

# UNITED STATES AIR FORCE RESEARCH LABORATORY

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## BIOLOGICAL EFFECTS OF DIRECTED ENERGY

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## TABLE OF CONTENTS

1.0 INTRODUCTION AND HISTORY .....	1
2.0 ACTIVE DENIAL TECHNOLOGY (ADT) RESEARCH .....	3
2.1 Use of Animal Models .....	4
2.1.1 Description of Work.....	4
2.1.2 Funding.....	4
2.1.3 Relevance .....	4
2.1.4 Products .....	5
2.2 Human Studies for ADT .....	8
2.2.1 Description of Work.....	8
2.2.3 Funding.....	9
2.2.4 Relevance.....	9
2.2.5 Products .....	9
2.3 Cancer Study (subcontract with Trinity University) .....	12
2.3.1 Description of Work.....	12
2.3.2 Funding .....	12
2.3.3 Relevance.....	12
2.3.4 Products.....	12
2.4 Dosimetry (subcontract with Trinity University) .....	13
2.4.1 Description of Work.....	13
2.4.2 Funding.....	13
2.4.3 Relevance .....	13
2.4.4 Products .....	13
3.0 NON-LETHAL WEAPONS RESEARCH.....	16
3.1 Acoustic Research.....	17
3.1.1 Description of Work.....	17
3.1.2 Funding.....	17
3.1.3 Relevance.....	17
3.1.4 Products .....	17
3.2 Blunt Impact Research .....	19
3.2.1 Description of Work.....	19
3.2.2 Funding.....	19
3.2.3 Relevance .....	19
3.2.3 Products .....	19
3.3 TASER Research.....	20
3.3.1 Description of Work.....	20
3.3.2 Funding.....	20

3.3.3	Relevance .....	20
3.3.4	Products .....	20
3.4	Radio Frequency Radiation NLW Research (other than ADT) .....	21
3.4.1	Description of Work.....	21
3.4.2	Funding .....	22
3.4.3	Relevance.....	22
3.4.4	Products .....	22
4.0	HUMAN EFFECTS CENTER OF EXCELLENCE (HECOE) .....	23
4.1.1	Description of Work.....	23
4.1.2	Funding.....	24
4.1.3	Relevance .....	24
4.1.4	Products .....	24
5.0	HEALTH AND SAFETY EFFECTS RESEARCH.....	25
5.1	Dosimetry and Modeling.....	25
5.1.1	Description of Work.....	25
5.1.2	Funding Sources.....	26
5.1.3	Relevance.....	26
5.1.4	Products .....	26
5.2	RFR Standards Support.....	35
5.2.1	Description of Work.....	35
5.2.2	Funding.....	36
5.2.3	Relevance .....	36
5.2.4	Products .....	36
5.3	Effects of Microwave Radiation Combined with Stress on the Integrity of the Blood Brain Barrier .....	37
5.3.1	Description of Work.....	37
5.3.2	Funding.....	37
5.3.3	Relevance.....	37
5.3.4	Products .....	37
5.4	HPM Electrophysiology Program [Ultrawideband (UWB) RFR Studies].....	39
5.4.1	Description of Work.....	39
5.4.2	Funding Source.....	40
5.4.3	Relevance .....	40
5.4.4	Products .....	40
5.5	Genetic Susceptibility of the Laboratory Rat ( <i>Rattus Novigicus</i> ) to a Model of Post-Traumatic Stress Disorder (PTSD).....	42
5.5.1	Description of Work.....	42
5.5.2	Funding .....	42
5.5.3	Relevance .....	42

5.5.4	Products .....	42
5.6	Radial-Arm Maze Performance Of Rats Following Repeated Low Level Microwave Radiation .....	43
5.6.1	Description Of Work .....	43
5.6.2	Funding.....	43
5.6.3	Relevance .....	43
5.6.4	Products .....	43
6.0	BIOTECHNOLOGY.....	44
6.1.	Biomarkers of Radio Frequency Radiation (RFR) Exposure.....	44
6.1.1	Description of Work.....	44
6.1.2	Funding source .....	46
6.1.3	Relevance .....	46
6.1.4	Products .....	47
6.2	Weapon Performance Measurement for Bio-Agent Defeat.....	49
6.2.1	Description of Work.....	49
6.2.2	Funding.....	49
6.2.3	Relevance .....	49
6.2.4	Products .....	49
6.3	Bio-Agent Simulant Evaluation and Development .....	50
6.3.1	Description of Work.....	50
6.3.2	Funding.....	50
6.3.3	Relevance .....	50
6.3.4	Products .....	50
6.4.	Bio-Agent Detection and Identification .....	51
6.4.1	Description of Work.....	51
6.4.2	Funding.....	51
6.4.3	Relevance .....	51
6.4.4	Products .....	51
6.5	Passive Radio Frequency Radiation Detection Methodology .....	53
6.5.1	Description of Work.....	53
6.5.2	Funding.....	53
6.5.3	Relevance .....	53
6.5.4	Products .....	53
6.6	The role of protein nitration in anthrax life cycle .....	55
6.6.1	Description of Work.....	55
6.6.2	Funding.....	55
6.6.3	Relevance .....	55
6.6.4	Products .....	55

## 1.0 INTRODUCTION AND HISTORY

Veridian Engineering, (originally Systems Research Laboratory), began work on contract F41624-96-C-9009, "Biological Effects of Directed Energy," in April 1997. The overall goal of this effort was to provide an experienced, qualified research team to work alongside government scientists to conduct biological effects, to include human effects, research of directed energy. To accomplish this, Veridian was to provide effective research program management, establish integrated research teams of contractor, subcontractor, and government scientists and technicians with experience in the field of directed energy biological effects, and perform meaningful, quality research in conformance with pertinent regulations. Veridian scientists provided scientific and technical support in five areas: Active Denial System (also known as Vehicle Mounted Active Denial System), radio frequency radiation (RFR) health and safety, non-lethal weapon biological effects research, the newly formed Joint Non-Lethal Weapons Human Effects Center of Excellence, and Biotechnology.

As needed, Veridian acquired scientific and technical staff, hired consultants, designed and built special fixtures and equipment to conduct both laboratory and field experiments in support of these five research areas. The assembled scientific staff represent an interdisciplinary research team with both knowledge of the literature on the biological effects of RFR and a working knowledge, including physiological and behavioral effects, long-term effects, and dosimetry. The scientific staff possessed the experience needed to perform physiological and behavioral effects research on a number of diverse non-lethal technologies, beginning with Active Denial Technology (ADT) and then extending to acoustics, kinetics, and TASERS. This subject matter expertise was valuable as the ADT program transitioned to the acquisition stage. Increased requests for evaluations of other non-lethal technologies were received from customers inside and outside the Department of Defense (DoD), as well as the consultation support required by the Human Effects Center of Excellence (HECOE) located at Brooks. Veridian responded to a number of special projects and needs of the customer; providing expertise in electrophysiology to investigate the response of excitable tissue to ultrawide band RFR; designing, modifying, and constructing unique test fixtures and equipment for lab and field experiments of non-lethal weapons; and working with operational units and warfighting labs to demonstrate the feasibility and utility of advanced technology concepts. Veridian also provided critical expertise to conduct research involving bioagent detection, protection, and neutralization, as well as RFR bio-dosimetry.

The Veridian/Air Force Research Laboratory (AFRL) team made a significant contribution to the non-lethal capabilities of the DoD by successfully demonstrating the robust utility of ADT to warfighters and senior level DoD officials in FY01. The Veridian team continued to perform thorough, relevant biological effects and safety-related research for the current active denial

concept, Vehicle Mounted Active Denial System (VMADS). In 1997, the Veridian/Radio Frequency Radiation Branch (HEDR) team planned and executed the first successful field demonstration for the concept which would become known as VMADS at Kirtland Air Force Base, NM. Veridian scientists designed protocols, equipment, and experimental setups for the field experiments and collected data during the field experiments to prove the utility of ADT to the warfighter.

As the need for, and the interest in, non-lethal technologies grew, Veridian was funded to plan and execute experiments investigating the effectiveness and risks of other non-lethal technologies, such as acoustics (to include infrasound), kinetic projectiles, and TASERS. In many instances, the bioeffects research programs were initiated to collect effectiveness data for systems already in development. Special sources, data collection equipment, and test fixtures were constructed, particularly for acoustics and kinetics; examples of these are the Infrasound Test System, a tunable infrasound chamber, and a compressed air-driven kinetic launcher. For the acoustic research, some sources could not be brought to the lab, so the research team had to design experiments to collect data in the field and then travel to remote locations to collect the data.

The report that follows is intended to summarize the research conducted under the five research areas. The format to be followed for each write-up is as follows:

- Research Area Overview
  - o Title of Work
  - o Description of work (the objectives)
  - o Funding
  - o Relevance
  - o Products (journal article, technical report, abstract, presentation, proceedings) Note that not all the publications have Veridian authors; these publications are listed to show all relevant publications in one place. Veridian (to include subcontractor and consultant) authors are in bold.



## **2.0 ACTIVE DENIAL TECHNOLOGY (ADT) RESEARCH**

Veridian Engineering scientists have been on the forefront of the research investigating the use of radio frequency radiation as a non-lethal technology since 1990. Several concepts emerged from the exploratory research efforts of the Veridian-Government research team in the early 90s. Veridian efforts over the past several years have concentrated on performing the required research to help prove the operational utility and public acceptability of the concept known for the Vehicle Mounted Active Denial System (VMADS). Numerous animal and human laboratory research efforts established effectiveness parameters with which an operational demonstrator could be designed and built. Other experiments determined damage thresholds to establish a safety index and carefully and thoroughly investigated the long-term effects of millimeter wave RF exposure. All of these effectiveness and safety data were collected to feed into the design of a demonstration system that could be demonstrated on human subjects at operationally relevant parameters. Veridian and AFRL/HEDR researchers teamed up with AFRL/DEH scientists/engineers to successfully demonstrate both the operational utility and technical feasibility of the VMADS concept to the Joint Non-Lethal Weapons Directorate, the AF Force Protection Battlelab, and other joint service customers through a series of field demonstrations and human experiments. Preparation for these remote demonstrations/experiments took three years; Veridian scientists participated in the design of the VMADS demonstration unit and in the design of the test site. Transition of the VMADS program out of the lab and into the acquisition stage will occur within the next year and may be on a fast track; ongoing lab and field experiments will need to continue through the transition. A substantial amount of critical research remains to be performed such as whole body frontal exposures, effects of environmental conditions on the effectiveness of VMADS, identification of countermeasures, development of optimum exposure parameters for system operation modes, and conduct of additional safety and long-term studies.

Special equipment and techniques were developed and/or modified to collect data required to establish dose response curves. Early in the contract period, millimeter waves (MMW) source power limited the research to investigate exposure levels that had operational utility; spot size was greatly restricted due to the use of a focusing lens to increase exposures to effective levels. Special restraint devices for animals and enclosures for humans had to be built to conduct the research. Due to space restrictions and exposure configurations, reflecting systems had to be built in labs. Dosimetry of such small spot sizes was challenging to both Veridian and government RF technicians; counsel was sought from AFRL/High-Power Microwave Division, Directed Energy Directorate (DEH) to develop an acceptable technique. Attempts were made to acquire other higher power MMW sources and get them operational but to no avail. Finally, the arrival of a higher power system from AFRL/DEH allowed experimenters to increase both spot size and power density to better answer operational utility questions. Infrared (IR) thermography was employed by

experimenters to develop temperature rise curves and to establish thresholds for effectiveness (ED50); however, data analysis was cumbersome until Veridian scientists developed software to automate the analysis of the IR camera. For field experiments, structures were designed to allow for controlled acquisition of data and to mitigate confounding environmental and procedural problems.

