developed a draft of the laboratory Quality Assurance Plan (QAP).
- Co-authored one presentation for the March 1996 meeting of the International Association for Dental Research. [Ovsey, V., Roddy, W., Cailas, M., Drummond, J., Cohen, M., Stone, M., Barka, M., Perry, R., Toepke, T., and Ralls, S.: Silver Generation Rates from Dental Waste Water. Joint Symposium of IADR, AADR and CADR. San Francisco, CA, March, 1996.]
- Co-authored one abstract published in Join Symposium of IADR, AADR, and CADR. San Francisco, CA, March, 1996. By J.L. Drummond, et al., ICP Analysis of Dental Waste Water.



Miller

- Relative to the project Evaluation of the Influence of Superantigens and Polyclonal B-cell Activators in periodontal Disease (Work Unit: 0601152N.MR0001.001-0063) two manuscripts reporting these results are in the final stages of preparation.
- Work Unit: 0601152N.MR0001.001-0063. Cytokine production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions. Tissue samples have now been collected from 24 donors and thin sections have been evaluated for IL-6 production utilizing an enzyme immunoassay procedure as well as by *in situ* hybridization. A paper has been accepted for presentation at the Annual Meeting of the American Association of Immunologists in New Orleans in June, 1996.
- Work Unit: 0601152N.MR0001.001-0063. Other than the evaluation of data, no additional work has been done concerning the project involved with the influence of *Treponema denticola* on cytokine production by cells obtained from periodontally diseased and non-diseased individuals. Final results from this study have been accepted for presentation at the Annual Meeting of the International Association of Dental Research in San Francisco in March, 1996.
- Relative to the project "Influence of growth factors on gingival and periodontal ligament fibroblasts." we have completed all of the planned research and a rough draft of a manuscript has been completed.
- Relative to the program entitled "Evaluation of disproportionate expression of T-cell receptor V β regions in lymphocytes from patients with advanced periodontitis", all control and experimental subjects been identified. It is anticipated that mRNA isolations will be completed during the current quarter.
- Relative to the development of a 2-D electrophoresis procedure to study components of gingival crevicular fluid from individuals with severe periodontitis, preliminary studies have been incorporated into a research proposal which will shortly be submitted for the IR program.

Beck

- Lymphocytes subjected to the antigens such as Td 33521, Td 33520, and anti CD3 were isolated for PCR studies. Cells in each group were frozen away for future experiments of RNA analysis.
- In addition, blastogenesis experiments were performed on each cell group to measure cellular activation upon stimulation.



- Several cell lines are being maintained for continuous growth such that cells from these lines can be used for further experiments.
- Activities of Il-6 and IL-10 were detected within intact cells using *in situ* and immunohistochemical staining experiments.
- Begun participating in NIH epidemiology experiments. These experiments deals with family study of inherited genetic disorder, Cleft-Lip Palate (CLP), and screening for loci associated with this disease.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Turner

- To test certain neutrophil factors for their abilities to kill differnt strains of the spirchete *Treponema denticola*.
- To fractionate the extracts of neutrophils by gel filtration chromatography.

Lamberts

- Complete, if possible, work on the medium manuscript for E. Pederson et al.
- Continue work on the manuscript of S. Schade et al.
- Assist NDRI investigators editorially whenever requested.

Ovsey

- Near the QAP toward completion.
- Successfully participate in the WSO37 study.
- Continue to improve laboratory operations.
- Support efforts in treatment technology installation, evaluation and optimization.



Miller

- Relative to the 2-D PAGE evaluation of components of gingival crevicular fluid, to await IR funding of this program. It is unlikely that additional work will be done without such funding.
- The Naval Dental Research Institute, Detachment Bethesda, the National Naval Dental Center, and the National Institute of Dental Research, National Institutes of Health entered into an agreement to identify, refine, develop, test and evaluate molecular, genetic, and cellular biomarkers for the epidemiological study of premalignant and malignant oral lesions. Oral lesions to be evaluated will be provided by a study entitled "Biomarkers for Oral Cancer," which involves recruitment of subjects for biopsy of oral lesions and collection of epidemiological data at collaborating Veterans Administrations hospitals, GEO-CENTERS, INC. is responsible for providing necessary personnel for this project to work principally in the areas of evaluation of p53, papillomavirus, and retinoic acid receptor expression as initial examples of biomarkers. During the next quarter on scientist will be hired. Considerable effort will be devoted to this program which is expected to have a duration of 4 years. During the next quarter an *in situ* hybridization procedure for the identification of retinoic acid oral receptor mRNA is to be developed as well as to study p53 expression in oral cancer.

Beck

- Main objective of this upcoming quarter is to continue with the NIH CLP project and learn the PCR technique.
- continue gathering necessary samples for the RNA analysis.
- Carryout more *in situ* hybridization and immunohistochemical staining experiments.



IV. NMRI TOX/DET, Dayton, OH

A. TOXICOLOGICAL STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Briggs

- Continues to assure that contract requirements for correlating toxicology research being conducted at the Naval Medical Research Institute (Toxicology, NMRI/TD) at WPAFB, Ohio. This position requires coordination with the Biomedical Operations staff at Geo-Centers, Inc. And planning and coordinating resources with the Executive Steering Committee members at NMRI/TD. This position also requires performing as a toxicologist and risk assessor as tasked. Another position requirement is to serve as the Quality Management Officer to develop the Quality Management Program and assure compliance with that International standard.

Bowen, Kimmel, Reboulet

- Complete inhalation toxicity exposure of test animals to spectronix Fire Extinguishant (SFE) for the edema section of the study.
- Develop analytical methodology for mixed combustion gases inhalation toxicology project.
- Develop facility infrastructure for mixed combustion gases inhalation toxicology project.
- Conduct inhalation toxicology exposures for the ozone depleting substances replacement (ODSR) and ozone depleting substance (ODS) neurobehavioral studies.
- Preparation for characterization of 10.0mm - 0.5mm five-stage cyclone aerosol sampling instrument.
- Preparation of Society of Toxicology (SOT) poster presentations.
- Preparation of Naval Environmental Health Center (NEHC) poste presentations.
- Preparation of journal articles for peer review and publication.
- Preparation of AutoCad™ drawings for SOT poster presentations and journal publications.



Smith, Zepp

- Conduct blood gas studies with regard to homeostasis effects following serial blood collection/transfusions.
- Complete data books for SFE range-finding/multiple-dose, edema and blood gas studies.
- Submit final reports for technical reports and publication of SFE Formulation A pilot studies: Part I and Part II.
- Complete studies on developing a new screening procedure for the determination of cardiac sensitization.

Prues

- Finding replacements for the current ozone depleting fire extinguishants has prompted the Navy to perform studies on alternative products, such as, the Spectrex fire extinguishant (SFE).
- Because of the toxic effects of chemicals on one's ability to perform their duty, the Navy is involved in trying to ascertain the mode of action of certain toxicants (i.e. TMPP), the presence with which their personnel are likely to come into contact.

Narayanan, T.K., Jung

Trimethylolpropane (TMPP) Evaluation:

- Preparation of a paper on TMPP metabolism, clearance, and metabolism.

Cell Model Project:

- Culture of liver cells (WB 344) to be used for the cell model project
- Measurement of the amount of time it takes for the plates to reach confluency and how long they stay there.
- Assessment of the toxicity of acetoamidophenol to cells and the reversibility of those effects.

