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Effects of high power pulsed and continuous wave microwave fields and electromagnetic pulse (EMP) fields were studied using various physiological and behavioral paradigms. Engineering progress included modifications to improve the EMP simulator and TEMPO exposure system, and developments of a tri-axial scanner for densitometry and dosimetry methods, including calorimetry, thermometry and the finite difference time domain method. Behavioral studies included effects on operant behavior, memory retention, circadian rhythmicity of the food consumption, time perception, motor activity and microwave evoked whole-body movements. Cardiovascular effects, cutaneous temperature changes, neural damages and ocular injuries were also studied. Except the absence of a demonstrable retinal injury, all these biological endpoints could be altered by the electromagnetic fields in the radiofrequency range. Details and accomplishments of research are presented in this report.

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INTRODUCTION

The following report summarizes the performance of OGDEN BioServices Corporation's (BSC) staff at Walter Reed Army Institute of Research at Department of Microwave Research (DMR) under the contract DAMD17-89-C-9021.

OGDEN's understanding of technical issues and insight into scientific principles has been the keystone of success for Department of Microwave's research program. Due to our efforts in planning and managing the research program, the research productivity of the Department has risen dramatically. In the past year alone, dozens of scientific papers have been published or submitted for publication. Moreover, in this period of time our research program has achieved international stature: our staff participates regularly and frequently in international conferences as session chairs and speakers.

We are determined to continue to provide high-quality scientific research and to represent U.S. Army's Microwave Bioeffects program under the guidance of U.S. Army Medical Research, Development, Acquisition and Logistics Command in its new location at Brooks Air Force Base in San Antonio, Texas.

Modifications to Improve the EMP (Electromagnetic Pulse) Simulator

The simulation of even a smallest nuclear detonation is almost impossible without using actual nuclear devices. The nuclear blast generates intense heat, enormous amount of radioactive fallout, and tremendous strength of electromagnetic pulse (EMP). The heat and radioactive fallout effects on human are well known to public; however, the biological effects on the EMP are least known subject. The known effects of EMP are mostly on electronic hardware. And for this reason, the majority study on EMP has been conducted from many different government agencies and private industries throughout world on the hardware field. The biological effect on the EMP has become a public concern recently and the experiments in the this field are becoming more active

lately.

The characteristic of the nuclear EMP is very complicated to reproduce inside laboratories. The outdoor EMP simulators in some laboratories are very bulky in size and extremely expensive to maintain and operate. The DMR is using a small, bench-size, simple and inexpensive system to maintain and operate for EMP simulator research. The EMP simulator system consists of a parallel plate transmission line which acts as an antenna (Fig. 1) and an EMP waveform generator. The parallel plate antenna has dimension of 118 X 20 X 40 cm (L x H x W) with 45 X 10 X 40 cm working volume which has fairly uniform electromagnetic field. The working volume is large enough to expose small organisms such as rats to study the biological effects. The EMP simulator waveform generator can produce 7 nanosecond risetime EM pulses with 100 kilovolts/meter peak electric (E) field strength which is comparable to a big outdoor EMP simulator. However, the EMP simulator waveform generator uses standard 110 V outlet for its power source; therefore, the maintenance and operation cost are insignificant compare to outdoor EMP simulator.

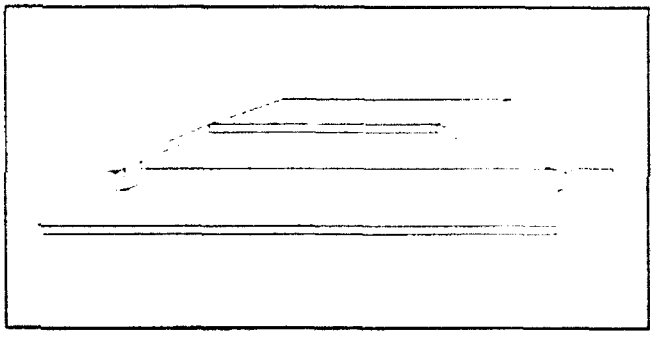

Figure 1. Parallel Plate Antenna.

The EMP simulator was delivered to WRAIR 1988, since then OGDEN engineering staff has modified it to improve its performances. The original EMP simulator delivered to WRAIR had a 10 ns risetime. However, the nuclear EMP in a real blast has less than 10 ns risetime. The OGDEN engineering staff made many alterations to eliminate the risetime problem and drastically shorten to 7 ns. This modification allowed to researchers to get more comparable EMP experiments. A special type of animal holder was developed for this simulator. It has a plastic base with a metal plate attached to it. A series of current-sampling resistors between the metal base and the bottom plate of parallel plate antenna allowed the investigators to gather more data around the experimental subject. The DMR purchased new measuring

instruments to fully utilize and characterize the EMP simulator, and these new instrument gave more precise data measurement. Moreover, OGDEN engineering staff modified the EMP simulator and the data acquisition system to be operational by only one person which in its turn saves many valuable man-hours and frees our staff to attend to other responsibilities.

The Tri-Axial Scanner

The tri-axial volume scanner was uniquely designed and developed by OGDEN technical staff with the guidance of the COR and the government scientists for WRAIR Department of Microwave Research. The tri-axial scanner was designed to assist researchers in studying the electromagnetic field patterns in an exposure site. Using the data acquired with the scanner, a contour map of field pattern can be developed. Such a map is a powerful tool used by researchers to best optimize the unique exposure system under consideration for each experiment. This field map enables the researcher to visualize the exposure field patterns.

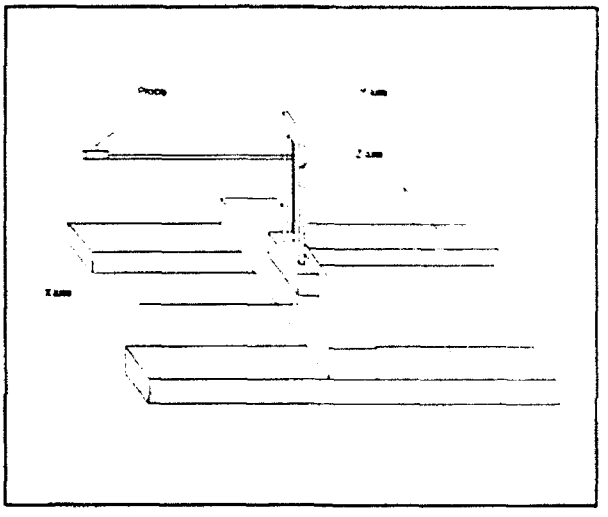

Figure 2. Scanner Diagram

The scanner accurately moves the probe and simultaneously takes field density, position and microwave source power measurements in real time. This fully automatic tri-axial scanning system is much faster, more accurate and more efficient than the manual mapping method. In performing this task, the scanner utilizes its three main subsystems, the three axial positioning arms (moving mechanism), the hardware controller box and the computer controller/data acquisition system.