## **2.1 Use of Animal Models**

### **2.1.1 Description of Work**

Veridian personnel have participated in a variety of studies using a variety of animal models to understand the consequences of ADT exposures. We have supported primate studies by the Naval Medical Research Detachment including multifrequency effects in monkeys (D'Andrea et al., in progress), effects of repeated, low-level exposure to MMW on visual acuity in monkeys (D'Andrea et al., in progress), threshold for MMW-induced corneal lesions in monkeys (Chalfin et al. 1999, 2002; D'Andrea & Chalfin, 2002), facial detection thresholds for MMW in monkeys (D'Andrea et al., 1999), and eye aversion thresholds in monkeys (D'Andrea et al., in progress). We have conducted studies comparing skin-heating rates in several species (Walters et al., 1998a, 2000). We have studied brain heating and changes in exercise capacity associated with microwave exposure (Walters et al. 1997; 1998e). We have collaborated in a variety of work on brain markers resulting from MMW exposure in rats (Mason et al., 2000; Ryan et al. 1998a,b; Walters et al., 1996; 1998e; 1999; 2001), and in work on plasma catecholamines in response to MMW exposures (Ryan et al. 1999a,b). We have compared microwave-induced heating patterns in the brains of rats to predictions from computational models (Walters et al., 1998d; Gajšek et al, 2002), as well as to patterns induced by conventional heating and exercise (Walters et al., 1997; 1998b). We have also examined the induction of thermal tolerance by microwave exposure (Mason et al., 1998), and collaborated in studies of circulatory shock induced by MMW exposures (Ryan et al., 2000). Work that Veridian personnel initiated on thermal injury is continuing (Mason et al., in progress).

### **2.1.2 Funding**

These efforts were funded by the Joint Non-Lethal Weapons Directorate (JNLWD) (USMC), AFRL 7757 project funds, and limited Navy funding.

### **2.1.3 Relevance**

DOD Directive 3000.3 identifies three conditions that must be met to successfully field a non-lethal technology - technical feasibility, operational utility, and policy acceptability. To satisfy these conditions, the biological effects of ADT, both intended and unintended, must be determined

through lab and field experiments. Research investigating the effects of MMW RFR on the eyes and skin (i.e., damage thresholds, aversion levels, repeated low-level exposures, spot size or scaling effects, etc.) is critical to addressing both operational utility and policy acceptability.

#### **2.1.4 Products**

The work described above has resulted in the publications and presentations listed below.

Chalfin, S., D'Andrea, J. A., Comeau, P. D., Belt, M. E., and Hatcher, D.J. 35 GHz and 94 GHz microwave absorption in the primate eye. *Health Physics*, 2002, in press.

Chalfin, S., D'Andrea, J. A., Comeau, P. D., Belt, M. E., and Hatcher, D.J. 35 GHz and 94 GHz microwave absorption in the primate eye. *Investigative Ophthalmology and Visual Science* 40(4):S447, 1999.

D'Andrea, J. A. and Chalfin, S. Effects of microwave and millimeter wave radiation on the visual system. In: B.J. Klauenberg and D. Miklavic (Eds.), *Radio Frequency Radiation Dosimetry*, Dordrecht, The Netherlands: Kluwer Academic Publishers 2002, pp. 395 - 407.

D'Andrea, J.A. Microwave effects on the visual system. Invited review paper presented at XXVI<sup>th</sup> General Assembly, Of The International Union, Of Radio Science (URSI), University of Toronto, Toronto, Canada, August 13-21, 1999.

D'Andrea, J.A., Hatcher, D.J., **Walters, T.J.**, Ziriak J.M., Hurt, W.D., Kosub, K.R., and **Weathersby, F.** Facial detection of 94 GHz millimeter waves by the nonhuman primate. Presented at 21<sup>st</sup> Annual Meeting of the *Bioelectromagnetics Society*, Long Beach, CA June 20-24, 1999.

**Gajšek, P.**, D'Andrea, J.A., Mason, P.A., Ziriak, J.M., **Walters, T.J.**, and Hurt, W.D., Mathematical modeling of EMF absorption in biological systems. Chapter 3 in: *Biological Effects of Electromagnetic Fields*, Peter Stavroulakis, ed., IEEE Press, In press.

**Kalns, J.E., Ryan, K.L., Mason, P.A.**, and Kiel, J.L. Can exposure to thermal levels of millimeter waves (MMW) alter cell behavior. Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, WA, 2000.

**Mason, P.A.**, Blystone, R.V., **Kalns, J.E., Ryan, K.L., Walters, T.J.**, and Kiel, J.L. Biomarkers of radio frequency radiation exposure. Presented at

*Seventh Annual Michaelson Research Conference*, Gig Harbor, WA, 2000.

**Mason, P.A., Walters, T.J., Lehnert, H.M., Mahajan, K., Skitek, E.B., Scholin, T.L., Taylor, R.L., and Ryan, K.L.** Thermal tolerance induced by 2.06-GHz microwave heating. *Experimental Biology Abstract*, San Francisco, CA, 1998.

**Ryan, K.L., Kalns, J.E., Mason, P.A., and Kiel, J.L.** Protein nitration precedes circulatory shock induced by millimeter wave (MMW) exposure. Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, WA, 2000.

**Ryan, K.L., Mahajan, K., Lehnert, H.M., Tehrany, M.R., Jauchem, J.R., and Mason, P.A.** Brain c-fos induced by 35-GHz radio frequency radiation (RFR) exposure in ketamine- and pentobarbital-anesthetized rats. *20<sup>th</sup> Annual Meeting of the Bioelectromagnetics*, St. Petersburg Beach, Florida, 1998a.

**Ryan, K.L., Mahajan, K., Lehnert, H.M., Tehrany, M.R., Jauchem, J.R., and Mason, P.A.** Brain c-fos induced by 35-GHz microwave (MW) heating, environmental heating (EH), and cutaneous noxious thermal stimulation. *Experimental Biology Abstract*, San Francisco, CA, 1998b.

**Ryan, K.L., Taylor, R.L., Lehnert, H.M., Mahajan, K., Walters, T.J., and Mason, P.A.** Plasma and adrenal catecholamines during 35-GHz microwave (MH) heating. *Experimental Biology Abstract*, Washington, D.C., *FASEB J.*,13:A743, 1999a.

**Ryan, K.L., Taylor, R.L., Lehnert, H.M., Mahajan, K., and Mason, P.** Effects of 35-GHz microwave (MW) heating on catecholamine content in specific regions of the rat brain. *21<sup>st</sup> Annual Meeting of the Bioelectromagnetics Society*, Long Beach, CA, June, 1999b.

**Ryan, K.L., Walters, T.J., Paulus, L.A., and Mason, P.A.** Determination of regional brain heating using biological indicators. Presented at *Fifth Annual Michaelson Research Conference*, Montana, August 1998.

**Walters, T.J., Mason, P.A., Lehnert, H.M., Mahajan, K., Scholin, T.L., and Ryan, K.L.** HSP expression in the CNS may be dissociated from whole-body thermotolerance. *Experimental Biology Abstract*, Washington, D.C., *FASEB J.*,13:A743, 1999.

**Walters, T.J., Nelson, D.A., Blick, D.W., Johnson, L.R., and D'Andrea, J.A.** The rate of skin heating in response to 94 GHz mm-wave irradiation

in humans, rhesus monkeys, and rats. Presented at *20<sup>th</sup> Annual Meeting of the Bioelectromagnetics Society*, St. Petersburg, FL, June 7-11, 1998

**Walters, T.J.**, Nelson, D.A., **Ryan, K.L.**, J.D'Andrea, J., **Blick, D.W.**, Johnson, L.R., and **Mason, P.A.** Surface heating from millimeter wave irradiation: Modeling inter-species variations. Presented at *22nd Annual Bioelectromagnetics Society Meeting*, Munich, Germany, June 2000.

**Walters, T.J.**, **Ryan, K.L.**, and **Mason, P.A.** The influence of brain and core temperature on endurance performance. *Experimental Biology Abstract*, San Francisco, CA, 1998.

**Walters, T.J.**, **Ryan, K.L.**, and Mason, P.A. Regional CNS distribution of hsp70 in the CNS of young and old food-restricted rats following hyperthermia. *Brain Research Bulletin* 55:367-374, 2001.

**Walters, T.J.**, **Ryan, K.L.**, Scholin, T.L., and **Mason, P.A.** Neuronal damage following thermal stress. *Experimental Biology Abstract*, San Diego, CA, 2000.

**Walters, T.J.**, **Ryan, K.L.**, Tate, L.M., and **Mason, P.A.** Microwave-induced regional heating as a function of orientation: Relationship to FD-TD predictions. Presented at *Fifth Annual Michaelson Research Conference*, Montana, August 1998.

**Walters, T.J.**, **Ryan, K.L.**, Tehrany, M.R., Jones, M.B., Paulus, L.A., and **Mason, P.A.** HSP70 expression in the CNS in response to exercise and heat stress in rats. *J. Applied Physiol.* 84:1269-1277, 1998.

## **2.2 Human Studies for ADT**

### **2.2.1 Description of Work**

Building on previous animal work which demonstrated that MMWs did create an effect potentially useful as a non-lethal technology and which determined effective, yet safe, range of exposure parameters for humans, several human studies were conducted. The objective of these studies was to (1) measure sensory and behavioral effects of exposure to MMWs to show that the system is both effective and safe as a non-lethal antipersonnel weapon; (2) determine pain thresholds as a function of duration and area of stimulus, as well as environmental conditions (e.g., ambient temperature, initial skin temperature); and (3) determine pain intolerability level in the laboratory, as well as in the field with a prototype system.