Neurotransmitter Analysis:

- Set up an HPLC for the Analysis of the neurotransmitter samples.
- Preparation of a standard curve.
- Analysis of homogenized brain samples for neurotransmitter levels.



DBNP Project:

- Preparation of a paper on the clearance, excretion, and metabolites of DBNP.

Ritchie

- Assistant Group Leader for the Neurobehavioral Toxicology Group at the Tri-Service Toxicology Consortium and NMRI/TD. Associate Principal Investigator (API) for all currently funded neurobehavioral toxicology-related work units (FY96 funding of \$465K).
- Purpose is to assist in all areas of program management, budgetary control and procurement, research design, protocol preparation, research supervision, statistical analysis and preparation of scientific papers and abstracts in the area of neurobehavioral toxicology research.

Neurobehavioral Toxicity Assessment Battery (NTAB): Assessing Animal Responses to Pharmacological Challenge (WU.1605):

- Predictive validation of the NTAB by comparison of animal and (known) human responses to identical pharmacological challenges on neurobehavioral tests with topographical similarity.

Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU.1408):

- Neurobehavioral effects of exposure to low concentrations of Ozone-Depleting Substances (ODSs) and Non-Ozone Depleting Substance Replacements (ODSRs) of military interest.

Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU.1420):

- Neurobehavioral effects of exposure to low concentrations of single and mixed combustion gases as might be encountered in military scenarios.

TMPP Mechanisms Of Action: Development Of Neurobehavioral Molecularization Techniques (WU.1512):

- Development of a number of neuro-molecular (cellular-level) analytical techniques for eventual inclusion in the Navy Neuro-Molecular Toxicity Assessment System. (The NTAS); anatomical disposition and effects of trimethylolpropane phosphate (TMPP), a potent neurotoxicant produced through the pyrolysis of synthetic lubricants used in military ships and aircraft.



Persian Gulf War (PGW) Simulation Using Sprague-Dawley Rats (U.S. Army and NMRI/TD):

- Development of an animal model to simulate exposure encountered by Persian Gulf War veterans.

Binole, Rix

- Begin conversion of Windows For Workgroups (WFW) systems to Windows 95.
- Rollout System Management Server (SMS)
- Build SQL database of library holdings.
- Continue maintenance of servers.
- Continue backup of data files on servers.
- Continue to provide hardware/software technical support for TOXDET personnel.
- Develop support/scientific software as needed.

Ademujohn

- The purpose of the neurobehavioral laboratory coordinator at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter the coordinator has been and will be involved in testing the effects of Ozone Depleting Substances (ODS) refrigerants, specifically, Halon 1301 on animal models via computer-aided qualitative and quantitative methods. The coordinator also supervises animal training protocols for the modified Wahmann chamber inhalation studies and roto-wheel studies.

Connolly

- Cataloging print and non-print materials for circulation.
- Ordering and maintaining serials collection.
- Handling reference questions.
- Providing interlibrary loan assistance.
- Preparing book orders.
- Locating needed materials in other libraries.



Walsh

- Member of the Pharmacodynamics Technical Area Group.
- Perform as Project Director for the Inhalation Toxicity of Vapor Phase Lubrication Project.
- Support *in vitro* toxicology research.
- Contract Representative to the Research Management Council.

Geiss

- Group Supervisor II/Scientist III.
- Group Administrator for the Pharmacodynamics (PD) Technical Area Group, Tri-Service Toxicology Consortium.
- Ensure the PD support is provided to project leaders.
- Continue methods and protocol development for the molecular biology laboratory.
- Perform ongoing experiments for the 6-day Trichlorethylene (TCE) study and Environmental Initiative (EI).

McDougal

- Lead basic and applied research in dermal penetration and biologically-based mathematical modeling.
- Responsible for all aspects of quantitative dermal toxicology and dermal risk assessment for Army, Navy and Air force at Tri-Service Toxicology Center.
- Focal Point for Explosives and Munitions Toxicology.
- Primary investigator on an Air Force Office of Scientific Research project entitled, "Species Differences in Skin Penetration".
- Project Leader for Modular Artillery Charge System (MACS).
- Mathematical modeler for Total Petroleum Hydrocarbon Project.
- Lead multidisciplinary research group (University of Michigan, North Carolina State University, Colorado School of Mines, and University of California at San Francisco) addressing quantitative dermal toxicology for Air Force Chemicals.



Grabau

- Member of Pathology Technical Area Group.
- Provide scientific image processing and analysis to ongoing research efforts.
- Member of Tri-Service Marketing/Program Development Team.

Narayanan, L.

- Member of the Hazard Assessment Group.
- Support combustion and *in vitro* toxicology research.
- Support studies involving toxicity evaluation of explosives and propellants.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Briggs

- Assisted with the collaboration of toxicology research by attending two Tri-Service Toxicology Management Council meetings and was voted to be a member of the TST management Council as the Geo-Centers, Inc. Representative. Also attended six NMRI/TD Steering Committee meetings and six Correlation Committee Meetings during this period to provide leadership in the deployment of resources and assuring the quality and integrity of the data collected and presented.
- Collaborated in the development of the Strategic Plan for NMRI/TD, helped to prepare and finalize six preproposals and four proposals to seek funding for 6.1, 6.2 or 6.3 Category funding for NMRI/TD research.
- Provided toxicology expertise for reviewing the 90-Day Inhalation Toxicology Draft Report in Rats with HFC 236-fa, the halon replacement refrigerant that is being categorized for toxicity and risk assessment. Provided information to the Command and the contractor which was included in the Draft Report. Reviewed the Cardiac Sensitization report on a HFC 236 isomer and presented a report. Reviewed and approved the Rabbit Developmental Toxicology Protocol that is currently being used to complete the toxicology profile for the HFC 236-fa refrigerant.
- Assisted in the preparation of the Annual Animal Care and Use Report to Congress.
- Prepared and submitted a review of the per diem process for determining animal use costs as tasked.
- Completed the preliminary plan for the renovation of the current inhalation resource in Building 433, and have gathered data for inclusion in the facilities needs report



relating to expanding the inhalation capacity and capabilities within Tri-Service Toxicology.

- Continued to support program development activities at NMRI/TD by presenting a paper at the Journal Club, preparing a Draft of the Quality Management Program, prepared and got clearance for a platform presentation at the Society of Toxicology (SOT) meeting in March, and co-authored two separate Poster presentations to be made at SOT and NEHC during March.
- Assisted with the toxicology studies being conducted to enhance the current cardiac sensitization model. These improvements were confirmed in a preliminary feasibility research study which was coordinated and reviewed during this period.
- Presented more than 50 Draft SOP's to the Executive Procedure to be included in the Quality Management Program.

Bowen, Kimmel, Reboulet

- Implement engineering plan for the construction of inhalation laboratory 203 for the mixed combustion gases inhalation toxicology study.
- Set up Fourier Transformation Infrared Spectrophotometer (FTIR).
- Preparation of posters for 1996 SOT (Anaheim, CA), 1996 Pre-SOT (WPAFB, OH).
- Preparation of posters for 1996 NEHC (Virginia Beach, VA).
- Neurobehavioral roto-wheel inhalation toxicology exposures of test animals to Halon 1301.
- Neurobehavioral operant inhalation toxicology exposures of test animals to Halon 1301.
- Preparation of peer reviewed journal publications.
- Prepare analytical instrumentation and methodology for characterization five-stage cyclone aerosol sampling instrument.
- Conduct edema study of SFE inhalation toxicity project.