The positioning arms are driven by three stepper motors, one for each axial direction. The utilization of these three axial arms enables the operator to perform linear, planar and spatial scans (three dimensional). The movements of each axial arm are independent and accurate to within 1 mm over a range of 90 cm. The two horizontal axes are worm gear driven on steel rails. The vertical axis is driven by a non-metallic wire in a wooden frame to minimize RF reflections. Each axis has a 1 meter long scale mounted along the arm for visual verification of the probe position. The scanning range for the X-axis and the Y-axis movements is -45cm to +45cm. The scanning range for the Z-axis is -30cm to +45cm. These ranges are enforced by mechanical interlocks to prevent stepper motor damage. The probe is attached to the wooden slider using the wooden probe mount with velcro fasteners. The probe can also be mounted on a pole if necessary in order to provide sufficient distance from the moving mechanism and work successfully within the geometrical confines and requirements of the exposure site.

Incident and reflected power data are communicated to the controller box via two BNC input cables. The power density probe data are collected via a third BNC input cable. The computer is interfaced with the controller box through a ribbon cable. The controller box is housed in the top half of a standard 19" wide, half height equipment rack. The motor power supplies are located in the bottom half of the equipment rack.

The requirements for the controller computer are a 286 or more advanced processor, EGA or VGA graphics card, 5 MB of free hard disk space and at least one floppy drive to store and transfer the acquired data. The computer interfaces the controller box through a computer interface card.

OGDEN technical staff is committed to continue to develop and modify this simple, cost-effective yet very reliable system to meet principal investigators requirements.

Modifications to Improve TEMPO Exposure System

The Transformer Energized Megavolt Output (TEMPO) transmitter was developed for the WRAIR Department of Microwave Research by the Sandia National Laboratory. This transmitter was designed to provide scientists and researchers with a means of studying biological effects and potential health hazards associated with exposure to high-power pulsed microwaves. The unique exposure parameters which TEMPO provides the research community is an extremely high-peak-power pulse which is comprised of a very intense electrical field. The manner in which the system operates allows for this high power level to be delivered to the test subject without the thermal effects associated with a high powered Continuous-Wave (CW) source. This provides a means for the researchers to obtain an exposure environment which will allow the examination of effects the electrical fields have on a subject and concurrently minimize the thermal effects associated with other high-power transmitters.

Since the delivery and installation of TEMPO by Sandia National Labs, the OGDEN engineering staff has been constantly improving and enhancing the transmitter system. The objectives of the improvements were: to increase the safety of the personnel operating and using the transmitter, to minimize the possibilities of machine failures, and to increase the operating performance of the transmitter. The performance modifications can be further categorized in the following areas: stability and reliability, higher peak power, higher energy levels, reduction in maintenance and the minimization of the time required for repairs to be completed once the system has been rendered inoperable. The following are improvements that the OGDEN engineering staff has accomplished since the delivery of TEMPO, demonstrating their expertise in operating, maintaining and modifying this highly complicated transmitter system in the interest of the DMR program.

Personnel Safety of the TEMPO Exposure System

Although most design modifications are driven to obtain a singular result, they often inherently influence other aspects, categories or reasons for consideration. The modifications outlined below were undertaken strictly in the interest of personnel welfare.

As a result of high voltage operation, TEMPO generates X- ray radiation as a byproduct. This phenomenon is a common characteristic of Virtual Cathode Resonators (VIRCATORS). The levels of X-rays, which personnel and test subjects are exposed to, have been attenuated to a safe level as determined by WRAIR Health Physics Department. This has been accomplished by installing an interlocking lead shield around the VIRCATOR. Recognizing that X-ray radiation is difficult to detect, and the threat of exposure continuously exists, the OGDEN staff has acquired additional services of Seimens Gammasonics Inc, Health Physics Services, to monitor the levels of radiation. Seimens continuously monitors the levels of radiation in and around the TEMPO transmitter in addition to monitoring personnel and recording their exposure history. In an effort to minimize the risk of exposure and as a result of the relocation of the DMR to Brooks AFB, the engineering staff has been able to remove the secondary operator from the transmitter room. The secondary operator and scientists utilizing TEMPO now can occupy a shielded room which is located adjacent to the transmitter room. Therefore, the only personnel in the transmitter room during operation is the primary operator. The primary operator has also been relocated into a Faraday screen room, located within the transmitter room, which protects the operator from spurious EMI fields produced by TEMPO.

The OGDEN staff has also been successful in containing yet another type of radiation which is inherent during high voltage operation of TEMPO, Ultra Violet (UV) light. UV light is generated during the operation of the high voltage switches. There are four such switches in use during the firing of TEMPO, three of which are visually monitored continuously by the primary operator. To filter the UV light which is

produced, the Faraday cage which houses the three switches, has been enclosed in polycarbonate sheeting, which in turn has been chemically treated to retard the UV light. In an effort to totally obscure the UV light, the OGDEN staff is investigating the properties of the chemical treatment which, barring the incompatibility of the chemical as a dielectric, will be used in the future on the switch housings when they are manufactured. This process will eliminate this potentially hazardous operating condition. It is also being recommended that the DMR invest in safety glasses which have been chemically treated to attenuate the UV light, as from time to time the primary switches described above are operated without the polycarbonate shield in place (ie. troubleshooting, routine maintenance, etc..) The fourth switch mentioned is not visible to the operator, as it is mounted inside of the TEMPO water tank.

As stated earlier, the secondary operator has been moved to a remote location in an effort to minimize the risk of injury to staff members as well as the researchers. This posed many inconveniences and safety concerns in itself, as the primary operator has now been severed from required information critical to the safe operation of the transmitter. To alleviate this problem, communication systems have been installed between the transmitter room and the diagnostics room where the secondary operator is stationed. Video cameras and monitors have been installed in both positions which allow the operators to view all pertinent data from either position. Likewise, voice activated intercoms have also been utilized.

The primary responsibility of the secondary operator is to perform as a safety person for the primary operator. To provide a means of performing these duties, a parallel inhibit line has been installed in the secondary position. This prevents the transmitter from firing until both operators have cleared the inhibit line. Beyond this, in the event of an emergency the secondary operator (in addition to the primary operator) also has control of the emergency shut-down line which has been designed to completely disconnect the transmitter from its supply voltage. Prior to this modification, the secondary operator was required to leave their position and cross the room to activate the shut-down procedure (in the event that the primary operator was incapacitated). Relocating the secondary operator out of view of the primary operator necessitates the

need for door interlocks on the shield room, screen room and chamber so that the doors must be closed prior to the transmitter entering the READY condition. In the event any of the three doors are opened, the transmitter will automatically return and be locked into the STANDBY condition until all doors are again closed.

The data acquisition system has become an instrumental safety tool in determining and characterizing the operating conditions of TEMPO. Initially the data acquisition system recorded data from sensors and monitors providing a means of investigating the conditions and circumstances which led to a failure. It also stored the RF data for an exposure series, providing the scientists with the parameters and conditions to which the subjects were exposed. The OBC engineers and technicians, through numerous revisions, compiled a system and computer program which allows the operators to observe the operating conditions and performance of the transmitter live. This enables the operators to recognize, through experience, potentially hazardous conditions immediately, vice post failure. In providing this service, the operators are able to terminate the operation of the transmitter prior to failure which often results in damaged equipment or hazardous conditions for operators. The "live-time" diagnostics also allow the operators to adjust operating parameters of the transmitter to optimum conditions during an exposure series. This in turn results in the recovery of a potentially unsatisfactory exposure for a given protocol.