In December 1997, a classified human use protocol (F-BR-1998-0003-H) was approved, allowing testing of pain threshold responses to MMW under a variety of conditions (different durations, areas, etc.). Previously, Dr. Dennis Blick (Veridian) had tested pain threshold for 3-sec duration under a protocol (AL-ACHE 95-25) by Dr. Clifford Sherry (Veridian) that had been approved earlier. Work began immediately on the classified protocol, but was suspended in March, 1998, under a presidential directive to revise the "Common Rule" as it affected federally sponsored classified research using human subjects. Dr. Blick then wrote and got approval of an omnibus health and safety protocol (F-BR-1998-0026-H) to measure pain thresholds at a number of different frequencies (including MMW and IR). This allowed continuation of the work that would have been done under the classified protocol. Under this protocol, testing of pain threshold and pain intolerability continued until March 2001 when the MMW transmitter failed. Considerable effort on the part of Dr. Zibignew Wojcik (Veridian) resulted in a program that provided automated processing of IR thermographic data, saving thousands of person-hours of data analysis. Data processing continued, in parallel with work on a new, classified protocol to permit testing with the prototype system at Kirtland AFB. Work on the new classified protocol began in November 1999. In May 2000, Dr. Blick met with the Institutional Review Board (IRB) at Wright-Patterson AFB; the protocol was approved, and the process of getting the approval of the Secretary of Defense (SECDEF) began. Since this was the first classified human use protocol to seek approval under the new Common Rule, procedures for staffing it up to SECDEF were not in place, and it failed to get the necessary approval within the required time (30 days after IRB approval). In hope that the DoD approval process was now established, Dr. Blick met with the IRB at Wright-Patterson AFB again in August, 2000. The classified protocol was again unanimously approved, and forwarded to the USAF Surgeon General's Research Oversight Committee (SGROC), which approved it immediately, and sent it on. In

spite of a memo from SECAF to Director, Defense Research and Engineering, Office of the Secretary of Defense (DDR&E) strongly recommending approval, the protocol again failed to receive timely SECDEF approval. Shortly thereafter, it was decided that declassification of parts of the program would allow the work to be done under an unclassified protocol. Dr. Blick prepared an amendment to the omnibus health/safety protocol which was approved by the AFRL IRB on 17 January 2001; it received final SGROC approval on 22 January 2001. Human subject testing at Kirtland AFB began 04 May 2001. Between 4 May 2001 and 27 September 2001 full body exposures for the repel effect were conducted. The data have been analyzed, and the results presented to the customer. A Technical Report summarizing the effects is in preparation. The IRB approved extension of the protocol for 1 year at its meeting in January 2002. A protocol "Effects of Skin and Environmental Conditions on Sensations Evoked by Millimeter Waves" (F-WR-2002-0016-H) was approved by the AFRL IRB at its meeting 21 February 2002; SGROC approval was received 27 February. Two more protocols: "Facial Sensitivity and Eye Aversion Response to Millimeter Waves" and "Effects of Ethanol on Millimeter-Wave-Induced Pain" have been submitted by Dr. Blick for approval. Work on these 3 protocols will be conducted under the follow-on contract.

### **2.2.3 Funding**

JNLWD (USMC) and AFRL 7757 project funds.

### **2.2.4 Relevance**

Initial laboratory results with humans helped prove initial operational utility and were fed into the prototype system design, as they indicated the power levels needed for effectiveness at range. Later field tests in humans were necessary to demonstrate both operational utility and policy acceptability.

### **2.2.5 Products**

Summaries of the data have been provided to the system developers for their use in system design and evaluation. Numerous graphs, charts, and pictures have been prepared for use in classified briefings, both by the investigators and the Government. Since nearly all of the results are either sensitive or classified, few open-literature papers or presentations have resulted. Data from our MMW studies have been used to develop an empirical model of thermal response of the skin. The resulting publications are:

Mason, P.A., Hurt, W.D., Ziriach, J.M., **Walters, T.J., Ryan, K.L., Nelson, D.A.**, Smith, K.I., Townsend, D.S., and D'Andrea, J.S. Models used to determine the bioeffects of directed energy exposure. *Proceedings of the NATO Research and Technology Agency Symposium on Countering the*

*Directed Energy Threat: Are Closed Cockpits the Ultimate Answer?* April, 1999, Antalya, Turkey, NATO-RTO-MP-30: Chapter 7.

**Nelson, M.T., Nelson, D.A., Walters, T.J., and Mason, P.A.** Thermal effects of millimeter wave irradiation of the primate head: Model results. *IEEE Transactions on Microwave Theory and Techniques* 48(11):2111-2120, 2000.

**Nelson, D.A., Walters, T.J., Mason, P.A., and Nelson, M.T.** Modeling thermal effects of millimeter wave exposure in primate head. Presented at *21<sup>st</sup> Annual Meeting of the Bioelectromagnetics Society*, Long Beach, CA, June 1999.

**Walters, T.J., Blick, D.W., Johnson, L.R., Adair, E.R., and Foster, K.R.** Heating and pain sensation produced in human skin by millimeter waves: Validation of a simple thermal model. *Health Physics* 78(3):259-267, 2000.

#### Other Papers and Presentations Related to Human Testing

**Adair, E.R., Blick, D.W., Walters, T.J., Mylacraine, K.S., and Johnson, L.R.** Laser doppler imaging of skin blood flow is a useful adjunctive tool for RF research. Presented at *21<sup>st</sup> Annual Meeting of the Bioelectromagnetics Society*, Long Beach, CA, June 20-24, 1999.

**Blick, D.W.** The sensory effects of millimeter waves in humans. Presented at *U.S.A.F. Special Session: Biological Effects of Millimeter Waves, 23d Annual Meeting of the Bioelectromagnetics Meeting*, St. Paul, Minnesota, June 10, 2001.

**Blick, D.W., Foster, K.R., Riu, P.J., Walters, T.J., and Adair, E.R.** Skin heating and sensations of warmth and pain produced by microwaves: data and thermal modeling. Presented at *20<sup>th</sup> Annual meeting of the Bioelectromagnetics Society*, St. Petersburg, FL, June 7-11, 1998

**Blick, D.W., Foster, K.R., Walters, T.J., and Adair, E.R.** Millimeter waves, sensation, and thermal models: Implications for safety standards. Presented at *Sixth Annual Michaelson Research Conference*, Cloudcroft, NM, August 16, 1999.

**Blick, D. W., Foster, K. R., Walters, T. J., and Adair, E. R.** Sensory and Thermal Effects of Microwaves in Human Skin: Implications of Thermal Models. Presented at *AFRL/Association of Old Crows Ninth National High-Power Microwave and Radio frequency Electromagnetics Symposium*, May 11-13, 1999, Sandia National Laboratories, Kirtland AFB, NM.



**Blick, D. W., Walters, T. J., and Johnson, L. R.** Effects of ambient temperature on the heating of human skin by millimeter-waves. Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, WA, August 11-15, 2000.

**Blick, D. W., Walters, T. J., and Johnson, L. R.** Millimeter-wave heating of human skin: effects of ambient temperature. Presented at *22d Annual Meeting of the Bioelectromagnetics Society Annual Meeting*, Munich, Germany, June 9-16, 2000.

**Mason, P.A. and Walters, T.J.** IR mapping for greater radiation safety. *NASA Tech Briefs* 23(4):1a-4a, 1999.

Murphy, M. R., **Blick, D. W.**, Mason, P. A., Merritt, J. H., and **Beason, C. W.** Recent research on the biological effects of millimeter waves. Presented at *European Bio Electromagnetics Association Annual Meeting*, Helsinki, Finland, September 6-8, 2001

**Walters, T. J., Ryan, K. L., Nelson, D. A., Blick, D. W., and Mason, P. A.** Effects of Cutaneous Bloodflow on 94 GHz Microwave Induced Skin Heating in Humans: Preliminary Studies. U.S. Air Force Special Session, "Dosimetry," Presented at *21st Annual Meeting of the Bioelectromagnetics Society*, Long Beach, CA, June 20-24, 1999.

**Walters, T. J., Ryan, K. L., Nelson, D. A., Mason, P. A., and Blick, D. W.** Critical avenues for heat transfer during 94-GHz irradiation in humans. Presented at *Sixth Annual Michaelson Research Conference*, Cloudcroft, NM, August 16, 1999.

## **2.3 Cancer Study (subcontract with Trinity University)**

### **2.3.1 Description of Work**

In collaboration with HEDR and Trinity University, Veridian personnel (Kavita Mahajan, Dr. Patrick Mason, Dr. Thomas Walters) and a Veridian Consultant (Dr. J. DiGiovanni) designed and conducted an exhaustive study to determine whether exposure to 94 GHz MMW could promote or co-promote carcinogenesis in a scientifically accepted animal model of skin cancer.

The SENCAR mouse model of skin carcinogenesis was chosen, based on the recommendation of our consultant, a leading expert in the study of skin cancer. Both single (1.0 W/cm<sup>2</sup>, 10 sec.) and repeated (0.33 W/cm<sup>2</sup>, 10 sec., 2 exposures per week, 12 weeks) exposures were tested for their ability to promote (in animals initiated with 7, 12-Dimethylbenz (a)-anthracene (DMBA) or co-promote (in animals initiated with DMBA and promoted with Tetradecanoylphorbol Acetate (TPA) the formation or growth of papillomas. Controls included similar skin heating with infrared, sham exposure, and a positive control (promotion with TPA) to demonstrate the sensitivity of the model.

### **2.3.2 Funding**

JNLWD (USMC)

### **2.3.3 Relevance**

There is no evidence in the literature to suggest that MMWs at high power can cause skin cancer. However, to address an important policy acceptability question sure to be asked, a well-thought out, scientifically sound experiment needed to be designed using accepted models and in consultation with outside experts. This study provides the best possible scientific evidence to support the safety of ADT.

### **2.3.4 Products**

Findings (i.e., exposure to 94 GHz MMW under the conditions tested does not promote or co-promote papilloma development in this accepted animal model of skin carcinogenesis) were published in the peer-reviewed literature.

Peer-reviewed publication:

**Mason, P.A., Walters, T.J., DiGiovanni, J., Beason, C.W., Jauchem, J.R., Dick, Jr., E.J., Mahajan, K, Dusch, S.J., Shields, B.A., Merritt, J.H., Murphy, M.R., and Ryan, K.L.** Lack of Effect of 94-GHz radio frequency radiation exposure in an animal model of skin carcinogenesis. *Carcinogenesis* 22:701-1708, 2001.

## 2.4 Dosimetry (subcontract with Trinity University)

### 2.4.1 Description of Work

Veridian personnel, in collaboration with Trinity University, San Antonio, Texas, conducted a number of studies aimed at understanding and modeling the effects of MMW on the skin and eyes. The development of the techniques using infrared thermography and data analysis (Mason and Walters, 1999) was critical to the success of these efforts, which included studies of the effects of ambient conditions (Blick et al., 2000) and skin blood flow (Walters et al., 1999) on MMW-induced skin heating. We also worked with computational models to predict heating effects in the eye (D'Andrea et al., 2000) and skin (Hurt and Mason, 1997; Mason et al., 1999). Some of our data were also used in thermal modeling efforts for human skin (e.g., Nelson et al., 1999; Nelson et al., 2000, Walters et al., 2000), and for comparing heating rates of human skin and skin of common laboratory animals (Walters et al., 2000). Finally, we contributed to a published review of health and safety issues related to MMW exposure (Ryan et al., 2000).

### 2.4.2 Funding

JNLWD (USMC) and AFRL 7757 project funds.

### 2.4.3 Relevance

Detailed understanding of the effects of MMW absorption in tissues is critical to the design and operation of ADT. Since not all exposure orientations can be tested in the lab or in the field, one must have a data-based model to predict energy absorption. The ability to predict the outcomes of various exposure scenarios will contribute greatly to future efforts in the Concept of Operations (CONOPS) area.

### 2.4.4 Products

The results of our studies were presented in the publications and presentations listed below.

**Blick, D. W., Walters, T. J.,** and Johnson, L. R. Millimeter-wave heating of human skin: effects of ambient temperature. Presented at *22d Annual Meeting of the Bioelectromagnetics Society*, Munich, Germany, June 9-16, 2000.

D'Andrea, J.A., Ziriak, J.M., Hurt, W.D., **Mason, P.A.,** and Chalfin, S. Modeling microwave absorption in the eye. *Proceedings of the Millennium Workshop on Biological Effects of Electromagnetic Fields*, Crete, Greece, 2000.

Hurt, W.D. and **Mason, P.A.** Dosimetry at millimeter wavelengths. *Proceedings of the Dosimetry of Lasers and Millimeter Waves Meeting*,

Technical Report AFRL-HE-BR-PC-1999-0002, Brooks AFB, TX, p. 79-82, 1997.