Smith, Zepp

SFE Formulation A:

- The objective of this research is to evaluate the potential health effects of exposure to the by-products of pyrolyzed SFE. SFE is fire suppressant and a potential replacement for Halon 1301.



Cardiac Sensitization:

- The objective of this research is to develop an in vitro test for the determination of cardiac sensitization. These initial studies will set the basic background needed for future studies.

Prues

Technical support for the following NMRI/TD Projects is to be provided:

- Spectrex fire extinguishant (SFE).
- Trimethylolpropane phosphate (TMPP).
- Serve as contract representative on the Safety Policy Committee.

Narayanan, T.K., Jung

Trimethylolpropane (TMPP) Evaluation:

- To prepare a paper on the metabolism, clearance, and possible metabolites of TMPP.

Cell Model Project:

- Maintain the cell line (WB 344) to be used for the cell model experiment.
- Monitor the growth of cells in the plates and determine how long they remain confluent before the numbers begin to decline.
- Measure the viability of cells after short term exposure to acetoamidophenol see if the cells will begin growing again after part of them have been killed by the acetoamidophenol.

Neurotransmitter Analysis:

- Set up the Beckman HPLC for the chromatographic analysis of the neurotransmitter studies.

DBNP Project:

- Prepare a paper on the metabolism, clearance, and excretion of DBNP.



Ritchie

Neurobehavioral Toxicity Assessment Battery (NTAB): Assessing animal Responses to Pharmacological Challenge (WU.1605):

- To meet milestone objectives related to evaluation of 500+ rats on various NTAB tests during various pharmacological challenges, as described in a recently approved (2/96) animal use protocol. The overall purposes of this work unit is validation of several new NTAB tests, and predictive validation of the NTAB for human risk assessment.
- To complete development of juvenile play, audiogenic startle, Porsolt F.S.T. and Morris Water Maze NTAB tests.
- To continue orientation and training of newly-assigned Neuropharmacologist.
- To complete and submit or present related abstracts and scientific posters at Pre-SOT (ManTech), SOT, NEHC, and Society for Neuroscience Annual Meetings.
- To completely rewrite and submit, in the new DoD format, a comprehensive protocol for all work to be completed under this work unit during the next two fiscal years.

Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU.1408):

- To begin the physical construction, testing and validation of a totally computerized system to deliver to rats (during performance of NTAB tests) up to five mixed fire gases. Construction of the system was begun in February 96 in NMRI/TD Laboratories 202/203.
- To completely rewrite and submit, in the new DoD format, a comprehensive protocol for all work to be completed under this work unit during the next two fiscal years.

Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU.1420):

- To complete all neurobehavioral testing of ODS Halon-1301 and Halon-1211, and ODSR candidate HFC-227ea using the Navy Roto-Wheel and tow operant tests.
- To submit a journal publication comparing the neurobehavioral toxicities of HFC-134a and CFC-12.
- To complete and submit or present related abstracts and scientific posters at Pre-SOT (ManTech), SOT, NEHC, and Society for Neuroscience Annual Meetings.

TMPP Mechanisms Of Action: Development Of Neurobehavioral Molecularization Techniques (WU.1512):



- To assist with surgical techniques for implantation of cannulas and electrodes in rats for EEG/microdialysis or Flow Injection Analysis recording during direct microinfusion of TMPP and/or d-amphetamine into the nucleus accumbens.
- To assist with surgical implantation of multi-unit stimulating and recording electrodes in the nucleus accumbens and ventral tegmental area in rats.
- To assist with investigation, using competitive radiolabeled binding techniques, of the specific CNS receptor binding of TMPP.
- To complete study of the effects of low dose exposures to TMPP on performance of well-learned operant habit in rats.
- To complete investigation of the effects of single or repeated administration of very low doses of TMPP on development of long-term CNS sensitization in rats.
- To begin a major scientific investigation of the effects of various human pharmaceutical drugs and anti-epileptic treatments on prevention of convulsive response to TMPP administration in rats.
- To complete and submit or present related abstracts and scientific posters at Pre-SOT, (ManTech), SOT, NEHC, and Society for Neuroscience Annual Meetings.

Persian Gulf War (PGW) Simulation Using Sprague-Dawley Rats (U.S. Army and NMRI/TD):

- To manage (as API) all phases of the inhalation exposure, drug treatment and neurobehavioral evaluation of rats used in the forthcoming Persian Gulf war exposure simulation study to be conducted in conjunction with the Tri-Service Toxicology Consortium.

Binole, Rix

- Continue to add to and improve our network capabilities.
- Pending guidelines from the Tri-Service program development task force.
- Rework/add to internet services.
- Pending system access install SMS.
- Pending input from end users convert current Navy Supply database to SQL or FoxPro.
- Continue to provide technical support for TOXDET personnel.
- Develop support software where needed.
- Pending funding install additional CD-ROM capabilities.



Ademujohn

- Testing various ODS substances (Halon 1301) on animal models using diminished capacity as the endpoint in Carneaux pigeons and Wistar rats.
- Range finding using operant - trained animals and measuring subsequent stages of diminished capacity.
- To compile, catalog and computerize the above mentioned data.
- To train pigeons and rats for problem solving protocols.
- Daily maintenance of pigeon intake and logging performance results.
- To obtain operant testing and training data for animals used in operant exposure testing.
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement of all materials used in testing and training protocols.
- Responsible for documenting and maintaining operant weights.
- Responsible for writing standard operating procedures for pigeon training protocols.
- Responsible for making daily accurate and detained entries and updates of all work unit laboratory books.
- Responsible for compiling information for and conducting weekly meetings with/between work unit P.I.'s and laboratory technicians.

Connolly

- Catalog materials as received.
- Catalog materials not yet cataloged.
- Provide library service to the toxicology community at WPAFB.
- Continue working on a manual card catalog.

Walsh

- Prepare and submit for review and acceptance, documents to support the Vapor Phase Project.
- Provide technical support to the Regulation of Metallothionein Gene Expression Project.
- Contract Representative observer to the Research Management Council (RMC).



Geiss

- Identify needs in molecular biology research support and design a technical approach to fulfill those needs.
- Develop protocols and research methods for the evaluation of biological effects of Air Force-related materials.
- Cooperate in current research relating to the toxicological effects of trichloroethylene (TCE), its metabolites and other compounds.
- Develop molecular methods for the Predictive Toxicology project.
- Train other scientist in Molecular Biology research methods.

McDougal

- Continue pharmacokinetic studies for 3 chemicals in hairless guinea pigs.
- Continue development of mathematical models for the diffusion cells.
- Measure diffusion of MACS across human skin.
- Begin estimating permeability constants for MACS components.

Grabau

- Support species differences in Skin Penetration Project
- Support combustion Toxicology of Advanced Materials (ACM) Project.
- Support Ammonium Perchlorate study.
- Assist Tri-Service Marketing/Program Development Team.

Narayanan, L.

- Standardize the elution conditions using an anion exchange column for perchlorate detection and quantitation of Ammonium Perchlorate using HPLC coupled with conductivity detector.
- Estimate the total circulating thyroxine (T4) levels in control and Ammonium Perchlorate exposed rats, using Radioimmunoassay (RIA).
- Quantitate neurotransmitters and their major metabolites' levels in control and quadricyclane exposed rats using HPLC.
- Continue communication with The Center for *InVivo* Microscopy regarding collaborative efforts to develop Magnetic Resonance Microscopy as a research tool to study the cutaneous penetration of model chemicals.



SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Briggs

- An effective management program has been developed that provides leadership for both NMRI/TD and Tri-Service Toxicology.
- Planning for the future and the additional preproposals and proposals are essential for creating a future for NMRI/TD. These Work Unit proposals and planning documents are critical to the collaboration of resources and securing funding for both NMRI/TD and the TST.
- As a toxicologist, reviews and evaluates study reports and communications with funding sponsors. These functions require expertise and awareness of the military relevance in the solutions to human health risk assessments.
- Reports to Congress relating to Animal Care and Use must be prepared annually to assure that NMRI/TD is in compliance with Animal Care Regulations and assurances that the rights of the animals are assured.
- The need for compliance with a Quality Management Program is an essential part of our development of a Center for Excellence at WPAFB in toxicology. Tasked to develop this program and perform the function of the Quality Management Officer.

Presentations prepared and delivered during this period:

- Quality management Plan - Presented at the Journal Club at NMRI/TD on January 25, 1996.
- Cardiac Sensitization Model Development Using Biochemical Markers - Abstract approved and platform presentation cleared. This presentation will be made during the Society of Toxicology Meeting on March 14, 1996.
- Toxicological Evaluations of the CFC Alternative HFC 236ea. Poster presentation at the Society of Toxicology Meeting on March 14, 1996.
- Cardiac Sensitization Model Development using Biomedical Markers - Poster presentation at the NEHC Workshop, 22-29 March 1996.



Bowen, Kimmel, Reboulet

- The Perkin Elmer FTIR was installed and the associated software was loaded. Preliminary tests were conducted to verify compliance. Operator training classes have been scheduled for April 9, 1996.
- Construction was started for the mixed gases inhalation toxicity laboratory (lab 203). The laboratory space has been cleared and instrumentation is being put in place.
- The edema study for the SFE inhalation toxicity exposure using guinea pigs was completed.
- A novel inlet aerosol diffusion vent was designed, constructed and retro-fitted into the 0.76 m³ inhalation exposure chamber to support the delivery of test material.
- Four posters were completed for presentation at the annual SOT, NEHC and Pre-SOT symposiums. Refer to attachments 1-6. Several AutoCad™ drawings were made to be incorporated into the posters.
- Neurobehavioral operant toxicity inhalation exposures of test animals to Halon 1301 were initiated. Test animals have been exposed to concentrations of 11,15,20 and 25%.
- Five journal articles have been submitted for peer review an/or publication. Refer to attachments 7-9.
- Instrumentation is being assembled to characterize the newly purchased cyclone sampling train. A fluorescent dye (Uranine) incorporated onto a monodispersed aerosol (salt) will be used to determine collection efficiency fraction versus particle size for the instrument.

Attachments:

1. Physical And Chemical Characteristics of SFE Fire Suppressant Atmospheres in Small vs Large Scale Tests: Implications for Pulmonary Deposition and Toxicity. EC Kimmel¹, EA Smith¹, JE Reboulet¹, and RL Carpenter²; ¹Geo-Centers, Inc., ²Tri-Services Toxicology consortium, Wright-Patterson AFB, OH.
2. Calculation of Exposure Chamber Leak Rate With Therman Correction: A Measure of Chamber Integrity. JE Reboulet and EC Kimmel. Sponsor EA Smith. Geo-Centers, Inc. And Tri-Services Consortium, Wright-Patterson AFB, OH.
3. The Toxicological Assessment of Inhaled Spectrex Fire Extinguishant (SFE), Dry Powder Extinguishing Agent. Eldon A. Smith², Edgar C. Kimmel², Larry E. Bowen², James E. Reboulet², and Robert L. Carpenter¹; ¹ Naval Medical Research Institute Detachment (Toxicology) and ² Geo-Centers, Inc. Building 433 AreaB, 2612 Fifth Street, Wright-Patterson AFB, OH, 45433-7903, USA.



4. The Assessment of Toxicity after exposure to a Pyrotechnically-generated aerosol. Eldon A. Smith², Edgar C. Kimmel², MAJ Jeffery H. English³, Larry E. Bowen², James E. Reboulet², CAPT (S) Kenneth R. Still⁴ and Robert L. Carpenter¹; ¹ Naval Medical Research Institute Detachment (Toxicology), building 433 Area B, 2612 Fifth Street, Wright-Patterson AFB, OH 45433-7903, USA, ² Geo-Centers, Inc. ³ Army Medical Research Unit--Toxicology Division and ⁴Officer-In-Charge, Naval Medical Research Institute Detachment (Toxicology).
5. A Method to Assess Motivational Factors In Complex Neurobehavioral Performance Deficits Induced by Toxic Exposures. LCDR John Rossi III, MSC, USN, Glenn D. Ritchie, HM3 Cynthia Onyika, HM3 Jay Smith, Cynthia Ademujohn, and Larry Bowen. Naval Medical Research Institute Detachment (Toxicology) and Geo-Centers, Inc., Wright-Patterson AFB, Ohio.
6. A Method to Assess Motivational Factors in Complex Neurobehavioral Performance Deficits Induced By Toxic Exposures. GD Ritchie, CY Ademujohn, J Rossi III, and LE Bowen. Tri-Services Toxicology Consortium: Naval Medical Research Institute Detachment (Toxicology) and Geo-Centers, Inc. Wright-Patterson AFB, OH, USA Sponsor: D. Dodd.
7. Comparison Of The Physical And Chemical Characteristics of SFE, A Dry Powder Fire Suppressant, Atmospheres in Small vs Large Scale Laboratory Tests. Edgar C. Kimmel Ph.D., Eldon a. Smith Ph.D., James E. Reboulet M.S., Bruce H. Black Ph.D., Ron S. Sheinson Ph.D., and Robert L. Carpenter Ph.D.
8. The Physicochemical Properties of SFE Fire Suppressant Atmospheres in Toxicity vs. Fire Extinguishment tests: Implications for aerosol deposition and toxicity. Edgar C. Kimmel, Eldon A. Smith, James E. Reboulet and Robert L. Carpenter.
9. The Navy Roto-Wheel: A new apparatus for multiple measures of physical incapacitation. Glenn D. Ritchie, Geo-Centers, Inc.; John Rossi III, Naval Medical Research Institute Detachment (Toxicology); Larry E. Bowen, Geo-Centers, Inc.; William B. Sonntag, ManTech Environmental Services; William J. Binole, Geo-Centers, Inc.; Robert L. Carpenter, Naval Medical Research Institute Detachment (Toxicology), Wright Patterson AFB, OHIO.

Smith, Zepp

- Organization of data books for SFE range-finding/multiple-dose, edema and homeostasis studies.
- Several cardiac sensitization studies were initiated to study the mechanical and electrophysiological events leading to ventricular fibrillation.



- Closed study books for all areas associated with SFE Formulation A pilot studies: Part I and Part II.
- Submitted final drafts for technical reports and publication on SFE Formulation A pilot studies: Part I and Part II.
- Constructed posters for Society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996.
- Start the following SFE protocols: Protocol 1-Evaluation of Blood Gas, Blood pH, Hemoglobin, Bicarbonate and Glucose Levels after Exposure to the Pyrolyzed By-products of SFE Formulation A.
- Start studies on the development of an in vitro method for the determination of cardiac sensitization.