Maintenance Modifications of the TEMPO Exposure System

The following modifications were driven in an effort to minimize maintenance, increases intervals between routine maintenance and to decrease the "off-line" time due to repairs. Through these alterations, the OGDEN staff has been able to save time on maintenance and repairs which in turn provides more time for research and development.

The high voltage (HV), generated by TEMPO, begins in an enclosed power

supply which utilizes transformer oil (UNIVOLT-N61) to serve as a dielectric insulator. Routine maintenance mandates the removal of this oil (350 gals). This has been a necessary procedure which evacuates carbon from the oil which in turn will build-up on the HV components and conductors in the power supply. This practice is both economically and environmentally disastrous. OGDEN, therefore, designed a recycling system which filters the oil, removing the carbon, returning the oil to its original state. Until the recent relocation of the DMR to Brooks AFB, the oil had not been changed in 4 years, surpassing the previous duration of use by 2 times. Furthermore, the oil did not need to be replaced at this time, but at the request of the Air Force the oil was disposed of in Maryland, prior to the transportation of TEMPO to Brooks AFB. The oil recycling equipment has proven to be a beneficial investment, as it is used annually on the COBER transmitter in addition to semiannually on TEMPO.

The mechanism which begins TEMPO's high voltage pulse operation is the ignition of the primary switches. Two primary switches are each charged with 20 kilovolts and discharged in a fraction of a second. The switches are pressurized statically with 40 psi of air. During the ignition of the switch, the air pressure increases in a burst, at this time the switch is in its most volatile state. It is during this time that most failures occur, the explosion of the switch being the most severe. Ogden has taken numerous steps to protect operators from this hazardous situation. After the first evidence of this condition, the Faraday cage which houses the switches was completely encased in polycarbonate. This protects all personnel from projectiles in the event of such a failure. Secondly, different material is being used in the manufacturing of new switches which were structurally reengineered. The switches are now mounted on aluminum standoffs which increase the distance from the metal surfaces to which the switches have arced in the past. Since this modification, the switches have operated continuously (with the exception of routine maintenance) without failure, increasing the previous longevity of the switch by more than ten-fold. The engineering staff is continuing to modify the switch housings in an attempt to eliminate the UV light mentioned earlier in this section. The staff is also investigating the possibility of replacing the aluminum HV components with stainless steel duplicates, as aluminum has been observed oxidizing. This oxidation results in poor conduction between

components, providing a potential HV failure if not continuously maintained. The stainless steel which does not oxidize, will reduce the maintenance effort required to ensure continuous operation of TEMPO.

The HV pulse, generated by the primary switches, travels through a transformer which raises the voltage to 1.5 million volts (1.5 Megavolt). This pulse is delivered to the resonant charge line. The charge line is housed within a 3' x 4' x 10' water tank. The treated water functions as a dielectric insulator. It is in this section of the transmitter that, due to the presence of extremely high voltages, many profound improvements were required. Just as the primary switches initiate the HV pulse, the output switch initiates the 1.5 MV pulse once the line has been charged. The output switch is the heart of the MV pulse section, and like the primary switch, the most delicate. The output switch was intentionally designed to be mounted in line with the charging line. This allows the horseshoe shaped charging line to discharge through the switch uniformly. This configuration of the switch proved to be inadequate, as it forced the HV line to pass close to the ground planes in the water tank. This in turn stressed the dielectric characteristics of the water in the immediate vicinity of the switch, causing an arc, and subsequently leading to the failure of the transmitter. This type of failure required a minimum of three days to repair, as the recovery time of the dielectric water required 3 days. This condition resulted in many modifications of TEMPO in an attempt to minimize the frequency of failures and decrease the "off-line" period after such a failure. The most radical and consequently the most advantageous modification was to mount the output switch perpendicular to the base of the charge line. This provided a means of relocating the HV line to the center of the water tank consequently maximizing the distance between the HV line to ground. By decreasing the stress placed on the insulating properties of the water, the transmitter is capable of operating for longer durations and at faster rates. These additional operating parameters have been extended into the scientific community utilizing TEMPO, permitting extended exposures and condensing the required scheduling times of the transmitter.

Further investigations indicated that improved operation of the switch could be attained. The air lines which pressurize the switch, were removed from the water tank

eliminating the possibility of HV arcing along the surface of the air tube. Accomplishing this change required the manufacturing of a new electrode assembly in addition to modifications of the trigger blade located inside the switch. Individual airways were machined into the trigger blade to accommodate the necessary air flow patterns required for correct operation of the switch. According to fluid dynamic modeling, this new configuration provided an increase in air flow as well as a more efficient exhaust of contaminated air. As demonstrated in the diagrams the air captured behind the trigger blade in the original design could not be evacuated entirely between bursts of HV current. The contaminated air remaining in the switch provides a potential conduction path for the HV, which often lead to a premature ignition of the switch or an arc across the electrodes.

Typically, as recorded in the operation logs for TEMPO, the life expectancy of the output switch was designed for approximately 300 shots. Upon reaching the 300 shot point, a failure of the switch was guaranteed before reaching 500 shots. This mandated an overhaul of the switch in the routine maintenance program once the 300 shot mark was obtained. The overhaul of the switch required a minimum of 3 days as outlined above. The primary reason responsible for failure of the switch, at this point, was the dulling of the trigger blade. The output switch design relies on the biasing level of the trigger blade for given potentials of HV. The biasing level is determined by the static air pressure applied to the switch by the primary operator. The air pressure maintained for optimal operation of the transmitter is that of a threshold just below the level at which the output switch is kept from "ringing over". Ringover is a function of the HV on the output switch arcing to the grounded electrode. The operation of the switch in this condition impedes the HV pulse from coupling onto the centerline of the charging line, hence, producing no RF. Proper operation of the switch requires the air pressure to be set just below the level of ringover. This allows the switch to self-bias causing an initial arc to develop between the trigger blade and the grounded electrode. This arc produces UV light which ionizes the internal chamber of the switch. The ionized air essentially functions as a conductor for the HV, allowing the switch to conduct current from the HV electrode to the grounded electrode. This symbolizes a closure condition of the switch which extinguishes once the HV is bled from the charging line. The conduction of the

HV in this manor provides the mechanism for a HV pulse to be coupled onto the centerline, which is delivered to the VIRCATOR section of the transmitter. The mechanism which allows the trigger blade to arc to the grounded electrode, is the sharp edge maintained on the inner surface of the blade. The original trigger blade was machined out of brass. It is assumed that brass was used due to its machinability characteristics. Unfortunately this same attribute proved to cause the switch blade to loose its edge prematurely. Now that the switch is mounted to the side of the water tank, the switch housing is able to be opened from outside the tank and the trigger blade sharpened without disturbing the resistivity of the water. Beyond this modification, the OGDEN engineering staff has manufactured a trigger blade fabricated of stainless steel. The stainless steel trigger blade has proven to be operated in excess of 20,000 shots without the need of maintenance, due to its ability to hold an edge. Since stainless steel is difficult and time consuming to machine, the OGDEN staff is currently designing an insert for the trigger blade. The insert will provide interchangeable blade edges to the trigger blade, which will be able to be installed without dismantling the output switch, minimizing the "off-line" duration of TEMPO.