**Mason, P.A.,** Hurt, W.D., Ziriach, J.M., **Walters, T.J.,** Ryan, K.L, **Nelson, D.A.,** Smith, K.I., Townsend, D.S., and D'Andrea, J.A. Models used to determine the bioeffects of directed energy exposure. *Proceedings of the NATO Research and Technology Agency Symposium on Countering the Directed Energy Threat: Are Closed Cockpits the Ultimate Answer? April 1999, Antalya, Turkey, NATO-RTO-MP-30: Chapter 7.*

**Mason, P.A. and Walters, T.J.** IR mapping for greater radiation safety. *NASA Tech Briefs* 23(4):1a-4a, 1999.

**Nelson, D.A., Nelson, T.J., Walters, T.J., Mason, P.A.,** and Nelson, M.T. Modeling thermal effects of millimeter wave exposure in primate head. 21<sup>st</sup> *Annual Meeting of the Bioelectromagnetics Society, Long Beach, CA, June 1999.*

**Nelson, M.T., Nelson, D.A., Walters, T.J.,** and **Mason, P.A.** Thermal effects of millimeter wave irradiation of the primate head: Model results. *IEEE Transactions on Microwave Theory and Techniques* 48(11):2111-2120, 2000.

**Ryan, K.L., D'Andrea, J.A., Jauchem, J.R.,** and **Mason, P.A.** Radio frequency radiation of millimeter wave length: Potential occupational safety issues relating to surface heating. *Health Physics* 78(2):170-181, 2000.

**Walters, T. J., Blick, D. W., Johnson, L. R., Adair, E. R.,** and **Foster, K. R.** Heating and pain sensation produced in human skin by millimeter waves: Validation of a simple thermal model. *Health Physics* 78(3):259-267, 2000.

**Walters, T. J., Blick, D. W., Johnson, L. R.,** and Hurt, W. D. Specific absorption rates of human skin during 94 GHz mm wave irradiation. Presented at: *Fourth Annual Michaelson Research Conference, Canandaigua, NY, August 15-18, 1997.*

**Walters, T.J., Nelson, D.A., Ryan, K.L., D'Andrea, J., Blick, D.W., Johnson, L.R.,** and **Mason, P.A.** Surface heating from millimeter wave irradiation: Modeling inter-species variations. Presented at *22d Annual Meeting of the Bioelectromagnetics Society, Munich, Germany, June 9-16, 2000.*

**Walters, T. J., Ryan, K. L., Nelson, D. A., Blick, D. W.,** and **Mason, P. A.** Effects of Cutaneous Bloodflow on 94 GHz Microwave Induced Skin

Heating in Humans: Preliminary Studies. Presented at *U.S. Air Force Special Session, "Dosimetry," 21<sup>st</sup> Annual Meeting of the Bioelectromagnetics Society*, Long Beach, CA, June 20-24, 1999.

### 3.0 NON-LETHAL WEAPONS RESEARCH

Veridian's experience in testing the effectiveness of directed energy non-lethal technologies led to the testing other potential non-lethal technologies, such as acoustic, blunt impact, and TASERS. Effectiveness data for these technologies was sparse to non-existent. Since these technologies were already in the field or about to be fielded, the luxury of conducting a thorough research program like ADT was not afforded. Veridian researchers designed and conducted pilot studies to provide quick answers as to the effectiveness of these technologies. Veridian's involvement in acoustics began in 1996 when the U.S. Army Armament Research, Development, and Engineering Center, Picatinny Arsenal, NJ, funded research into the effectiveness of continuous wave and pulsed sonic devices. The acoustics work was extended into the infrasound region at the request of the National Institute of Justice; a special test device known as the Infrasound Test System was designed and built. The Veridian team investigated every acoustic device it could find to thoroughly cover the acoustic parameter space searching for an effect; numerous lab tests and field tests were conducted to collect data. A tunable resonance chamber had to be designed and constructed to perform experiments with high intensity infrasound. The result of all the testing was that no robust effect was shown and the development of acoustic Non-Lethal Weapons (NLWs) stopped by the Joint Non-Lethal Weapons Directorate (JNLWD). Veridian scientists submitted a proposal to the JNLWD for funding to conduct initial effectiveness testing on small, high velocity projectiles, beginning with the 0.32 caliber PVC balls used in several fielded blunt impact munitions. As with acoustics, research data to support effectiveness of the impact of the balls or the potential for injury did not exist. Experiments on the projectiles will continue into the next contract to study multiple impacts and other size and mass projectiles, supported by the JNLWD. As the interest from the NIJ, the DOD, and FAA in the incapacitating effects of TASERS grows, the existence of effects research is drawing interest. The NIJ funded Veridian through AFRL/HEDR to conduct experiments to determine the effectiveness of existing TASER devices. This effort is spanning the old and new contract so the report has not yet been written. Research with TASERS continues into the next contract with JNLWD funding to look at optimizing the output of the TASER devices. Building on the effects data from ADT, Veridian researchers developed and conducted a series of experiments to determine how well a hand-held MMW device could be aimed. A program was written to integrate aiming data to determine the spot size needed on target to produce an effect. Thus, effects data and human factors data collected in the lab will help the hardware developers design the man-portable system.

## 3.1 Acoustic Research

### 3.1.1 Description of Work

Beginning in 1996 (and ending in 2000), Veridian Engineering was asked to assess the biological impact of an array of acoustic weapon prototypes in a number of scenarios employing various bio-behavioral endpoints. Bio-behavioral endpoints examined were equivalent to a robust, immediate (or a fast acting) effect on the target. The possibility of a subtle effect of acoustic energy may have some military utility; however, the charge from the Government was to look at the acute incapacitating or behavioral disruption effects of acoustic energy absorption. Startle is a useful first exposure effect; that was acknowledged but not studied.

### 3.1.2 Funding

U.S. Army's Armament Research, Development, and Engineering Center (ARDEC), National Institute of Justice (NIJ).

### 3.1.3 Relevance

Both military and law enforcement agencies have a compelling interest in non-lethal weapons technology designed to disorient, incapacitate, confuse, or repel individuals or groups, without causing acute or long-term injury. Such weapons offer the potential of a more appropriate response in support of certain peacekeeping and tactical combat operations. A strong effects research base must underlie and support the operational requirements of the user and withstand the scrutiny of policymakers.

### 3.1.4 Products

The results of our studies were presented in the publications and presentations listed below

**Cook, M.C., Sherry, C.J., Brown, G.C.,** and Jauchem, J.R. Lack of effects on goal-directed behavior of high-intensity infrasound in a resonant reverberant chamber: U.S. Air Force Research Laboratory; Technical Report: AFRL-HE-BR-TR-2001-0154, Brooks Air Force Base, TX, Nov. 2001.

Murphy, M.R., Jauchem, J.R., Merritt J.H., **Sherry, C.J., Cook, M.C.,** and **Brown, G.C.** Acoustic bioeffects research for non-lethal applications. In: *Fraunhofer Institut Chemische Technologie, ed. New options facing the future. Proceedings of the First European Symposium on Non-Lethal Weapons; 2001 Sep 25-26, Ettlingen, Germany.* Karlsruhe, Germany: DWS Werbeagentur und Verlag GmGH; 2001:9.1-9.13.

**Sherry, C.J., Cook, M.C., Brown, G.C.,** Jauchem, J.R., Merritt, J.H., and Murphy, M.R. An assessment of the effects of four acoustic energy devices on animal behavior: U.S. Air Force Research Laboratory;

Technical Report: AFRL-HE-BR-TR-2000-0153, Brooks Air Force Base,  
TX, Oct. 2000.

**Sherry, C.J., Cook, M.C., Jauchem, J.R., Brown, G.C., and Edris, R.** The effects of infrasound on rhesus monkey performance of a continuous compensatory tracking task. *Aviation, Space and Environment Medicine* (In press)



## **3.2 Blunt Impact Research**

### **3.2.1 Description of Work**

Recognizing the need to establish a human effects database for blunt impact munitions, the JNLWD funded an initial study to determine the effective impact velocity required to change the behavior of a goal-directed target. The study was entitled "Bio-Behavioral Effects of Kinetic Munitions on Swine (*Sus scrofa*).” The swine were trained to bar-press for a food reward; the objective of the study was to determine at what impact velocity 50% of the swine stop pressing for food (EV50) for at least 15 seconds after being impacted by a single 0.32 caliber PVC ball weighing 0.4 gram. The location of all impacts was just behind the front shoulder. Each swine was impacted just once to minimize any habituation. These same balls are the primary payload of two NLWs – the Modular Crowd Control Munition (MCCM) and the 66mm Non-Lethal Grenade. A custom-built compressed air driven launcher had to be constructed and chamber pressures calibrated to launch velocities. Maximum launch velocities were limited to approximately 120% (~625 ft/sec) of the reported launch (muzzle) velocities of the MCCM (~520 ft/sec). The up-down method of Dixon and Massey was used to estimate the EV50 with a relatively small group of animals. Particularly challenging was the selection of an initial velocity and an incremental velocity step in the absence of relevant literature; a pilot study was conducted to establish the initial velocity and incremental step size.

### **3.2.2 Funding**

JNLWD (USMC).

### **3.2.3 Relevance**

Research data on the effectiveness of blunt impact munitions with which to assess operational utility is sparse. Blunt impact weapon systems are being developed and fielded without any measures of effectiveness. This initial study (and follow-on studies) will form the basis upon which to develop meaningful measures of effectiveness.

### **3.2.3 Products**

Single impacts did not produce the robust effects set as effectiveness criterion (i.e., stop behavior for 15 seconds or longer). Results and recommendations are documented in an unpublished report to the JNLWD ("Effects of the Impact of a Single .32 Caliber, Vinyl PVC Projectile at Various Velocities on Trained Behavior in an Animal Model," dated 17 July 2001); results of the study were briefed at the JNLW Director's Review and to the Human Effects Advisory Panel (HEAP).

### **3.3 TASER Research**

#### **3.3.1 Description of Work**

The objectives of this study were to compare the effectiveness of existing TASER-like devices side by side in a simulated operational employment using the same behavioral task and to collect physiological data to enhance our understanding of the mechanisms which contribute to effectiveness and unintended consequences. Two models of TASER devices from both TASER International and Tasertron are being tested on each of ten swine trained to bar-press for a food reward. This conduct of this study spanned the old and new contract; data collection and analysis is ongoing. Interest in the results of this study has drawn more attention with the announcement that the Federal Aviation Administration is considering use of TASERs onboard aircraft. The DOD (in particular the USMC) has also shown an active interest in using TASERs as a non-lethal technology. Hence, future work has been funded to continue studying the biophysical mechanisms and optimizing the output parameters for improved effectiveness and minimizing risks.

#### **3.3.2 Funding**

NIJ

#### **3.3.3 Relevance**

Like other NLWs such as acoustics and kinetics, there is little coordinated research which forms the basis for determining the effectiveness and risk of injury of TASERs. Unlike acoustics and kinetics, though, there is an experience database for operational use of TASERs to subdue uncooperative targets. TASER-like devices have been used effectively by local law enforcement for a number of years. Hence, they must have some usefulness in subduing individuals.

#### **3.3.4 Products**

Informal presentations to the HEDR staff have been made. A formal report, requested by the HECOIE, was prepared for the Human Effects Review Board (HERB) on the current understanding concerning effectiveness and risk of the use of TASERs. Preliminary results of the characterization of the outputs of each of the TASER devices being tested, using a standard test setup, were presented in the report. This report and a briefing to the HERB are archived in the HECOIE database.