List of publications, abstracts, technical reports, speeches, and poster presentations for this quarter:

- Evaluation of Bronchoalveolar Lavage From Rats Exposed To A Pyrotechnically-Generated Aerosol.; KS Zepp¹, EA Smith¹, SL Prues¹, EC Kimmel¹, JE Reboulet¹, and RL Carpenter².; ¹ Geo-Centers, Inc., ² Tri-Services Toxicology Consortium, Wright Patterson AFB, OH.
- Poster : presented at the Society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996.
- The Use of Push/Pull cannulation As A Viable Alternative In Multiple Blood Sampling.; SL Prues¹, EA Smith¹, HMC SM Bulger², KS Zepp¹, and HM² JL Cassell².; ¹ Geo-Centers, Inc., ² Tri-Services Toxicology Consortium, Wright Patterson AFB, OH.
- Poster: presented at the society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996; also presented at the Naval Environmental Health Conference held in Virginia Beach, Virginia, on March 22, 1996.
- Toxicological Evaluation of Exposure To Two Formulations of A Pyrotechnically-Generated Aerosol.; EA Smith¹, EC Kimmel¹, HM² JL Cassell², JE Reboulet² and RL Carpenter².; ¹ Geo-Centers, Inc., ² Tri-Services Toxicology Consortium, Wright Patterson AFB, OH.
- Poster: presented at the Society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996.
- Crossmatching Blood Types From Four Rat Strains.; HMC SM Bulger² and EA Smith¹.; ¹ Geo-Centers, Inc., ² Tri-Services Toxicology Consortium, Wright Patterson AFB, OH.
- Poster: presented at the Society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996.



- Physical and Chemical Characteristics of SFE Fire Suppressant Atmospheres in Small vs Large Scale Tests: Implications for Pulmonary Deposition and Toxicity.; EC Kimmel¹, EA Smith¹, JE Reboulet¹ and RL Carpenter². ¹Geo-Centers, Inc., ²Tri-Services Toxicology Consortium, Wright Patterson AFB, OH.

Prues

- SFE Project--Blood gas, pH, hemoglobin, bicarbonate, and glucose level monitoring after exposure to by-products of SFE formulation A.
- Assisted in a cannula validation study which was required to evaluate the effects of blood withdrawal and replacement through the femoral artery and vein of a test animal. In order to show that a test animal would not be compromised by this technique, a preliminary stress test was conducted.
- Prepared poster for its presentation at his years 35th Annual Society of Toxicology Meeting held in Anaheim, California this March.
- TMPP project--Simultaneous microdialysis perfusion and EEG recording with behavioral observations to determine its effects.
- Performed surgical procedures for the precise implantation of microdialysis guide cannulae and placement of EEG electrodes.
- Setup and collected data during microdialysis perfusion/EEG experiments.
- Assisted in data spreadsheet analysis.
- Prepared graphs for use in NEHC poster.
- Maintained and stocked laboratory.

List of Publications, Abstracts, Technical Reports, Poster Presentations, Speeches, etc. for this quarter:

- SL Prues, EA Smith, SM Bulger, KS Zepp, JL Cassell: The Use of Push/Pull Cannulation as a Viable Alternative in Multiple Blood Sampling. Quarterly Progress Report (Abstracts for the Society of Toxicology) 1995.
- KS Zepp, EA Smith, SL Prues, EC Kimmel, JE Reboulet, and RL Carpenter: Evaluation of Bronchoalveolar Lavage from Rats Exposed to a Pyrotechnically-Generated Aerosol. Quarterly Progress Report (Abstracts for the Society of Toxicology) 1995.
- JW Lindsey, S Prues, C Alva, GD Ritchie, J Rossi III, HN Davis: Simultaneous Microdialysis, EEG, and Behavioral Assessment of the Effects of Trimetholpropane Phosphate on Sprague-Dawley Rats. Society for Neuroscience Abstracts, Vol. 21, part 2, 1995.



Narayanan, T.K., Jung

Trimethylolpropane (TMPP) Evaluation:

- Prepared a paper entitled "Trimethylolpropane Phosphate (TMPP) Absorption, Distribution, Metabolism, and Excretion in Fischer 344 rats."

Cell Model Project:

- A known amount of cells were added to plates and the number of cells was measured each day by a non-radioactive cell proliferation assay. The cells were counted to monitor their growth, to see when they reach confluency, and to see how long they remained confluent before some of the cells started to die. It was determined that 500,000 cells seeded initially would be confluent in 3 days and maintain their number for about 1 week. Confluent plates were exposed to acetoamidophenol for differing lengths of time and at different concentrations. Higher concentrations and longer times resulted in a greater amount of cell death. A concentration of 8 mg/ml that was left on the plates for 3 hours caused about 50% of the cells to die. A concentration of

6 mg/ml that was exposed for the same length of time and resulted in about 70% cell death. These plates also recovered much faster. The plates that had been exposed to 8 mg/ml acetoamidophenol did not regenerate cells as quickly as the ones that had been exposed to a lesser concentration. However, both sets of cells did begin to divide again after a portion of them had been killed off by acetoamidophenol.

Neurotransmitter Analysis:

- Samples for neurotransmitter analysis were prepared by homogenizing the brain in 0.17 M HC104 (100 mg tissue/ 1 ml). This solution was centrifuged and the resulting supernatant was filtered through 0.45 micron HPLC filters. The samples (5 ml) were applied to a reverse phase C18 column that was run with a 1.2 ml/min flow of mobile phase [32 mM citric acid, 12.5 mM disodium hydrogen o-phosphate, 0.5 mM octyl sodium sulfate, 0.05 mM EDTA, 0.03 M sodium hydroxide, pH 3.5] with 15% methanol for 30 min.

DNBP Project:

- Prepared a paper entitled "The Absorption, Distribution, Metabolism and Excretion of 2, 6-di-tertiary-butyl-4-nitrophenol in Fischer 344 rats." (Sent for publication clearance)

Reference listing of Publications/Abstracts for this Reporting Period:



- "Trimethylolpropane Phosphate (TMPP) Absorption, Distribution, Metabolism, and Excretion in Fischer-344 rats" TK Narayanan, AE Jung, GD Ritchie, J Rossi III and KR Still. Sent for clearance for publication.

Ritchie

- A major ACUC animal use protocol was written, submitted and approved supporting the majority of NTAB validation research planned by the Neurobehavioral Group for the next two fiscal years.
- A major ACUC animal use protocol was written, submitted and approved supporting the majority of TMPP research planned by the Neurobehavioral Group for the next two fiscal years.
- A protocol addendum was written and submitted to include a classical conditioning test (conditioned paw withdrawal and eyeblink) in rats in the NTAB; equipment was ordered.
- Two addenda were written, submitted and approved to support TMPP ligand binding research requirements.
- Twenty-one White Carneaux pigeons are completing extensive spectral and pattern operant discrimination training in preparation for pharmacological challenges during performance of NTAB tests.
- Four highly trained rats are being tested for cognitive or motivational deficits induced during exposure to progressively increasing concentrations of Halon-1301.
- TMPP-induced CNS sensitization studies (nucleus accumbens/ventral tegmental area) were completed using 20 rats.