The recovery time of the treated water in the tank was still an issue. Failures continued to occur when the transmitter was operated for an extended period of time (more than 200 shots) without sufficient time for water rejuvenation. As the research demands on TEMPO increased, it was made clear that this problem needed to be addressed. The OGDEN staff has been able to reduce the period of recovery from 3 days to 1.5 hours. In accomplishing this, the resistivity of the water has also risen from 6 Megohms (6,000,000) to 16 Megohms. This in turn allows the transmitter to operate indefinitely without a failure due to the resistivity of the water.

The VIRCATOR component of TEMPO is housed within an extremely high vacuum chamber. The vacuum functions much like the treated water, preventing the HV from arcing to the walls of the vacuum chamber. The centerline continues into this section as the cathode shaft. The HV is applied to the cathode, creating a great potential difference to the anode, which is at ground potential. The space between the cathode and anode becomes charged, forming a plasma cloud. The plasma cloud

functions as a conductor,(much like the UV light in the switches). The HV on the centerline travels to the cathode in search of ground potential. As the electrons leave the cathode, towards the anode, only 10 percent of the electrons intersect with the anode, the remaining electrons pass into the resonator. In search of ground, these electrons reverse direction and travel toward the anode. This action is repetitive until all electrons intersect with the anode. This repetitive action is the mechanism which produces the Radio Frequency (RF) at 3 GHz.

The original anode was fabricated with bronze screen which would burn through after 30 shots, requiring the VIRCATOR to be rebuilt. This maintenance required 6 hours to complete. Two major steps have been taken to minimize the time required for this procedure and to increase the interval at which it needed to be performed. First, the lead bricks which encased the VIRCATOR were replaced with large interlocking blocks, decreasing the removal and installation of the lead from 5.5 hours to 15 minutes, thus decreasing the overhaul of the anode to 45 minutes. Secondly, the material used for the anode has been replaced with a stainless steel alloy, which permits TEMPO to be operated for durations of up to 3000 shots without anode fatigue. These modifications have resulted in an extended life expectancy of the anode by a factor of 100. They have also reduced the scheduled maintenance time required for an overhaul of the anode by 730%.

Performance Modifications of the TEMPO Exposure System

Performance modifications are addressed with the intent of enhancing the operating parameters of the transmitter. TEMPO has been designed as a prototype high pulse transmitter of which three have been manufactured. The transmitters have been distributed to the following organizations: Armstrong Labs at Brooks Air Force Base, Harry Diamond Labs and WRAIR. The OGDEN engineering staff has led the development of TEMPO through an evolution of change, which has clearly established the WRAIR TEMPO as the most reliable and productive machine of the three.

The primary concentration of research and development which accounts for the elevation of the output power of the VIRCATOR has been the cathode configuration. These developments have yielded an increase in output power of the VIRCATOR by more than four-fold. This research has also lengthened the duration of operation without the previously required recovery cycle of the cathode.

The operation of the VIRCATOR requires the cathode to release electrons accelerated by the HV pulse. As stated earlier these electrons are in search of the anode which is at ground potential. The original aluminum cathode proved to be inefficient in releasing the electrons. A stainless steel cathode has been used improving the level and quality of RF generated. The primary disadvantage to using stainless steel is its inability to dissipate heat, which, as it escalates, impedes the generation of RF. Further experimentation led the OGDEN staff to the current cathode material, carbon. The carbon has proven to maintain the high level of RF for longer durations. Attaching an aluminum backplate to the cathode further extended the duration of use by dissipating the heat generated more efficiently. These modifications of the cathode have provided the research staff with a transmitter capable of producing an average of 600 MW per pulse for a durations well beyond the original design.

Along with the composition of the cathode the engineering staff has refined the configuration of the cathode to bring the level of RF to yet another level. TEMPO is currently capable of delivering 800 MW of RF energy. This has been accomplished by tapering the back of the cathode, making the transmission line more uniform to the HV pulse. To exploit this theory to its fullest, the engineering staff has increased the diameter of the cathode shaft to match the diameter of the cathode. In theory the HV pulse, which is a high frequency pulse, propagates along the surface of the shaft. This is quite different than conventional current flow which travels through the shaft (conductor). By fabricating the shaft as a cylindrical conductor and also increasing the diameter, the surface area has increased tremendously which should decrease the induction of the transmission line. As proven in the past, the reduction of inductance provides a means for a more efficient propagation of the HV pulse. The investigation of this modification has been temporarily interrupted by the relocation of the facility to

Brooks AFB. The preliminary results have indicated that the quality of RF has been improved by producing a wider, squarer envelope of energy. Along the same lines, the next approach is to make the ground plane surrounding the cathode shaft appear to be electrically homogeneous.

Since the delivery of TEMPO to DMR, the transmitter has evolved from a maintenance intense machine into an instrumental research tool. As delivered, TEMPO was only capable of firing 30 shots of RF at a level of 200 MW. Through their persevering efforts, the OGDEN staff has enhanced the operating parameters of the transmitter which now fires 1000 shots continuously maintaining a power level of 800 MW.

Rationale for CNS/Behavioral Studies

Concern about biological effects of microwave irradiation has increased as applications have proliferated. Much of this interest has focused on the effects of low-level, non-ionizing radiation on the nervous system. These effects are often assessed through measurement of behavioral changes in organisms following exposure to continuous wave (CW) microwave irradiation. Current safety guidelines specify exposure levels of the presumed harmful effects of electric fields that RF fields induce. Civilian standards, on the other hand, do not address the unique RF fields produced by military equipment such as radars and directed energy weapon systems. High-peak, low average power microwave fields produced by military equipment may satisfy civilian safety standards, however, peak power levels are high enough to cause concern and their possible bioeffects on the Army personnel need to be studied.

The effects of RF fields depend on the exposure level, power density, and specific absorption rate. Because behavior is equally as important a determinant of change as is the RF field itself, sufficient characterization and comparison requires analysis that extends over a range of behavior. During the duration of the contract, OGDEN research staff teamed with government investigators studied the effects of high power pulsed, CW microwave fields and electromagnetic pulses on operant behavior, memory, circadian rhythms, physical endurance, general motor activity level, startle and avoidance behavior of the organisms. Also, a preliminary experiment studied the interaction of a wide spectrum of pharmacological agents and pulsed microwaves.