### **3.4 Radio Frequency Radiation NLW Research**

#### **3.4.1 Description of Work**

Beginning in 2000, Veridian, working in concert with AFRL/HEDR, performed work that attempts to model one such potential non-lethal weapon. AFRL funded a collaborative effort to determine the design constraints for a handheld (or crew-served) RFR weapon with a defined spot diameter. Using effectiveness parameters from the ADT program, Veridian researchers investigated the effects of system shape and weight distribution on an operator's ability to hold the directed energy, speed-of-light device on the target delivering an effective energy exposure. The title of the study is "Aiming a Handheld Non-Lethal Weapon: Variables Affecting Hold Time on Target." The model employed is a modified, commercially available laser tag gun. Subjects were instructed to aim the gun at a target point 25 m away for a period of 1 minute under a variety of experimental conditions. Accuracy was monitored throughout the aiming session and the results digitized. Three phases have been completed to date, viz., varying weight, weight distributions of the gun and exercise on aiming accuracy. Conclusions reached thus far include: (a) mild aerobic exercise had a sustained negative impact on the ability of subjects to aim the weapon; (b) other factors such as weight of the weapon and randomly-presented loud noise had little or no impact; and (c) subjects' aerobic capacity (as indexed by estimated  $VO_2\text{max}$ ) was only minimally related to their general success in aiming the weapon.

A computer program (HEATER) was written to simulate the resultant skin heating from a moving spot of MMWs. The HEATER code uses the results of the human aiming study as input for spot movement, and predicts skin temperature change with time. Peak power and 3 dB spot size are also used as input parameters. Currently, crude empirical skin heating and cooling models are used. Veridian will work with AFRL/DEH to develop better physics based models, which will allow HEATER to better predict system the effectiveness of a human carried and pointed ADT-type NLW.

After better skin heating and cooling models are inserted into HEATER, a prediction of skin temperature will be made for a moving spot on human skin. A human study with an RF source and mirror system that mimics aiming movements used in the prediction will then be performed, with the results used to verify both skin temperatures achieved, and intolerable pain thresholds. HEATER can then be used to design a human carried and pointed ADT-type NLW.

Possible follow-on efforts include (a) modifying various characteristics of the weapon (e.g., adding additional weight, adding a stock, etc.); (b) imposing additional challenges on the subject during the aiming sessions

(e.g., wearing a weighted pack); (c) modifying the performance of human exposures in the laboratory with a Veridian-constructed, computer-controlled mirror system that mimics the average of all operators' aiming data. This will determine intensity and duration thresholds and verify the RFR effects model. The current RFR heating model used is empirical. The database used for this model is being expanded, which will allow use of the model across a greater range of system relevant parameters. This updated model will be compared with the physics based model being developed by Veridian and AFRL/DEH. These effects data will feed into the development of the hardware, source, and antenna.

### **3.4.2 Funding**

AFRL 7757 project funds

### **3.4.3 Relevance**

New directed energy non-lethal weapons are currently under development, including some that may eventually be handheld. For a directed energy weapon requiring energy on target for some duration to be effective, we must understand the effects of weapon design characteristics (e.g., weight) on both random and systematic errors in aiming.

### **3.4.4 Products**

**Brown, G.C., Scholl, D., Fines, D.A., Theis, C.F., Beason, C., and Cook, M.C.** Aiming a model of a hand-held, non-lethal weapon: Variables affecting time on target. Interim Report to AFRL/HEDR, February 2002.

**Brown, G.C., Scholl, D., Fines, D.A., Theis, C.F., Beason, C., and Cook, M.C.** Aiming a model of a hand-held, non-lethal weapon: Variables affecting time on target. In preparation for submission to Military Psychology.

## **4.0 HUMAN EFFECTS CENTER OF EXCELLENCE (HECOE)**

In 1999, the Joint Non-Lethal Weapons Directorate (JNLWD) funded AFRL/HEDR to establish the Joint Non-Lethal Weapons Program Human Effects Center of Excellence. In 2000, the HECOE was activated. The mission of the HECOE was to provide human effects support to the program managers of new and ongoing NLW programs and concepts. Veridian scientists were instrumental in activating the HECOE, providing subject matter expert consultation for a number of non-lethal technologies, and shaping the future direction of the HECOE.

### **4.1.1 Description of Work**

Initial efforts of the HECOE and Veridian scientists focused on supporting legacy NLW programs, many of them Army and many of them blunt impact NLWs. Veridian scientists performed human effects analyses on two blunt trauma non-lethal weapon (NLW) systems, a TASER system, and a multi-sensory device for the Human Effects Review Board. Veridian's experience in conducting effectiveness testing on directed energy, kinetic, acoustic, and TASER technologies have proven valuable to the understanding of the available literature and the capability of related technologies. Veridian took the lead in organizing the first ever workshop on risk characterization of NLWs with worldwide experts in the field of risk assessment. The workshop was successful and the proposed risk characterization framework was presented at the Society for Risk Assessment 2001 annual meeting in New Orleans, LA, in addition to being published in a peer-reviewed journal on risk assessment. Veridian scientists served as subject matter experts in acoustics, radio frequency radiation, kinetics, and NLW modeling and simulation for the risk assessment expert panel. A second risk characterization workshop was conducted on a blunt impact system, the 66mm non-lethal hand grenade, currently in acquisition. Inadequacies of the predictive tools were identified. Monte Carlo methods for determining the probability of effectiveness and risk of injury were developed, however, accuracy of these methods depends upon dose response predictive models. Programs to improve the predictive tools were established; these programs include the Non-Penetrating Projectile (NPP) program and the Interim Total Body Model (ITBM). Blunt impact and TASER research efforts, sponsored by the Joint Non-Lethal Weapons Directorate, were initiated to fill research data gaps, establish effectiveness and injury criterion, and determine physiological mechanisms. The experience of

Veridian personnel in acquisition and in research and development test and evaluation (RDT&E) has allowed the HECOIE to provide valuable inputs into the Master Test Plan for NLW systems in development.

#### **4.1.2 Funding**

JNLWD (USMC) and Marine Corps Systems Command (MARCORSYSCOM).

#### **4.1.3 Relevance**

The foundation for effective non-lethal weapons is the ability to predict both the probability of effectiveness and injury based upon relevant biological, psychophysical, and behavioral research data. JNLWD recognized the need for effects-based NLWs and established the HECOIE to ensure that human effectiveness was addressed in each NLW system being developed or NLW concept being considered.

#### **4.1.4 Products**

The following Human Effects Analysis reports were prepared by HECOIE/Veridian and HECOIE/Conceptual Mindworks (CMI) staff: Modular Crowd Control Munition (MCCM), 40mm Non-Lethal Crowd Dispersal Cartridge, 66mm Vehicle-Launched Non-Lethal Grenade, Taser Area Denial Device, Clear-a-Space Concept Exploration Program – an assessment of flashbang devices. Other reports were prepared for the HECOIE by the Toxicology Excellence in Risk Assessment (TERA), a non-profit corporation located in Cincinnati, OH – Proposed Non-Lethal Weapon Risk Characterization Framework, and by Battelle Labs – a Preliminary Human Effects Analysis of the MCCM.

**Brence, A., Dayton, T.E., Sherry, C. J., Kosnik, D., Huantes, D., and Klauenberg, B.J.** Use of flashbang devices to clear a space. Submitted to the Clear Spaces Concept Exploration Phase (CEP) program. April 25, 2002.

**Gonzalez, D.L., Dayton, T.E., Widder, J., Constable, R., and Klauenberg, B.J.** The 66mm non-lethal grenade. August 20, 2001.

**Gonzalez, D.L., Sherry, C.J., Dayton, T.E., Constable, R., and Klauenberg, B.J.** The TASER area denial device. October 11, 2001.

**Sherry, C.J., Klauenberg, B.J., Brown, G.C., and Dayton, T.E.,** The TASER based electrical non-lethal weapons. May 15, 2002.

**Simonds, J., Dayton, T.E., and Klauenberg, B.J.** The 58 ft-lb Fragmentation Hazard Criterion. April 26, 2002

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**Widder, J., Dayton, T.E., Gonzalez, D.L., Constable, R., and Klauenberg, B.J.** The non-lethal 40mm crowd dispersal cartridge. February 22, 2001.

## **5.0 HEALTH AND SAFETY EFFECTS RESEARCH**

Veridian executed exploratory research into the mechanisms of RFR interaction with tissue and potential for affecting physiological functions. Three such studies, in which Veridian scientists are involved, are underway presently – the blood-brain barrier (BBB) study, the RFR biomarker study, and the ultrawide band (UWB) RFR study.

The RFR environment demands an ongoing research program to provide scientific evidence for setting standards for RFR exposure. The UWB study investigated the potential for UWB energy to stimulate excitable tissue. These are the first electrophysiological experiments aimed at characterizing the coupling of UWB RFR energy to electrically excitable tissue and its resulting response.

The blood-brain barrier (BBB) maintains the brain environment, controlling entry of chemicals from the blood, and insulating the brain from rapid changes in the concentration of hormones, ions, peptides, etc. Recent work published in the literature suggest low level RF exposure disrupts the BBB. Veridian employees, working with the AFRL/HED, completed the first stage in an ambitious project aimed at replicating these important findings.

### **5.1 Dosimetry and Modeling**

#### **5.1.1 Description of Work**

Veridian scientists and subcontractors (Trinity University) used computer FDTD (Finite-Difference Time Domain) and computational models to predict where RF energy is deposited in the body, and the resulting thermal consequences. Three-dimensional models of common laboratory animals were developed, using magnetic resonance imaging and computer graphics software to assign voxels to particular tissue types. Similar graphics techniques were used to improve greatly the accuracy and resolution of an existing man model. These models were then used with FDTD codes to investigate patterns of RF deposition, and how the results of such FDTD modeling depend on various assignments of tissue

electrical properties. The output of such computer models was also compared to actual measurement of tissue heating.

### **5.1.2 Funding Sources**

AFRL 7757 project funding and JNLWD funding.

### **5.1.3 Relevance**

Determining where RF energy is deposited in the human body is critical to understanding many of the health and safety questions regarding exposed (or potentially exposed) personnel to directed energy NLWs and various DOD radars. Furthermore, the development of accurate computer models of the bodies of humans and various laboratory animals greatly facilitates the experimental work regarding Active Denial System undertaken by the Air Force Research Laboratory. Thermal models of the deposition and distribution in tissues of heat resulting from RF radiation are also helpful in guiding the collection of data important to USAF health and safety issues.

### **5.1.4 Products**

The animal and human models (as well as the Radio frequency Radiation Dosimetry Handbook and other AFRL/HED-sponsored publications) have been made available to researchers world-wide on a website developed by VE personnel (Mason et al., 2000b). Experimental results are described in detail in the publications listed below and attached. VE personnel and their data also contributed to extensive thermal modeling work. Publications and presentations are listed below.

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D'Andrea, J.A., Ziriak, J.M., Hatcher, D.J., Cox, D.D., Henry, P.J., Kosub, K.R., and **Mason, P.A.** Effects of head resonant radio frequency radiation on time estimation behavior of rhesus monkeys. Presented at *22<sup>nd</sup> Annual Bioelectromagnetics Society Meeting*, Munich, Germany, June 2000.