Deliverable associated with above described work units:

- T.K. Narayanan, A.E. Jung, G.D. Ritchie, J.F. Wyman and J. Rossi III. Disposition, metabolism and clearance of the potent cage convulsant trimethylolpropane phosphate (TMPP). *Epilepsia*, submitted Feb. 1996.
- G.D. Ritchie, J. Rossi III, W.B. Sonntag, W. Binole and R.L. Carpenter. The Navy Roto-Wheel: A new apparatus for multiple measures of physical incapacitation. *Behavior Research Methods, Instruments, & Computers*, submitted Feb 1996.
- G.D. Ritchie, C. Oniyka, J. Smith, C. Ademujohn and L. Bowen. A method to assess motivational factors in complex neurobehavioral performance deficits induced by toxic exposures. Abstract, 1996 Navy Health Environmental Center (NHEC) annual conference, Virginia Beach, VA.



- G.D. Ritchie, C. Oniyka, J. Smith, C. Ademujohn and L. Bowen. A method to assess motivational factors in complex neurobehavioral performance deficits induced by toxic exposures. Poster Presentation, 1996 Navy Health Environmental Center (NHEC) annual conference, Virginia Beach, VA.
- G. D. Ritchie, J. Rossi III, L.E. Bowen and C.Y. Ademujohn. A method to assess motivational factors in complex neurobehavioral performance deficits induced by toxic exposures. Abstract, Society of Toxicology, Anaheim, CA.
- J. Rossi III, C.Y. Ademujohn, and G.D. Ritchie. The navy Neurobehavioral toxicity Assessment Battery (The NTAB). Abstract, 1996 Navy Health Environmental Center (NHEC) annual conference, Virginia Beach, VA.
- J. Rossi III, D. Pruitt, C. Nocjar, B. Knutson, C.Y. Ademujohn, G.D. Ritchie and J. Panksepp. Sensitization induced by sub-convulsive doses of the novel neurotoxicant trimethylolpropane phosphate (TMPP). Abstract, 1996 Navy Health Environmental Center (NHEC) annual conference, Norfolk, VA.
- G.D. Ritchie and J. Rossi III. Characterization of trimethylolpropane phosphate (TMPP), a chemical model for absence epilepsy. Abstract, Basic Mechanisms of the Epilepsies Meeting, San Diego, CA, 1996.
- J. Rossi III (Chairperson), and G.D. Ritchie (Presenter). Multiple Chemical sensitivity, CNS Sensitization and Special Populations. Conference on Advances in Toxicology and Applications to Risk Assessment, 30th Annual Toxicology conference, Hope Hotel, WPAFB, Dayton, OH, April 1996.

Binole, Rix

- SQL and SMS are functional. System wide rollout will proceed withing the constraints of system access.
- Web pages are in the process of being redesigned to provide an easier user interface.
- Procurement of all software/hardware for use at NMRI/TD.
- Technical support for all software/hardware installed.
- Complete rewrite of the Pigeon Neurobehavior training systems.
- Assistance in the design and printing of all posters/presentations.
- Supply database conversion is on hold due to time constraints.
- Upgrade of list servers and Web page to allow for code sharing by PBPK list members.



Ademujohn

- Compiled, organized, cataloged, via computer-aided graphics, the weekly data on Pigeon 'Match' and 'Shapes' protocols.
- Trained and conditioned new and incoming rodent and pigeon groups to protocol adaptation.
- Crucial in the reorganization overhaul of the Neurobehavioral laboratory to provide a professional appearance and more efficient use of space.
- Implemented an SOP on the procedures for pigeon "Match" protocol training.
- Wrote procedures for Negative Pressure Acquisition in the Wahmann Chamber for pigeon conditioning.
- Maintenance of all laboratory work unit notebooks.
- Implemented several data methods to compile training data and weight maintenance on the pigeon operants.
- Modified pigeon Wahmann chamber for uniform problem solving display.
- Implemented an effective Wahmann chamber disinfection program.

Cited as contributor in the publications listed below:

- GD Ritchie, J Rossi III, LE Bowen and CY Ademujohn. A method to assess motivational factors in complex neurobehavioral performance deficits induced by toxic exposures. *The Toxicologist*. 16 (1) 1996.
- GD Ritchie, D.Pruitt, C. Nocjar, B. Knutson, CY Ademujohn and J Panksepp. Sensitization Induced by Sub-Convulsive Doses of the NOEL Neurotoxicant Trimethylolpropane Phosphate (TMPP). Abstract. 35th Annual Meeting of the Society of Toxicology, San Diego, CA (March 1996).
- GD Ritchie, J Rossi III, LE Bowen and CY Ademujohn. A Method To Assess Motivational Factors In Complex Neurobehavioral Performance Deficits Induced By Toxic Exposures. Poster Presentation. 35th Annual Meeting of the Society of Toxicology, San Diego, CA (March 1996).

Connolly

- 29 books cataloged and prepared for circulation.
- 209 articles obtained from local libraries.
- 25 articles entered into the reprint database and file.
- 29 interlibrary loans obtained.
- 19 books and/or slide sets obtained from local libraries.
- 9 literature searches conducted using in-house CDROM database capabilities.



- 6 searches successfully conducted on the Internet for customers, including downloading of documents as required.
- 35 reference questions answered.
- 9 telephone inquiries on journal locations in local area handled successfully.
- Journal routing slips prepared and system in place to route incoming journals to all interested parties.
- 46 requests for articles located and filled from in house resources.
- 6 articles obtained using the CARL UnCover system via the Internet.
- 2 interlibrary loan requests from other libraries filled using resources here.

Walsh

Toxicity of Vapor Phase Lubricants Project:

- Completed/submitted for internal review the animal use protocol.

Regulation of Metallothionein Gene Expression Project:

- Performed liver perfusions for primary hepatocyte isolation.
- Performed Cadmium and zinc toxicity range finding, using MTT as a biochemical endpoint.
- Performed a primary hepatocyte plating efficiency study.
- Performed analytical assays to include; Glutathione, microculture tetrazolium, protein and Ethoxyresorufin assays.
- Coordinated supply and animal orders.

Research Management Council

- Attended two RMC meetings as the Contract Representative.
- Attended a RMC one-day off site to discuss tactical and strategic plans for the Division.

Geiss

- Continue to assist in planning resource allocation, individual training and professional development for the 16 members of the PD group. I also communicate with other scientists and project leaders to identify their technical needs and to suggest solutions.
- Project Support: Gene probe for ornithine decarboxylase (ODC) was prepared for use in RNA analysis. The cDNA for ODC was cloned in a plasmid and amplified in bacteria for use as a probe. Oligonucleotide probes for hybridization analysis were designed for synthesis.



- Received a new technician in my group and am currently training him to support Toxicology Division projects.

McDougal

Air Force office of Scientific Research (AFSOR) Project (Dermal Penetration):

- Briefed Air Force Science Advisory Board on this project on 14 Feb.
- Developed extremely sensitive headspace assay for tridecafluoroiodohexane one of the three model chemicals used in this project.
- Reestablished proficiency required for surgical implantation of jugular cannulas.

Modular Artillery Charge System (MACS) Project (Dermal Penetration):

- Completed diffusion studies in three species (full thickness nad split thickness skin) with dibromoethane.
- Determined flux and permeability coefficients for each of these studies using both traditional methods and mathematical modeling.
- Completed model discrimination three physiologically based mathematical models for the static diffusion cells.
- Visited customer for this project at Picatinny Arsenal, NJ on 29 Feb to discuss progress and future needs.