Post-Exposure Effects of High-Peak Power Microwave Pulses On Operant Behavior

Behavioral effects of COBER transmitter on Wistar rats were studied by using operant schedules. Rats were trained on fixed-ratio, variable-interval, and differential reinforcement-of-low rate schedules. Ten minute exposure to 240, 720, 2160, and 6480

pulses at a 1 Megawatt peak power level caused a rectal temperature rise of 0.7 to 2.5 °C in the animals. Total doses (SAs) were set to 0.50, 1.5, 4.5, and 14 kJ/kg by adjusting the pulse repetition rate. Each pulse produced a peak whole-body SA and SAR of 2.1 J/kg and 0.21 MW/kg, respectively. A microwave-transparent animal holder was used to keep the animal's body axis parallel to the E-field. Regardless of their schedules of reinforcement, animals exposed to the highest dose level failed to respond, on the average, for 13 minutes after the exposure when they were placed in operant conditioning chambers. However, as soon as their rectal temperatures decreased, responding resumed and no further changes in response pattern were exhibited. No long-term effects were observed. No behavioral effects were found at the lower dose levels. It is concluded that the behavioral perturbations produced by pulsed microwave fields were thermal in nature [Akyel, 1991; Akyel, Hunt, Gambrill, and Vargas, 1991; Akyel, Hunt, and Vargas, 1989].

In-field Behavioral Effects of Pulsed Microwave Fields

A similar experiment studied in-field behavioral effects of 1.25 GHz, 1-MW peak power and 10 μs pulse width for 10 minutes. At high dose levels, 7.5 and 10kJ/kg rats' overall response rates were decreased by 30 - 40% relative to their baseline rates. Due to specific requirements of the schedules, only fixed-ratio animals which needed a high number of responses, collected fewer reinforcements, at the highest dose level [Akyel, Blair, Serafini, Akyel, Varle, 1991]. OGDEN staff, designed and built a microwave-transparent, fully functional computer operated operant chamber equipped with a response lever, stimulus lights, and a feeder for this unique in-field experiment. There were several requirements for a RF-transparent operant chamber. To reduce the perturbation of incidents fields, only polycarbonate and fiber optic materials were used in the design of this apparatus. To enhance RF absorption, the chamber was restrictive enough to keep the animal's body parallel to the electric (E) field but large enough not to induce stress to the animal. In order not to intensify thermal effects of microwave fields, the chamber had adequate ventilation to ensure heat dissipation. Finally, the

chamber was transparent for visual inspection of the animal's behavior and easy to service and clean. A paper describing this apparatus to the scientific community is published in the well-circulated and prestigious Physiology and Behavior Journal [Akyel and Belt, 1992].

Neuropathology in Rats Exposed to Pulsed and Continuous-Wave Radiofrequency Radiation

With the leadership of government scientist Dr. Campbell and the help of OGDEN staff, a preliminary study examining the neuropathology of the rats exposed to pulsed and continuous-wave RF radiation was completed using COBER transmitter. In this study, rats were exposed to 25 kJ of RF energy, either in the form of pulsed radiation (930 kW peak output power for approximately 95 s, 2716 - 2803 pulses, 10 μs pulse width, 29.2 Hz pulse re[petition rate) or as continuous wave radiation (963 W for 95 s). Examinations of the brains of these animals using routine neuropathological tissue techniques and a more sensitive silver-impregnation method revealed considerably more evidence of neurological damage in animals exposed to the pulsed radiation than in those exposed to continuous wave radiation [Campbell, Hunt, Bates, and Gambrill, 1991].

Mechanisms of Microwave-Evoked Whole-Body Movements

To determine the mechanisms underlying a reflex-like whole-body movement caused by a high peak power pulsed and low power continuous microwaves that was reported by a series of experiment done at Department of Microwave Research, OGDEN researchers and technicians, collaborating with government scientists, designed a unique automated measurement system. The exposure device consisted of a downward pointing WR 650 waveguide with an endplate short and hole in one board wall to accept the animal holder. The holder was a ventilated plastic cylinder which restricted the rat's extraneous motions and detected evoked body movements. The floor of the holder

contained a piezoelectric film that detected the movement. The whole experiment was programmed through computer to eliminate experimenter bias. Using this automated system a wide spectrum of neuroactive drugs from six categories including serotonin (5-HT), dopamine, norepinephrine, acetylcholine, opiates, and benzodiazepine were used to determine the mechanism of microwave-evoked whole-body movement. Drug or vehicle injected rats were sham-exposed or exposed to 1s, 120 W pulses at 1.25 GHz. Atropine (cholinergic) and clonidine (noradrenergic) reliably interfered with microwave effects so that were no differences in response incidence between sham and RF exposed rats [Akyel, Akyel, and Raslear, 1993; Akyel, Raslear, Akyel, Seaman, 1993; Raslear and Akyel, 1993].

Behavioral Effects of the High-Peak-Power, Pulsed Microwave Fields (TEMPO)

Behavioral effects of TEMPO transmitter which operates at 3 GHz level with a 700 MW peak transmitted power and produces 80 ns width pulses at a rate of 1 pulse per 8 s were studied in the following experiments.

Effects on Treadmill Performance (TEMPO)

To assess the physical endurance of organisms following an exposure to 200 TEMPO high-peak power microwave pulses treadmill performance of rats was studied. Although the exposure system produced a time-averaged midbrain specific absorption rate (SAR) of 0.21 W/kg and time-averaged whole-body SAR of 0.07 W/kg, both well within the present safety guidelines, the results indicated that exposure to such RF fields decreased the rats' endurance, as reflected in running time on the treadmill without faltering [Akyel, Belt, Raslear, Hammer, 1993].

Effects on Memory (TEMPO)

Memory consolidation in the rat following 200 TEMPO pulses were also studied. Water-deprived rats were trained in a single trial to find water in one arm of a Y-maze. Immediately following training rats were either exposed to high-peak power microwave pulses as described above or returned to their cages. Statistically significant differences were found between groups in errors made on a 24-hour retention test in the Y-maze [Raslear, Akyel, Serafini, Bates, and Belt, 1991].

Effects on Circadian Rhythmicity and Food Consumption (TEMPO)

Circadian rhythmicity and food demand following exposure to TEMPO pulses were studied by Raslear, Akyel, Serafini, Bates and Belt [1991]. In this study, rats were either exposed to 400 high-peak power pulses or were placed in the exposure chamber for an equivalent period of time. Immediately thereafter, the rats were placed in home cages equipped with response levers and pellet dispensers. Rats could press the lever to obtain food pellets, which were their only source of food. Each day the number of lever presses required to obtain a single food pellet (the price of food) was increased. The demand for food (consumption as a function of price) was not effected by microwave exposure, but the circadian pattern of food intake was affected.

Effects on Time Perception and Discrimination (TEMPO)

The effects of TEMPO pulses on a time perception and discrimination task were studied in rats. As determined by calorimetry, a maximal, whole-body-averaged, specific absorption rate of 0.072 W/kg was produced. Thus exposures were well below recommended SAR limit of 0.4 W/kg. Power levels of transmitted microwaves were varied over a 50 dB range to obtain ascending and descending dose-response functions for each of the behavioral measures. Measures of time perception, response bias, and

total trials did not change with power levels. Dose-response effects were observed for discriminability, ability to distinguish between durations, session time, and trial completions (failures to respond on a trial). The observation of repeatable dose-response effects on discriminability and null responses indicated that the microwave exposures were affecting cognitive function in the rats, particularly decision-making processes [Raslear, Akyel, Bates, Belt, and Lu, 1993].