Dick, E.J. Jr., **Kalns, J.E.**, **Ryan, K.L.**, **Mason, P.A.**, Barshay, A., Brown, J.L., Scruggs, J., Gooden, R., and Kiel, J.L. Differential protein nitration in



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**Gajšek, P., Walters, T.J.,** Hurt, W.D., Ziriak, J.M., **Nelson, D.A.,** and **Mason, P.A.** Validation of FDTD predictions with empirical data. Presented at 23<sup>rd</sup> *Annual Meeting of the Bioelectromagnetics Society, St. Paul, Minnesota, June 2001.*

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## 5.2 RFR Standards Support

### 5.2.1 Description of Work

Veridian scientists have been instrumental in supporting the International Committee for Electromagnetic Safety's (ICES) Subcommittee 4 in generating a revision of the C95.1 Safety Standard: "Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300GHz." Several consultants were hired to review the extensive literature on RFR biological effects – Louis Heynick, Dr. John Osepchuk, and Dr. Robert Adair.

Veridian personnel have contributed to the work of ICES SC4 in two major areas. First, we have developed a database application to facilitate the scientific literature review on which the new standard will be based. Working with the leadership of SC4, Dr. Star Ferdinand wrote a set of database applications that will result in a permanent database encompassing the entire published literature on radio frequency radiation (RFR) bioeffects. The application consists of 12 different stand alone database programs: Literature Surveillance, Risk Assessment, Epidemiology Chair and Reviewer, Engineering Chair and Reviewer, In Vitro Chair and Reviewer, In Vivo Chair and Reviewer, and Statistics Chair and Reviewer. The Literature Surveillance program allows the surveillance chair to produce and update a database containing the bibliographic data on all papers published on RFR bioeffects. The surveillance chair distributes this database, with periodic updates, to each of the other chairs. The other chairs (Epidemiology [EPI], Engineering [ENG], In Vitro [VIT], and In Vivo [VIV]) each have a database consisting of subsets of the master database. The chairs recruit reviewers to assess the scientific quality and relevance to standard setting of papers in their areas. Each paper is sent (along with digital evaluation forms and the reviewers' database program) to 2 independent reviewers. Each paper sent out for review by the biology chairs (EPI, VIT, and VIV) is also sent out for review by the ENG chair, so that quality of each gets 2 kinds of evaluation. When reviewers send back the completed electronic forms to their chairs, the chairs add the evaluations to their databases. Finally they forward the contents of their databases to the Risk Assessment Working Group (RAWG) Chair, which is responsible for determining threshold values for scientifically established adverse health effects. The final database allows the RAWG to select papers that have been judged by reviewers to be of adequate technical quality, as well as having relevance for standard setting, while eliminating from consideration papers of inadequate technical quality.

The second contribution to the standard setting process is the participation of Veridian scientists. During the course of this contract, Dr. Blick, Dr. Brown, Dr. Sherry, and Dr. Mason have regularly attended meetings of

ICES and SC4. In addition, Dr. Blick assumed the position of In Vivo Working Group Chair in 1997. Since then, he has added approximately 900 reviews to the database. Dr. Blick is also a member of the Editorial Working Group, which meets twice a year to work on a draft of the new standard.

### **5.2.2 Funding**

AFRL 7757 project funds.

### **5.2.3 Relevance**

The Air Force is one of the world's largest users of high power electromagnetic devices. In order to maximize the safe use of such devices, while maintaining the health and safety of its personnel, the Air Force needs a science-based safety standard that is protective, without being excessively conservative. Thus, AFRL/HED encouraged its employees and contractors to contribute their expertise to the IEEE/ICES consensus-based standard setting process. Veridian personnel have held and are holding leadership roles in national and international standards setting organizations.

### **5.2.4 Products**

More than 90% of the relevant in vivo papers have been sent out for review, and, except for a few reviewers who have been slow to respond, most of these reviews have been completed and forwarded to the RAWG for final evaluation. Dozens of reviewers scattered around the world have successfully used the Veridian-developed database application to contribute to the literature review. Dr. Blick has attended ICES and SC4 meetings in Bologna, Italy; Munich, Germany; Luxembourg; Victoria, BC, Canada; St. Petersburg, FL; San Antonio; Long Beach, CA; and Minneapolis, MN. Dr. Blick has also attended meetings of the Editorial Working Group in Ft. Lauderdale, FL (twice), Phoenix, AZ, and Washington, DC (twice). The Editorial Working Group will present a draft of the new standard to the entire Subcommittee 4 in June, 2002, at its meeting in Quebec City, Quebec, Canada.

## **5.3 Effects of Microwave Radiation Combined with Stress on the Integrity of the Blood Brain Barrier**

### **5.3.1 Description of Work**

The blood-brain barrier (BBB) maintains the brain environment, controlling entry of chemicals from the blood, insulating the brain from rapid changes in the concentration of hormones, ions, peptides and other items. Its integrity can be altered by disease states (e.g., tumors), physiological insults, (e.g., hyperthermia). Veridian employees (George Lantrip, Kavita Majahan, Patrick Mason, Alexander Salazar, Clarence Theis) nearly completed the first stage in an ambitious project aimed at illuminating the 1992 findings of L. Salford, who reported low levels of RFR allow albumin to pass through the BB into the brain and A. Friedman, in 1996 who reported that physical stress allows pyridostigmine (PYR) to enter the brain. Experimental subjects in Phase I of this program experienced stress in the form of 30 min of restraint. This was followed by a subcutaneous injection of 0.177 mg/kg PYR (ED99 dose for 40% serum cholinesterase [ChE] inhibition) and a 30-min exposure to continuous wave 915 MHz RFR at 20 W/kg (or sham exposure). Phase I results failed to indicate any effect of restraint stress or RFR on BBB leakage relative to sham-exposed subjects.

The second phase will attempt to replicate the Salford experiments, examining the effects of continuous wave and modulated 915 MHz RFR on the integrity of the BBB. Post-exposure, subjects will be anesthetized; perfused intracardially, and the brains removed and assayed using an albumin immunohistochemistry assay. To determine the effectiveness of assays in revealing albumin leakage, the results from the immunohistochemistry assay will be compared to those from labeling albumin with Evan's Blue or sodium fluorescein.

### **5.3.2 Funding**

AFRL 7757 project funds.

### **5.3.3 Relevance**

In the early 90s, work by Salford postulated that exposure to very low levels of RFR affected the BBB, allowing albumin to enter the brain. In addition, laboratory results by A. Friedman in 1996. suggested that physical stress allows PYR, a nerve agent prophylactic, to enter the brain. Such findings, if true, could have an impact on the RFR safe exposure standards and for U.S. military personnel exposed to stress and/or RFR.

### **5.3.4 Products**

Miller, S.A., Murphy, M.R., Merritt, J. H., and **Mason, P.A.** Effects of microwave exposure combined with stress on the integrity of the blood-

brain barrier (BBB). *Society for Neuroscience Meeting*, New Orleans, LA, 2000.

Miller, S.A., Murphy, M.R., Merritt, J. H., and **Mason, P.A.** Blood Brain Barrier (BBB) integrity following exposure to 915 MHz microwaves and restraint stress. *Seventh Annual Michaelson Research Conference*, Gig Harbor, WA, 2000

## 5.4 HPM Electrophysiology Program [Ultrawideband (UWB) RFR Studies]

### 5.4.1 Description of Work

The general programmatic question is "Are there any hazards associated with exposure to ultrawideband (UWB) pulses?" The specific question for this project is can brain or muscle be affected by exposure to UWB pulses? Work began on this project in September 2000 and has continued into the new contract.

For the initial screening study, the classic frog gastrocnemius muscle preparation was selected. This experimentally robust preparation has been used since the time of Galvani and Volta, and it has provided much of the information on how electrically excitable tissues respond to electrical stimulation. Thus the dependent variable is well characterized, and the novelty is in the independent variable.

One classic way of describing the response of electrically excitable tissue to stimulation is the strength-duration (S-D) curve. As the duration of the pulse is decreased, the current (and voltage) required to elicit a response is increased. Plotting the S-D curve for muscle contraction involves determining a series of thresholds for elicitation of a minimal-amplitude contraction at multiple pulse durations, starting with long-duration stimuli of about 100 msec and going as short as the available pulse source can achieve. In the conventional biomedical literature, many S-D curves for muscle or nerve have been described down to 10 usec, and some published data exist at 1 usec. However, the immediate goal of this project is to complete the S-D curve down to 1 nsec, increasing the known domain by a factor of 1,000.

Between September 2000 and October 2001, an electrophysiology laboratory was designed and equipped; a Review Committee was established to set the approach to be used in the first year; and biological and engineering methods were implemented. Four different sources, (1) a conventional Grass stimulator, and pulsers produced by the (2) Avtech, (3) Bournlea and (4) Velonex companies were used; each had its own pulse duration and voltage characteristics. A computerized experimental control and data acquisition system was used, and HP function generators provided control over signal timing. The isotonic contractions of an isolated gastrocnemius muscle were measured by a transducer that converted muscle movement into an electrical signal that could be recorded and measured by an objective mathematical criterion; there were no judges. Over a 10-hour experimental day, more than 300 data points could be acquired.

The project completed the voltage S-D curve down to c. 4 nsec, producing a classically shaped plot. However, when pulse duration got shorter than

about 200 nsec, the signal showed severe ringing and the instrumentation approach could not allow measurement of current or voltage. In November 2001, the Review Committee found the work so promising that they recommended taking the additional time to improve the approach used for signal delivery and measurement and then to reacquire the S-D curve in terms of current, the physiologically most relevant parameter. By the end of February 2002 substantial progress had been made on these tasks. Ringing had been reduced considerably, and an approach allowing current measure had been implemented.

The initial current S-D curve acquired with the new arrangement showed a single UWB pulse of c. 1 nsec and c. 30 A could elicit a contraction. This observation provides an experimentally determined "figure of merit" that could be used to set a safety standard. The Review Committee posed several other questions relating to determining the general mechanism for the observed effect and for establishing that possible artifacts were not responsible for the observed responses. In the next year, the newly implemented methods will be refined, more extensive data will be acquired, and additional questions will be examined.

#### **5.4.2 Funding Source**

AFRL 7757 project funding.

#### **5.4.3 Relevance**

The USAF is developing sources using UWB pulses.

As part of this effort, safety standards must be developed for these new sources. In the late 21<sup>st</sup> century, key general questions about safety most frequently have included studies of possible effects on genes and cancer and on reproduction and development. In this case the USAF also is asking about the possibility of effects on electrically excitable tissues, which include muscles and nerves.

#### **5.4.4 Products**

To date, four extensive project reports and a major briefing have been completed, and a presentation of the initial work will be made in June 2002, at the 24<sup>th</sup> Annual Meeting of the Bioelectromagnetics Society.

**Rogers W.R., Merritt, J.H., Murphy, M.R., Barker, T., Kuhnel, C., and Johnson, L.H.** Extension of the single-pulse, contact-stimulation strength-duration curve down to 5 nanoseconds. To be presented at the 24<sup>th</sup> *Annual Meeting of the Bioelectromagnetics Society*. Quebec City, Quebec, Canada; June 2002.

**Rogers, W.R.** Testing for Effects of High-Peak, Short-Pulse-Width Electromagnetic Signals on Frog Muscle. Presentation to Review

Committee, November 15 and 16, 2001. Five hours, including lab tour;  
101 PowerPoint slides.

**Rogers, W.R.** Testing for Effects of High-Peak, Short-Pulse-Width  
Electromagnetic Signals on Frog Muscle. Internal USAF Report submitted  
November 11, 2001. 99 pp.