Total Petroleum Hydrocarbon (TPH) Project:

- Wrote a physiologically-based mathematical model for the inhalation and oral administration on nonane in rats.
- Planned an inhalation pharmacokinetic study to collect information required for metabolic constants.
- Used model to determine that pilot information from inhalation study would provide appropriate information.

Other Scientific and Regulatory Interactions:

- Traveled to Washington, DC to participate in International Life Science Institute's working group on "Estimation of Dermal and Inhalational Exposures to Contaminants in Drinking Water" on 1 Feb.
- Attended WSU School of Medicine Research Committee meeting on 16 Feb. To rank proposals for Biomedical Sciences Research Grants.
- Continued to act as scientific mentor for Capt. Wade Weismann with emphasis on mathematical modeling and laboratory data collection.



List of Publications, Abstracts, Technical Reports, Poster Presentations, speeches etc. for this Quarter:

- Jepson, G.W., J.D. Cafferty, Z. Liron, and J.N. McDougal: Stratum Corneum Permeability Coefficient Determination for Volatile Chemicals using Thermal Gravimetric Analysis Methods. *The Toxicologist* 30 (1):169 (1996).
- McDougal, J.N.: Mathematical Modeling of Skin diffusion Cells. *The Toxicologist* 31 (1):170 (1996).
- Dong, L., J.H. Grabau, D.R. Mattie, G.W. Jepson and J.N. McDougal: In Vivo Dermal Absorption of Dichlorobenzene, Chloropentafluorobenzene and Tridecafluoroiodohexane in Fischer 344 Rats. *The Toxicologist* 31 (1):172 (1996).

Grabau

Support Species Differences in Skin Penetration Project.

- Dermal image analysis provided for research animal specimens to quantitate epidermal and dermal end points following four *in vitro* exposures; methods to quantitate human cutaneous endpoints in a maner similar to previous animal studies is under development.
- Continued communication with the Center for *In Vivo* Microscopy regarding collaborative efforts to develop Magnetic Resonance Microscopy as a research tool to study the cutaneous penetration of model chemicals.

Support Combustion Toxicology of Advanced Materials (ACM) Project:

- Support efforts to complete final reports for phase I.: due to the loss of staff involved with key protions of the dreaft technical report, portions of previous research are being recompiled.
- Support efforts to plan and implement phase II.: Meeting and planning sessions are ongoing to an support in-life exposure, the role of image analysis is being evaluated and determined.

Support Ammonium Perchlorate Study (A10):

- Thyroid meorphometric analysis was completed and involved 120 images with over 8,000 follicles from 24 animals at 5 dosage groups.

Assist Tri-Service Marketing/Program Development Team:

- Co-authored a report detailing past, present and future directions of the Program Development Team titled "Program Development Strategic Plan".



- Co-authored a poster presentation titled "Risk Assessment Of Hazardous materials In The Department of Defense" for the Navy Occupational Health and Preventative Medicine Workshop (Virginia Beach, VA, March 1996).
- Attended a symposium on an advanced Internet programming language (Java) to determine its potential role in the Tri-Service Toxicology Internet site.
- Initiated joint effort to redesign and reorganize the Tri-Service Toxicology Web site.

Other duties as requested or required:

- A research proposal titled "Research Proposal: Developmental Toxicology Methods Development" was submitted on 22 February in response to a verbal request from the Chair, Research Management Council.
- A DOS-based image analysis system was successfully proted to the Windows 95 Operating System (OS) completing a 6 month effort to correct technical issues.
- Instillation of a Unix-based image analysis system was completed.

Presentations, Technical Reports:

- Abstract submission and Acceptance for Poster Presentation at the Navy Occupational Health and Preventative Medicine Workshop (Virginia Beach, VA, March 1996)
- Rick Assessment of Hazardous Materials In The Department of Defense, D.R. Mattie, L.S. Martin, J. Grabau, W.W. Jedderberg and K. R. Still, Tri-Service Toxicology Consortium, The Oxford Associates, Inc., GEO-CENTERS, INC., WPAFB, Ohio.
- Report to Tri-Service management Council- Program Development Strategic Plan, D.R. Mattie, L.S. Martin, J.H. Grabau, and J.H. Barkeloo; January 4, 1996.

Narayanan, L.

Ammonium Perchlorate Project

- The elution condition for Ammonium Perchlorate detection was standardized using Anion exchange column. Ammonium Perchlorate was quantitated using HPLC coupled with conductivity detector. Ammonium was not found to be stable both in low and high concentrations of Ammonium Perchlorate dose solutions over a period of 150 days.
- Circulating levels of thyroxine (T4) in control and Ammonium Perchlorate exposed rats were quantitated. Ammonium Perchlorate blocked the incorporation of free iodine into tetraiodothyronine (T4) by the thyroid gland.



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- Submitted an article titled "Perfluorodecanoic Acid, a Peroxisome Proliferator, Activates Phospholipase C, inhibits CTP: Phosphocholine Cytidylyltransferase, and Elevates Diacylglycerol in Rat liver" to *Toxicology Letters*.

Acute and Reproductive Toxicity of Quadricyclane:

- Neurotransmitters's analysis was performed in different regions of rat brain to assess the neurotoxicity of Quadricyclane.

Publications/Abstracts for this Reporting Period:

- Nicholas V. Reo, Latha Narayanan, Katrina B. Kling, and Mehdi Adinezhadeh. (1995) Perfluorodecanoic Acid, a Peroxisome Proliferator, Activates Phospholipase C, Inhibits CTP: Phosphocholine Cytidylyltransferase, and Elevates Diacylglycerol in Rat Liver. *Toxicol. Letters* (Accepted).

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Briggs

- Assist the Tri-Service Management Council in finalizing a Charter and establishing operating procedures.
- Continue to help the Executive Steering Committee in project management and planning for conducting toxicology studies.
- Assist with technical supervision and planning s tasked by the Officer In Charge at NMRI/TD.
- Respond as tasked to assure that the contractor resources are available to meet the requirements of the Work Units and study plans.
- Help to assure the compliance with standards for excellence that are universally practiced by toxicologists around the world.
- Complete the final toxicology profile studies with the HFC 236-fa refrigerant of Navy interest. Assist with the preparation of reports and providing expert advice to NAVSEA.
- Respond to the needs for proposals that are approved for future research.
- Finalize two technical reports relating to the Good Management Plan and cardiac sensitization.



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Bowen, Kimmel, Reboulet

- Initiation of the blood gases section of the SFE inhalation toxicity study of SFE.
- Initiation of the characterization of the cyclone sampling instrument.
- Completion of the operant inhalation toxicity exposures of test animals to Halon 1301.
- Initiation of the generation methodology and analytical calibration for the ozone depleting substance replacement HFC-227ea to be used in the neurobehavioral toxicology studies.
- Continued work on the preparation of laboratory 203 for the neurobehavioral mixed combustion gases inhalation toxicity project.
- Completion of FTIR application training class.

Smith, Zepp

- Initiate pulmonary physiology studies on with regard to aerosol effects on the lung.
- Begin writing new protocols in the area of cardiac sensitization.
- Begin developing assays for the neurobehavioral group to investigate subtle changes in the central nervous system.
- Complete protocol NAV292: Evaluation of Blood Gas, Blood pH, Hemoglobin, Bicarbonate and Glucose Levels after Exposure to the Pyrolyzed By-products of SFE Formulation A.
- Close data books for SFE range-finding/multiple-dose, edema nad blood gas studies.
- Poster: to be presented at the Society of Toxicology 35th Annual Meeting held in Anaheim, California, on March 10-14, 1996.