Behavioral Effects of EMP Fields

OGDEN researchers investigated behavioral effects of electromagnetic pulse fields using a distinctive EMP simulator that has been drastically improved beyond its original capacity by OGDEN's technical staff [Akyel, Raslear, Bates, and DeAngelis, 1992; Akyel, Raslear, and Serafini, 1990; Mathur, Bates, and Bassen, 1990]. Our research results have been presented as only EMP experiments accomplished in a controlled laboratory setting at the "EMP Human Health Effects Science Review Panel" sponsored by the U.S. Navy Theater Nuclear Warfare Program [Akyel and Raslear, 1993]. A spectrum of behavioral paradigms (memory consolidation, general motor activity level, behavioral despair, and preference for electromagnetic fields) were employed to study the effects of EMP fields on rats. DMR's parallel plate EMP generator was used to expose animals to 200 pulses (6 pulses/min) with a rise time of 7 ns and a peak field strength of 100 kV/m. No significant differences were found between exposed and sham-exposed animals in memory consolidation and motor activity tests. Porsolt's forced-swimming test did not reveal any reliable differences in immobility time (behavioral despair) of the rats that were sham-or EMP-exposed. However, rats, when given a choice reliably preferred the side of a Plexiglas tube that was exposed to EM pulses.

Modeling of the Radiofrequency Radiation Hazards

(The Finite Difference Time Domain Method for Electromagnetic Field Simulation)

Simulating electromagnetic fields inside an arbitrary object involves very complex and tedious calculations. Using conventional calculating methods to simulate electromagnetic field propagation through an object may take hundreds of man-hours to accomplish. Therefore, utilizing the HP work-station and the electromagnetic field simulation software to model and calculate the RF field generated in any arbitrary object is very critical and time saving for researchers and engineers.

The Finite Difference Time Domain (FDTD) method has been used extensively to calculate electromagnetic field inside and outside of any material. The FDTD is very simple in concept and execution. However, it is remarkably robust, providing highly accurate modeling predictions for a wide variety of electromagnetic wave interaction problems. The FDTD applications include human body dosimetry studies for electro-magnetic safety, studies of electro-magnetic pulses (EMP) interaction with arbitrary objects, radar cross section (RSC) studies and many other microwave circuit studies. The FDTD is known to be a very useful tool in microwave engineering to simulate electromagnetic waves and pulses. The following are some basic properties of the FDTD program.

- The FDTD is a direct solution of Maxwell's time-dependent curl equation.
- The FDTD is a marching-in-time procedure which simulates the continuous actual waves by sampled-data numerical analog propagating in a data space stored in a computer.
- The FDTD achieves sampled-data reduction of the continuous EM field in a volume space, over a period of time.
- Space and time discretizations are selected to bound errors in the sampling process to ensure numerical stability of the algorithm.
- Electric and magnetic fields are interleaved in space to permit a natural satisfaction of tangential field continuity at media interfaces.

The preliminary FDTD computer code used in the Department was developed by OGDEN contractor Prof. Om Gandhi was able to simulate electromagnetic field within an object with a limited volume and was able to use only limited electromagnetic pulse shapes. OGDEN engineers worked on these limitations, and made many modifications to improve the computer code. The preliminary FDTD code was debugged to understand the limitations of the program. Modifications wer made on the FDTD code to calculate the field within much larger objects with higher volume space. Expanding the number of cells to be calculated from 8,000 to 300,000 made possible to calculate more than 30 times greater volume than original code. Test runs on the modifications proved to be successful. The preliminary code was only designed to simulate far field effects. However, our investigators mostly use open-end waveguide systems designed to study near field effects. Improvements were made to simulate a WR 650 open-end waveguide exposure system used in near field experiments.

Currently Mr. Lee, our senior electrical engineer, compared the results produced by HP work-station using FDTD code on open-end waveguide exposure system and the results obtained by using computer controlled microwave tri-axial scanner. This will improve the reliability of both measurement systems. OGDEN staff is planning to improve further the FDTD code in order to be able to simulate the ultra wide band frequency generator which the Department purchased from Sandia National Laboratories.

Microwave Effects on Baroreceptor Function

(Protocol N-11-87)

The purpose of this study was to test the hypothesis that high peak power microwave radiation can induce a subtle resetting of the arterial pressure monitoring center, the baroreceptor. A resetting of the baroreceptor results in decreased heart rate (bradycardia) and decreased arterial blood pressure (hypotension). The hypothesis was tested in 35 ketamine (166 mg/kg, i.m.) anesthetized rats, whose heart rate, mean arterial pressure, systolic arterial pressure, diastolic arterial pressure, pulse pressure, respiration rate, colonic temperature and neck temperature (at baroreceptor) were monitored continuously through the use of an indwelling arterial catheter, blood pressure transducer, pneumatic cuff, pneumatic transducer and a microwave transparent Luxtron temperature measurement system. Fifty-eight exposures were performed. Several types of exposures were implemented: sham-exposures (S), continuous wave (CW) and pulsed microwave exposures. The pulsed microwaves were 1.25 GHz microwaves operated at 400 kW peak power pulse modulated at 0.5 Hz (10 μs pulse width, 2 watts average) and 16 Hz (1 μs pulse width, 6.4 watts average). The CW microwaves were 2 watts (equivalent to 0.5 Hz pulses) and 6.4 watts (equivalent to 16 Hz pulses). These exposures were ventral head and neck exposures for 5 minutes in a L-band waveguide exposure system powered by a Cober transmitter. The average specific absorption rate (SAR) per watt transmitted was 4.75 W/kg at the medulla oblongata and 17.15 W/kg at the neck. The estimated whole-body average SAR was 1.51 W/kg per watt transmitted. Data were collected at 50 cycles/second for pre-exposure equilibration, exposure and post-exposure recovery periods composed of 5 minutes each.

Respiration rate and mean arterial pressure were not altered by any of these four exposures. Changes in heart rate and pulse pressure (difference between systolic and diastolic pressures as an indicator of the stroke volume) were observed. These cardiac inhibitions (bradycardia and possible decreased stroke volume) evolved in 1.5 to 3 minutes. The difference between pulsed and CW microwaves on cardiac functions was subtle, namely a minor difference in average exposure time to development of

bradycardia and bradycardia incidence. A quantitative relationship between bradycardia incidence and microwave power was noted. The incidence was 0 % in sham (0/12), 22 % in rats exposed at a lower microwave power (0.5 Hz and 2 watts, 5/23) and 57 % in rats exposed at a higher power (16 Hz and 6.4 watts, 13/23). The magnitude of bradycardia was also power dependent: 20 bpm (beats per minute, 5 % of the baseline) in low microwave power and 35 bpm (9 % of the baseline) in higher microwave power. Post exposure recovery in 5 minutes was complete in lower microwave power but incomplete in higher microwave power. The pulse pressure inhibition was noted in rats exposed at higher microwave powers only. The magnitude of change is approximately 4 mm Hg (12 % of the baseline). The microwave pulse pressure inhibition was persistent without any sign of recovery in 5 minutes after the microwave exposure. At the appearance of cardiac inhibition, the colonic temperature increased by 0.38 °C and the neck temperature by 2.25 °C in rats exposed to a higher microwave power.