**Rogers, W.R.** Testing for Effects of High-Peak, Short-Pulse-Width  
Electromagnetic Signals on Frog Muscle. Internal USAF Report submitted  
August 3, 2001. 27 pp.

**Rogers, W.R.** Testing for Effects of High-Peak, Short-Pulse-Width  
Electromagnetic Signals on Frog Muscle. Internal USAF Report submitted  
June 26, 2001. 63 pp.

**Rogers, W.R.** Testing for Effects of High-Peak, Short-Pulse-Width  
Electromagnetic Signals on Frog Muscle. Internal USAF Report submitted  
February 15, 21, 2001. 25 pp.

## **5.5 Genetic Susceptibility of the Laboratory Rat (*Rattus Novigicus*) to a Model of Post-Traumatic Stress Disorder (PTSD)**

### **5.5.1 Description of Work**

Brain systems involved in the development of PTSD include those responsible for the response to stress, and also those involved in behavioral processes such as sensitization and fear conditioning. Little research has been directed at the mechanisms underlying the possible genetic susceptibility for this maladaptive stress response. This might be possible by examining stress responsivity in different strains of a given species. We hypothesized that the genetic susceptibility to PTSD lies in those brain systems and regions where the processes of behavioral plasticity (sensitization) and adaptation to stress converge. Beginning in 2000, Veridian and AFRL scientists worked with colleagues at the University of Texas Health Science Center at San Antonio (UTHSCSA) to test this hypothesis by examining genetically diverse strains of rats (Sprague Dawley, Lewis, Wistar, and Wistar Kyoto) in a fear-potentiated startle (FPS) paradigm. (The normal startle is a well-understood reflexive response to mildly noxious stimuli, such as a burst of white noise.) The FPS response involves classically conditioning fear to a previously non-feared stimulus (such as a light) by presenting it in conjunction with mild electric footshock. Later, when the light is presented without the shock, the normal startle response is amplified.

Data from Phases 1 and 2 have all been collected as of November 2001. Data from Phase 1 showed the appearance of FPS for some strains (e.g., Sprague Dawley), but not others (e.g., Wistar Kyoto). Data from Phase 2, in which we tested the effect of a prior stressor (cold stressor task) on FPS in these same rat strains, yielded inconclusive results.

### **5.5.2 Funding**

Veterans Administration

### **5.5.3 Relevance**

Post-traumatic stress disorder may occur in individuals who experience a traumatic event. Although the incidence of PTSD among the general population is quite high (between 1% and 12%), the disorder is particularly prevalent among veterans who have experienced war-related aggression. It is estimated that as many as 35% of Vietnam veterans have developed PTSD at some time during their lives. However, there may be a genetic component to the disorder, since not all individuals subjected to a given trauma go on to develop PTSD.

### **5.5.4 Products**

The results of Phase 1 were presented at the annual Society for Neurosciences meeting, November 10-15, 2001. They will also be



presented at the 3rd Forum of European Neuroscience, July 13-17, 2002, and at *11th Annual Meeting of the International Behavioral Neuroscience Society*, June 19-23, 2002.

## **5.6 Radial-Arm Maze Performance Of Rats Following Repeated Low Level Microwave Radiation**

### **5.6.1 Description Of Work**

This work sought to replicate previous studies that by H. Lai in 1987 who reported a working memory deficit in rats exposed to low-level, 2450-MHz microwave (MW) irradiation when subsequently tested in a 12-arm, radial-arm maze. Lai reported an attenuation of this MW-induced learning deficit in subjects pretreated with either physostigmine or naltrexone hydrochloride, but not with naloxone methiodide. The present replication utilized the same exposure system (circular polarized waveguides), whole body SAR (0.6 W/kg), pulse regimen, pretreatment drugs, exposure time, and maze configuration used by Lai. Lai employed error rate (viz., re-entry into already-visited maze arms) as their dependent measure; the present study analyzed both error rate and time to criterion. Veridian supplied technician support for this study.

The present study failed to replicate the data of Lai et al. Analyses of the error rate dependent measure showed neither a drug nor an exposure effect, but did reveal a significant effect of time (i.e., performance improvement over consecutive test days). Analyses of the time-to-criterion data showed (a) no effect of exposure; (b) slower acquisition in subjects pretreated with physostigmine or naltrexone hydrochloride (as compared to naltrexone methiodide or saline); and (c) an effect of time similar to that found with the error rate data.

### **5.6.2 Funding**

AFRL 7757 project funds

### **5.6.3 Relevance**

The positive results of Lai et al have implications for the adequacy of RFR safe exposure standards. Prior to considering changing exposure standards Lai's results need to be replicated by independent researchers.

### **5.6.4 Products**

N/A

## **6.0 BIOTECHNOLOGY**

This ongoing program used pharmacology, toxicology, and tissue culture expertise for ongoing, national defense-critical research involving bio-agent detection, protection, and neutralization, as well as RFR bio-dosimetry. Veridian scientists evaluated the relative effectiveness of conventional high explosive weapons and conceptual high temperature incendiary weapons against storage and production facilities containing biological agents. They developed the test procedures and the post-test assay to measure the extent of neutralization of the bio-agent simulant. The data provided will aid the Air Force in down-selecting which concept(s) to develop.

Veridian scientists are participated in a research program (in progress) to assess the nature of various simulants with regard to accuracy in representing a particular agent as well as cataloging general characteristics. A spin-off of this program was the development of a new vaccine strain of *Bacillus anthracis* (Alls/Gifford strain). We anticipate that this strain will result in more sensitive detection sensor technology and better vaccines to protect U.S. troops.

Veridian scientists were also involved in a program to improve sensor technology for bio-agent detection to develop a novel approach to detect and identify a wide variety of bio-agents.

Veridian scientists conducted research into the biomechanisms of the effects of microwave exposure. Researchers have been searching for measurable biomarkers for some time. The team Veridian made what seems to be a breakthrough in biomarker research. Veridian scientists are on the cutting edge of microwave biomarker research and have published several articles in peer-reviewed journals describing this phenomenon.

### **6.1. Biomarkers of Radio Frequency Radiation (RFR) Exposure**

#### **6.1.1 Description of Work**

This effort has two objectives. Objective 1 is to find relevant biomarkers of MMW exposure. It was previously observed that levels of nitrated proteins increase during MMW exposure. This suggests that nitrated proteins, their precursors, or metabolites might be relevant endogenous biomarkers to determine MMW exposure in DOD and civilian personnel. Investigation of biomarker compounds in blood was performed following MMW exposure. Objective 2 is to determine how biomarker expression from MMW exposure compares to that from other stressors (environmental and infrared heating and hemorrhage). In developing biomarkers it is essential to know how the levels of expression in response to MMW exposure compare to that produced by other stressors. This will indicate if the biomarker is unique to MMW exposure and can be used to specifically determine occurrence and extent of MMW exposure.

Rats exposed to MMW for tens of minutes experience body temperature increases, circulatory collapse, and death. Since the energy from 35-GHz exposure is absorbed within the first 0.9 mm of the skin, we hypothesized that substances may be released from the skin during prolonged MMW heating that lead to subsequent effects in the internal organs and contribute to death. Investigation by our laboratory showed that prior to vascular collapse, nitrated proteins are increased in both the liver and lung suggesting that substances released from the skin trigger pro-inflammatory events in distant organs. Thus, we focused on determining the feasibility of using nitrated proteins, or their precursors, and inflammatory molecules (cytokines) as potential biomarkers from plasma, liver, lung and skin.

The well-established model of MMW-induced vascular collapse in rats was employed. The results were contrasted to data from experiments using environmental or infrared heating or hemorrhage. Although we anticipated that some of the released substances might be common to all stressors, our goal was to identify those genes, proteins, and nitration pathways that are uniquely associated with MMW exposure. To that end, rats were exposed to sub-lethal levels of the stressors and euthanized at various times following treatment. Organs and blood were collected and assayed for levels of nitrated proteins, nitration precursors and cytokines.

One aspect that was clear from initial results was that the search for mediators produced during exposures could be quite time consuming because most assays are very specific. An alternative strategy that was used is a global screening approach in which large numbers of genes and proteins are surveyed using state-of-the-science genomic and proteomic assays. These results were then used to design more focused, in depth studies of specific targets.

In addition to the above *in vivo* experiments, an *in vitro* MMW exposure system was also developed in order to facilitate the understanding of specific cellular responses evoked by MMW exposure. This includes theoretical and empirical dosimetry that is essential in order to minimize the perturbations produced by the culture flasks on the homogeneous MMW field and ensure consistent sampling.

Our original hypothesis was that nitration of proteins may contribute to early death during MMW heating. Under this hypothesis, nitration should increase steadily during heating reaching peak levels at the time of death. This pattern was observed in peripheral leukocytes, but not in other organs including the liver, lung and gut. In fact, the pattern of nitration is tissue specific and transient suggesting that nitration may serve an altogether different function that is unrelated to pathology. These data

indicate that nitrated proteins are not merely the consequence of uncontrolled metabolism or inflammation, but rather that nitration may play a role in protecting organs from injury. This hypothesis will be further tested in subsequent investigations.

We also hypothesized that exposures would cause a systemic up-regulation of inflammatory signaling molecules (e.g., cytokines – TNF  $\alpha$ , IL-1, IL-6) leading to injury in distant organs (e.g., lung, liver). Therefore, the levels of several cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$  and soluble phospholipase A-2 (sPLA2 - a rate-limiting enzyme of arachidonic acid pathway) in plasma were measured following exposure to environmental heat and MMW. The only significant change in these cytokines was an increase in the concentration of IL-1 $\beta$  following environmental heating. Thus, the pro-inflammatory cytokines we tested are not appropriate plasma biomarkers for MMW.

Initial data has been collected for the genomic and proteomic surveys. Genomics data from lung tissue revealed changes in expression for 28 genes after MMW treatment. For infrared treatment, expression of 12 genes changed, only three of which overlapped with MMW-induced genes. This indicates somewhat different kinetics or pathways are activated by the two different forms of heating. Most of the changes in gene expression support the hypothesis of a thermal induced immune response for MMW and IR exposures. This is indicated by the up-regulation of the pro-inflammatory mediator IL-1 $\beta$ , as well as other genes involved in inflammation and tissue remodeling. These data demonstrate the utility of this method for studying bioeffects and help lead the way to identifying potential candidate biomarkers for further study.

In proteomics analysis of plasma proteins, as many as 10 proteins were produced in response to prolonged MMW exposure. The data point to an acute phase-like response at 24 and 72 hour post-exposure and demonstrate the promise that proteomics may have for the identification of substances that are uniquely associated with MMW exposure.

### **6.1.2 Funding source**

AFOSR

### **6.1.3 Relevance**

Communication, military radar, weapon detection, and non-lethal weapon technologies are being developed that make use of the millimeter wave (MMW) range (3 - 300 GHz) of the electromagnetic spectrum. Some of these emerging technologies will require increasingly higher power outputs. There is increased possibility of prolonged overexposures, especially of the maintenance technicians or operators, as more of these systems are fielded. The proposed research will: 1) investigate the

biological effects of such exposures and 2) determine which endogenous substances (e.g., proteins, low molecular weight by-products, lipids) produced in response to MMW overexposure could be used as clinical biomarkers. These studies will enable assessment of possible health effects of MMW overexposure.