Prues

- There are other studies associated with the SFE Project that are to be performed to provide sufficient data with which to draw an informed conclusion. Continued technical support will be supplied to this project.
- The TMPP project will take off in a new direction; linking fast scan cyclic voltametry and microdialysis. Technical expertise will be supplied for the development of a microelectrode containing a carbon fiber which will allow for same site sampling collection.



Naryanan, T.K., Jung

- To increase the productivity in the lab.
- To continue the TMPP binding studies.
- To begin culturing mouse neuroblastoma cells and human cortical neurons for experiments with TMPP.
- To continue the work with the rat liver cells of the cell model project.
- To finish HPLC analysis of the neurotransmitter samples.
- Set up for the synthesis of DBNP.

Ritchie

Neurobehavioral Toxicity Assessment Battery (NTAB): Assessing Animal Responses to Pharmacological Challenge (WU.1605):

- To begin NTAB predictive validation procedures by comparison of effects of pharmacological challenges (three different drugs) on NTAB responses (five tests) in both rats and pigeons.
- To complete NTAB test development for juvenile rat play behavior, Porsolt F.S.T., Morris Water Maze, auditory startle, and classical conditioning (eye blink and paw withdrawal) paradigms.
- To re-write and submit the overall ACUC animal use protocol, in new DoD format, to allow three years of research under Navy Work Unit .1605, .1408, .1420.

Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU .1408):

- To begin re-engineering of the whole body inhalation exposure system in NMRI/TD Laboratories 202/203, to include construction of the Matheson 5-gas cabinet, chemical fume hood, high velocity evacuation system and the P-E System 2000 FTIR.
- To begin development and testing of the five fire gas delivery system.

Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU .1420):

- To complete neurobehavioral toxicity evaluation of Halon-1301 and HFC-227ea.
- To investigate possible neurobehavioral toxicity evaluation of the newly developed ODSR candidate Trioxide.
- To complete writing of one journal article related to neurobehavioral toxicity induced by brief exposure to CFC-12 versus HFC-134a.



TMPP Mechanisms Of Action: Development Of Neurobehavioral Molecularization Techniques (WU.1512):

- To begin a long-term study investigating the ability of various human CNA pharmaceutical drugs and anti-epileptic treatments to eliminate or alleviate TMPP-induced acute and long-term neurotoxicity.
- To assist in a large number of rat surgeries for FIA, microdialysis and multi-unit stimulation/recording studies related to TMPP acute an long-term neurotoxicity and CNA sensitization.
- To support development of tissue cultrue techniques for availability of human cortical neurons for singer cell analysis of TMPP-induced acute neurotoxicity.
- To complete writing of one journal article related to TMPP-induced neurotoxicity.

Persian Gulf War (PGW) Simulation Using Sprague-Dawley Rats (U.S. Army and NMRI/TD):

- To begin a 14-day pilot study, exposing three groups of naive rats to: (a) JP-4 vapors, DEET, pyridostigmine and stress or (b) JP-8 vapors, DEET, pyridostigmine and stress or; © diesel fuel vapors, DEET, pyridostigmine and stress to simulate environmental exposure conditions of U.S. military veterans during the Persian Gulf war.
- To oversee neurobehavioral evaluation of exposed rats on NTAB tests including: (a) grip strength analysis; (b) auditory startle response; © open field locomotion; and (d) CNS sensitization endpoints.

Ademujohn

- To accurately and efficiently compile, log organize and analyze all incoming data from inhalation studies.
- To accurately train rodents for various testing protocols.
- To accurately train pigeons for upcoming testing protocols.
- To maintain a clean and orderly laboratory environment.
- To provide technical assistance in modified Wahmann chamber studies.
- To provide technical support in testing relative toxicity of Halon 1301 in pigeons.
- To provide technical support to operant chamber analysis of rat exposures to low concentrations of Halon 1301, R-227ea.
- To provide technical support in streamlining operant training methods for upcoming pigeon and rodent training protocols/testing, i.e. in play behavior, swim testing and pharmacological studies.



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- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities, pre-testing and testing protocols.

Binole, Rix

In the next quarter ADP will continue to implement those software and hardware products which increase automation and productivity. Projects scheduled for the coming quarter include:

- Rollout SMS to all workstations
- Build SQL database of library holdings to improve access of this information over the internet.
- Continue to convert WFW machines to Windows 95
- Continue to add to and improve out network capabilities.
- Continue to provide technical support to TOXDET personnel.
- Develop support/scientific software as needed.
- Pending funding install additional CD-ROM capabilities.

Connolly

- Continue cataloging.
- Continue preparing cards for the manual card catalog.
- Continue training program.
- Continue providing document delivery services to customers.

Walsh

- Perform as Project Director for the toxicity of Vapor Phase Lubricants Project.
- Submit the Vapor Phaser animal use protocol to the Animal Care and Use Committee (ACUC).
- Provide technical support for a Cadmium dose response relationship study to support the Metallothionein Gene Expression Project.
- Continue as Contract Representative to the RMC.

Geiss

- Laboratory methods: Continue to develop molecular methods for using in our lab. Integrate new technologies into the battery of methods used to support Toxicology Division projects.



GEO-CENTERS, INC.

- Group Administrator: Continue to perform assigned tasks. Assist in the coordination of a chemical inventory and hazardous materials processing. Develop a Group mission statement and list of research objectives.
- Training: Continue to assist in training scientist in molecular methods.
- Project Support: continue to develop probes for use in hybridization experiments.

McDougal

- Continue pharmacokinetic studies in hairless guinea pigs, Hartley guinea pigs, and Fischer 344 rats with tridecafluoriodohexane.
- Continue development of mathematical models for whole animal experiments.
- Measure diffusion of MACS components across human skin.
- Estimate permeability constants for MACS components and provide information to customer.

Grabau

- Support Species Differences in Skin Penetration Project
- Quantitate epidermal and dermal end points following *in vitro* exposures
- Continue development of methods to quantitate human cutaneous endpoints in a manner similar to previous animal studies is under development.
- Support Combustion Toxicology of Advanced Materials (ACM) Project
- Continued support of phase II development and implementation.
- Support Ammonium Perchlorate Study
- A new morphologic endpoint, follicular epithelial height, will be evaluated
- Assist Tri-service Marketing/Program Development Team
- Continued revision of Tri-Service Toxicology Web page
- Implement the Program Development Strategic Plan

Narayanan, L.

Toxicity of Triaryl Phosphate Vapro Phase Lubricants:

- Measure and quantitate neurotransmitters and their major metabolites' levels in control and Triaryl Phosphate exposed rats using HPLC coupled with electrochemical detection.
- Measure and quantitate neurotoxic esterase and acetylcholine esterase enzyme levels in differencet regions of the rat brain in control and Triaryl Phosphate exposed rats.



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Toxicity Evaluation of Simulated Persian Gulf War (PGW) Exposure in Sprague-Dawley Rats, COET324:

- Measurement and quantitation of neurotransmitters and their major metabolites' levels in control and multiple chemicals exposed rats using HPLC coupled with electrochemical detection.
- Continue ongoing research projects by repeating some of the experiments and being involved in research projects that are of interest to Tri-Service.



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