It is apparent that significant acute cardiac deficit (up to 20 % from combined bradycardia and lower pulse pressure) can occur in individuals sensitive to microwave exposure. The probability of occurrence is dependent on microwave power although a high local SAR (109 W/kg at baroreceptor) and high whole-body average SAR (8.6 W/kg) was required to ascertain the occurrence of cardiac deficit in more than half of the experimental subjects and in the presence of significant local and whole-body hyperthermia. Nevertheless, these microwave induced cardiac deficits are abnormal and contrary to the anticipated hyperthermic responses in experimental animals. Acute cardiovascular inhibition was also noted by an independent group of investigators exposing rats' baroreceptors. Added significance should be attached to the current findings if one considers that hot spots in the neck region can occur in humans exposed to certain radiofrequency radiations [Lu, Brown, Johnson, Mathur, and Elson 1991a; 1991b; 1992].

Reflexive Responses Evoked by High Peak/Low Average Power Microwave Pulses (Protocol N-11-88)

One of the potential biohazards induced by a high peak/low average power pulsed microwaves is the neurophysiological responses evoked by pulsed microwaves. Evoked body movements is a representative of these neurophysiological responses. Results of a previous pilot study led to the hypothesis that a 2:1 enhancement of the Specific Absorption (SA) or dose/response relationship existed between pulsed and continuous wave (CW) microwaves of equal energy [Wachtel, Beblo, Vargas, Bassen, and Brown 1989]. This study was designed to provide detailed statistical analysis on this pulse enhancement effect and to examine the significance of factors as Specific Absorption Rate (SAR), peak power and exposure duration. In addition, microwave dosimetry and thermometry were performed to elucidate the primary site of microwave action.

A population of thirty-five female Balb-C mice, weighing 20 to 25 grams, were fitted with a tail-flick motion sensor (for identifying body movements) and placed in a low-Q cavity for exposure to a variety of microwave pulses. Microwave exposures for each mouse consisted of three sessions and each session of six randomly arranged test events. The first series of experiments were designed to test the two-fold increase in the response rate of mice by high peak power microwaves at an equivalent average power (160 $W_{average}$, 200 kW_{peak}, 10 μs pulse width and 80 Hz pulse repetition rate) in relation to the response rate evoked by gated CW (160 W_{peak}). Three different pulse burst events (4, 8, and 16 J corresponding to 2, 4, and 8 pulses in 25, 50, and 100 ms) and three different gated CW events (8, 16, and 32 J corresponding to a single pulse lasting 50, 100 and 200 ms). The second series of experiments were designed to supplement and to expand the dose-response curve of the first series of experiments. Five different microwave exposures and one sham exposure were included. These microwaves were 32 J pulse bursts with a 200 kW peak power (16 pulses in 200 ms), and a 20 kW peak (160 pulses in 2 s). The peak powers used in gated CW were 98.6 W (one 19.7 J pulse lasting 200 ms), 28.6 W (one 22.7 J pulse lasting 800 ms), and 7.2 W (one 23 J pulse lasting 3.2 s). Total number of test events studied were 507. In addition to incidence of

body movements, the latency and duration of evoked body movements were also evaluated. The mid-brain SAR was 96.0 W/kg per watt transmitted in pulsed microwave and 73.7 W/kg per watts transmitted in gated CW. This difference was caused by a fixed tuning of the system. Evoked body movements by acoustic and tactile stimuli were tested in half of the mice after microwave experiments to insure that the responsiveness of the mouse was not compromised and to obtain positive control for comparison.

Because of the care and design of the experiment, the incidence of the spontaneous body movements was very low 2.4 % or 1 out of 41 sham test events). The low incident rate in sham test events allowed us to associate the presence of body movements to the administration of microwave with confidence, especially when a high incidence rate of microwave evoked body movements (more than 80 %) was noted. Thus, the range of response rate is more than 30 folds. Body movements evoked by other stimuli, such as acoustic and tactile stimuli, provide additional confidence and positive control for the study.

The difference between incidence rates of the body movements evoked by pulse bursts and gated CWs of equivalent powers was not noted. Thus, current results indicated that 2:1 pulse enhancement hypothesis was not valid.

The incidence rate of the microwave evoked body movements increased proportionally with microwave dose (SA) for microwave dose rate (SAR) fixed at 8 W/kg. The incidence rate reached a plateau at 20 J or 1 kJ/kg if duration of microwave administration was not considered. For SA higher than 20 J or 1 kJ/kg, the incidence rate of the microwave evoked body movements increased proportionally with average incident power or whole-body average SAR. A response plateau was noted at approximately 20 W incident power or 1 W/kg. Evoked body movements was identified in mice exposed to a whole-body average SAR as low as 0.36 W/kg, the basis (0.4 W/kg) of the IEEE C95.1-1991 personnel protection guideline for a controlled environment. Therefore, IEEE personnel protection guideline may be invalid under certain circumstances [Brown, Lu, Mathur and Wachtel 1991; Lu, Brown, Mathur, Gambrill, Serafini and Wachtel 1991; Brown, Lu and Elson 1994].

Study of the latency and duration of the evoked body movements can be used to quantify the response strength of the evoked body movements. Difference in latency among mice subjected to microwave, acoustic and tactile stimuli was noted. The latency was also correlated with the incidence rate of evoked body movements. On the contrary, no such correlation was noted in the duration of response. It was clear that the more intense the stimulus perceived by the mouse the shorter the latency and the higher the incidence rate. However, once the body movements were initiated, the response would run a full course irrespective of how intense the stimulus was [Brown, Lu and Elson 1994].

Changes in rectal temperature as a result of any of the above exposures could not be discriminated easily. The minimum and maximum amounts of bulk heating without thermal loss in these microwave exposures were between 0.05 and 0.38 °C. The actual measurement of the rectal temperature increase in mice which received 20-32 J of microwave energy was between 0.08 and 0.17 °C, and the rate of increase was between 0.028 and 0.056 °C/s. These values were in the vicinity of biological noise and instrument errors. None of rectal temperature changes could be used to predict the occurrence of the microwave evoked body movements. On the contrary, skin temperature changes associated with microwave exposure could be determined easily in anesthetized mice. It was noted that the highest skin temperature associated with microwave energy in mice which received 20-32 J occurred at 0.5 cm from the tip of the nose. The range of skin temperature changes at this site was between 1.4 and 1.6 °C and the heating rate was between 0.4 and 0.7 °C/s. Therefore, skin could be the primary site in which the stimulus for the microwave evoked body movements originated. Due to high skin heating rates, these microwaves must have been perceived by the mice as an intense thermal sensation but not a pain sensation because the temperature increments were well below the threshold for thermal pain [Lu, Brown, Mathur, Gambrill, Serafini and Wachtel 1991; Brown, Lu and Elson 1994].