#### **6.1.4 Products**

Bruno, J. G., Parker, J., **Kalns, J.E.**, Darcy, T.J., Grubbs, T.R., Cox, A.B., Morales, P.J., **Alls, J.L.**, and Kiel, J.L. Characterization of NR10(2) macrophages. *In Vitro Cell Dev. Biol. Anim.* 34:613-616, 1998.

**Kalns, J.E.**, Parker, J.E, Bruno, J., Holwitt, E., Piepmeier, and Kiel, J.H. Nitrate reductase alters 3-nitrotyrosine accumulation and cell cycle progression in LPS + IFN-gamma-stimulated RAW 264.7 cells. *Nitric Oxide* 2: 366-374. 1998.

**Kalns, J.E., Ryan, K.L., Mason, P.A.**, Kiel, J.L. Can exposure to thermal levels of millimeter wave (MMW) alter cell behavior? Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, Washington, August 2000.

**Kalns, J.E., Millenbaugh, N.J.**, Blystone, R.V., Eggers, J., Lawrence, W., Soza, L., Kiel, J.L., Mason, P.A. Comparison of changes in colonic and skin temperatures during prolonged exposure to millimeter waves, environmental heat, or infrared heat lamps. . *24th Annual Meeting of the Bioelectromagnetics Society*, Quebec City, Quebec, Canada, 23-27 June 2002.

**Kalns, J.E.**, Mason, P.A., **Millenbaugh, N.J.**, Blystone, R.V., and Soza, L., Lawrence, W., Kiel, J.L., Eggers, J., Ryan, K.L. The effect of plasma from heated rats on macrophage activity in vitro. In preparation.

**Kalns, J.E.**, Mason, P.A., Soza, L., Lawrence, W., Scruggs, J., Kiel, J.L., Blystone, R.V., and Ryan, K.L. Environmental heat induced 3-nitrotyrosine increase in lung and liver. In preparation.

**Kalns, J.E., Millenbaugh, N.J.**, Blystone, R.V., Eggers, J., Lawrence, W., Soza, L., Kiel, J.L., Ryan, K.L., Mason, P.A., and Witzmann, F. Proteomics analysis of plasma reveals that prolonged 35-GHz exposure causes upregulation of acute phase proteins. *24<sup>th</sup> Annual Meeting of Bioelectromagnetics Society*, Quebec City, Quebec, Canada, 23-27 June 2002.

**Kalns, J.E., Ryan, K.L., Mason, P.A., Bruno, J.G., Gooden, R., and Kiel, J.L.** Oxidative stress precedes circulatory failure induced by 35-GHz microwave heating. *Shock*, 13:52-59, 2000.

**Kalns, J.E., Ryan, K.L., Soza, L., Lawrence, W., and Kiel, J.L.** Early peak in 3-nitrotyrosine (3-NT) accumulation does not require cytokine stimulus. *FASEB Journal* 15(5):A83, 2001.

**Kiel, J.L., Mason, P.A., Alls, J.L., P. Morales, and Seaman, R.L.** A mechanism of single-pulse radio frequency radiation interaction with biological molecules. *20<sup>th</sup> Annual Meeting of the Bioelectromagnetics Society*, St. Petersburg Beach, Florida, 1998.

**Mason, P.A., Blystone, R.V., Kalns, J.E., Ryan, K.L., Walters, T.J., and Kiel, J.L.** Biomarkers of radio frequency radiation exposure. Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, Washington, August 2000.

**Millenbaugh, N.J., Kalns, J.E., Blystone, R.V., Mason, P.A., Eggers, J., Frazier, J., Ryan, K.L., Lawrence, W., Soza, L., and Kiel, J.L.** Use of gene array technology to survey gene expression changes in tissues of rats exposed to 35-GHz or infrared heating. *24th Annual Meeting of the Bioelectromagnetics Society*, Quebec City, Quebec, Canada, 23-27 June 2002.

**Millenbaugh, N.J., Mason, P.A., Hurt, W., Kuhnel, C., Johnson, L.H., Kalns, J.E., Blystone, R.V., and Kiel, J.L.** Dosimetry for *in vitro* cell cultures during 35-GHz exposure. *24th Annual Meeting of the Bioelectromagnetics Society*, Quebec City, Quebec, Canada, 23-27 June 2002.

**Ryan, K.L., Kalns, J.E., Mason, P.A., and Kiel, J.L.** Protein nitration precedes circulatory shock induced by millimeter wave exposure. Presented at *Seventh Annual Michaelson Research Conference*, Gig Harbor, Washington, August 2000.

Ryan, K. L., Mason, P.A., Soza, L., Lawrence, W., Scruggs, J., Kiel, J., Blystone, R.V., and **Kalns, J.E.** Blood loss causes nitric oxide production but not 3-nitrotyrosine accumulation in tissue. In preparation

Ryan, K.L., **Kalns, J.E.**, Scruggs, J., Kiel, J.L. Nitric oxide (NO) synthesis inhibition does not inhibit nitration induced by environmental heating. *FASEB Journal* 15(5):A93, 2001.

## **6.2 Weapon Performance Measurement for Bio-Agent Defeat**

### **6.2.1 Description of Work**

This program (in progress) is a classified project evaluating the relative effectiveness of conventional high explosive weapons and conceptual high temperature incendiary weapons in neutralizing storage and production facilities containing biological agents. John Alls, Veridian, manages the AFRL/HEDB team conducting post-test assays of bio-agent simulants. Three weapon concepts have had initial testing using two different target configurations. The data provided will determine which concept(s) will be developed. Follow on studies will investigate additional neutralization concepts/techniques against additional target configurations.

### **6.2.2 Funding**

AFRL Munitions Directorate, Eglin AFB, FL

### **6.2.3 Relevance**

These are important, timely field tests that will help develop techniques to maximally neutralize an adversary's biological agent production capability. Weapon concepts are being evaluated as significant data points are accumulated. Effective concepts will be pursued further.

### **6.2.4 Products**

Classified reports have been developed for the customer. Data collection and analysis fed into AFRL/MN classified reports

## **6.3 Bio-Agent Simulant Evaluation and Development**

### **6.3.1 Description of Work**

This program (in progress) assesses the nature of various simulants with regard to accuracy in representing a particular agent as well as cataloging general characteristics. A spin-off of this program was the development of a new vaccine strain of *Bacillus anthracis* (AIs/Gifford strain). A database of simulants used, characteristics, vulnerabilities, and resemblance to actual agents is being compiled and updated. This database includes thermal sensitivity of *Bacillus anthracis* (Sterne strain), *Bacillus thuringiensis*, and *Bacillus globigii*. Additional simulants may be evaluated as well as relating thermal sensitivity to calorimetry data.

### **6.3.2 Funding**

Army/ ECBC

### **6.3.3 Relevance**

Developing simulants of biological agents that possess the same properties of the agents except for their virility is crucial to improving our ability to detect, protect against, and neutralize live agents.

### **6.3.4 Products**

Holwitt, E.A., Kiel, J.L., **AIs, J.L.**, Morales, P.J., and Gifford, H.D. Thermal Sensitivity of Biowarfare Simulants. *Chemical and Biological Sensing*, P.J. Gardner, Ed., *Proceedings of SPIE* 4036:31-39, 2000.

McCreary, R.P., Kiel, J.L., **AIs, J.L.**, Parker, J.E., and Gifford, H.D. Anticipating Technological Surprise In Biowarfare And Bioterrorism. Presented at *MASINT Biological Warfare Science And Technology Symposium*, 12-15 Feb 2001, Ft. Walton Beach, Fl.

Kiel, J.L., Gifford, H.D., **AIs, J.L.**, *Bacillus Anthracis* A/G (AIs/Gifford) As A New Vaccine Strain. U.S. patent pending.



## 6.4. Bio-Agent Detection and Identification

### 6.4.1 Description of Work

This program (in progress) is developing a novel approach to detect and identify a wide variety of bio-agents, and has the ability to amplify its sensitivity. It will also be capable of determining viability of agents. Specific aptomers have been produced for anthrax. They are currently being sequenced. Prototype flow cells are being tested. Aptomers will be developed for additional agents.

### 6.4.2 Funding

AFRL/MN

### 6.4.3 Relevance

The US military and federal/local agencies have a long-standing requirement to rapidly detect and identify a wide variety of bio-agents. The threat of chemical/biological agents to US troops in Iraq and in Afghanistan and to citizens in the US have underscored the urgency of developing and fielding such a technology. Current methods, although accurate and sensitive, require a dedicated lab and a lengthy assay time (24-48 hours). Development of a methodology that fast and mobile, yet just as accurate and sensitive, allows for quicker isolation and decontamination of infected areas and for more timely treatment of infected individuals. This technology has direct applications to Homeland Defense, Force Protection, and Weapon Neutralization (Neutralization of Weapons of Mass Destruction) efforts of the DoD.

### 6.4.4 Products

Kiel, J.L., Parker, J.E., **Alls, J.L.**, **Kalns, J.E.**, Holwitt, E.A., Stribling, L.J.V., Morales, P.J., and Bruno, J.G. Rapid Recovery and Identification Of Anthrax From The Environment. *Tropical Veterinary Disease*, J. House, K.M. Kocan, and P. Gibbs, Eds., *Annals Of The New York Academy Of Sciences* 916: Sept. 2000.

Kiel, J.L., Parker, J.E., Grubbs, T.R., and **Alls, J.L.** Growth Medium For Rapid Isolation And Identification Of Anthrax. *Chemical and Biological Sensing*, P. J. Gardner, Ed., *Proceedings of SPIE* 4036:92-102, 2000.

Kiel J.L., Parker, J.E., Holwitt, E.A., Sloan, M., Mason, P.A., **Alls, J.L.**, and Gifford, H.D. Biological Agent Detector. Presented at *Joint Aerospace Weapon Systems Support Symposium, Sensors & Simulation Working Group*, 26-30 June 2000, San Antonio, TX.

Kiel, J.L., Parker, J.E., **Alls, J.L.**, Holwitt, E.A., and Morales, P.J. Rapid Recovery And Identification Of Anthrax Bacteria From The Environment.

Presented at 5<sup>th</sup> *Biennial Conference Of The Society For Tropical Veterinary Medicine*, 12-16 June 1999, Key West, Fl.

Kiel, J.L., Parker, J.E., Holwitt, E.A., Sloan, M., Mason, P.A., **Ails, J.L.**, and Gifford, H.D. Pulsed Electromagnetic Radiation Biological Agent Detector. Presented at *Joint Aerospace Weapon Systems Support Symposium, Sensors & Simulation Working Group*, 26-30 June 2000, San Antonio, TX.

Kiel, J.L., Bruno, J.G., Parker, J.E., **Ails, J.L.**, Holwitt, E.A., and Batishko, C.R. The Artificial Immune System. U.S. patent pending.

Parker, J.E., **Ails, J.L.**, and Kiel, J.L. Diazodenitrification In The Manufacture Of Recombinant Bacterial Biosensors. U.S. patent 5,902,728 issued 11 May 99.

## 6.5 Passive Radio Frequency Radiation Detection Methodology

### 6.5.1 Description of Work

This program (in progress) has achieved proof of concept in the area of total exposure measurement as well as real time measurement using several frequencies of radio frequency radiation. Detection has occurred at transmission powers of 5 watts or less. Characterization and development of this quantitative photo-detection methodology is currently being pursued.

### 6.5.2 Funding

AFOSR

### 6.5.3 Relevance

This effort is aimed at developing an integrating RFR dose dosimeter for use in the field allowing health physicists to track exposures in work areas and radar sites.

### 6.5.4 Products

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