The incident rate of microwave-evoked body movements increased proportionally with the magnitude of skin temperature increment and skin heating rate. These relationships could be used to extrapolate for an absolute threshold (0 % incidence rate),

i.e., 1.21 °C and 0.24 °C/s. The equivalent SAR value for 0.24 °C/s is 834 W/kg which is 2 orders of magnitude larger than the whole-body average specific absorption rate resulted from these pulsed microwaves. Therefore, the microwave-evoked body movements represent a biological response from highly-localized microwave absorption [Lu, Brown, Mathur, Gambrill, Serafini and Wachtel 1991; Brown Lu and Elson 1994].

Preliminary Thermometric Studies on Microwave Exposure Reported to Cause Retinal Damage in the Rabbit (Protocol N-14-90)

Pulsed microwave-induced retinal injuries were noted in reports by different groups of investigators. The threshold for microwave-induced retinal injuries appeared to be lower than the threshold of an injurious whole-body averaged specific absorption rate (SAR, 4 W/kg) and the permissible local SAR (8 W/kg) for personnel protection. In addition, pulsed microwaves appeared to be more potent in inducing ocular injuries than the continuous wave microwaves. Due to deficits in these reports concerning microwave-induced retinal injuries, a study was initiated to evaluate the potential microwave induced injuries in Dutch belted rabbits.

Dutch belted rabbits were selected as an experimental model because of their pigmented eyes and because it is a good toxicological practice to be able to extrapolate from more than one species of animals to human. Due to the anatomical positions of rabbit's eyes, two microwave dose/dose rates can be delivered simultaneously to eyes of each rabbit. Therefore, the number of animals needed for evaluation of retinal injury and determination of threshold for such injury decreases. Dose determination is important in toxicological evaluation. However, microwave dose/dose rate is represented, depending on the underlying hypothesis, by absorbed energy, SAR, and by the change in tissue temperature resulting from the interaction between microwave absorption and thermoregulation. Thus, the microwave-induced tissue temperature changes are subjected to the influence of ambient conditions. The purpose of this study is to determine:

- the reliability of the dosimetric method,
- the microwave dose/dose rate delivered to the rabbits and
- the ocular and rectal temperature changes resulting from microwave energy absorption.

Prior to the initiation of planed research, microwave exposure equipment has to

be configured to suit research goals. The design criteria for the microwave exposure equipment calls for a narrow beam at 1.25 GHz to obtain a maximum ratio of the power density/power output of the transmitter and to avoid exposing surrounding tissue as much as possible. Dielectric lens and dielectric-loaded waveguide applicators were considered and evaluated by the engineering staff. Half-beam width and power density per watt output were used for evaluation. A 1" Teflon loaded WR650 open-end waveguide was selected because it provided most desirable characteristics of adequate focusing without appreciable attenuation along the beam path.

Five male Dutch belted rabbits between 1.5 and 2.0 kg were used. During thermometry (determination of thermal dose), rabbits were anesthetized with ketamine-xylazine anesthesia and local xylocaine infiltration. A fistula was placed below each eye immediately beneath the mucosal membrane of the conjunctiva for placement of the microwave transparent temperature probe (Luxtron) to a depth of 2.0 cm. Seven ocular temperatures (0, 0.5, 1.0 and 1.5 cm from the surface of the eyelid at the incident side and 0.5, 1.0 and 1.5 cm from the surface of the eyelid at the exit side of the microwave). An additional probe was inserted to 5.5 cm into rectum for rectal temperature measurement. The rabbit was maintained under anesthesia by continuous ketamine-xylazine anesthetics. The rabbit was exposed by placing the right eye at the geometric center of a Teflon-loaded waveguide at a distance of 7.6 cm. Four hour continuous exposures were used. The microwave parameters were 1.25 GHz, 1 MW peak power, 10 μs pulse width, 0.86 Hz pulse repetition rate and an 8.6 W average output power. The exposure was performed in an anechoic chamber which was maintained between 22 and 25 °C. The ocular temperatures and the rectal temperature were acquired at 0.1 Hz. Each rabbit was sacrificed with intracardic injection of sodium pentobarbital after thermometry and the carcass was used for ocular dosimetry. Results and discussion will be presented after ocular dosimetry.

A critical and essential element in a study of biological effects of microwave radiation is the determination of the dose and/or dose rate administered. The microwave dose rate is denoted by the local specific absorption rate (SAR) and the whole-body average specific absorption rate. Both of these SARs constitute the basis

for comparison among laboratories that employ different exposure methods and form the basis of all current standards for protecting personnel against radiofrequency radiation which include microwaves. Whole-body calorimetry is used to determine the whole-body specific absorption rate by determining the amount of energy within the object with known mass being exposed to radiofrequency radiation. The calorimetric dosimetry will be discussed in a different section. Thermometry is used to determine the local SAR(s) at the point(s) of measurement(s). Due to the dynamic nature of thermal diffusion through conduction and convection, and various ambient environments which interact with the conduction and convection, the tolerance and sources of experimental errors are to be accounted for to obtain reliable and replicable results. The thermometric dosimetry procedure used in this laboratory was first developed in the rat model [Lu, Brown, Johnson, Mathur and Elson 1991a; 1991b; 1992; Brown, Lu and Elson 1994], perfected in the rabbit ocular model [Gambrill and Lu 1992; 1993; Lu, DeAngelis and Gambrill 1992; 1993], and applied to an *in vitro* dosimetry for the rat lens [Lu, Gambrill and Brown 1994].

In principle, the local microwave dosimetry utilizes the thermal nature of the absorbed energy for determining the amount of energy absorbed. A microwave transparent temperature measurement system (Luxtron) was used to monitor changes in object temperature before, during and after microwave exposure. The rate of temperature change during microwave exposure was adjusted for pre-exposure profile and post-exposure cooling. Results of initial calculations based on thermal characteristics of the tissue revealed that deviation from a linear function (assuming a constant rate of energy deposition) was less than 1% if the exposure time was limited to 10 seconds (s). Therefore, linear functions are anticipated before, during and after microwave exposure if each period is limited to 10-20 s. Furthermore, the rate changes due to thermal diffusion is linear within a short period of time indicating that the average rate between pre-exposure and post-exposure period equals the average rate of changes in thermal gradients during microwave exposure. Thus, simple subtraction will eliminate the errors contributed by thermal diffusion/thermal gradient.

In order to obtain reliable data from the Luxtron microwave transparent

thermometry system, temperature data were acquired at 10 Hz, the maximum operational speed of the thermometry system. However, the 10 Hz output of the system was rather noisy. The oscillation frequency of the output noise was about 2 Hz and the amplitude of the output noise was about 0.8 °C. Infrequent sampling would have rendered the temperature data inaccurate and useless. Since the output noise revolved around an average value and did not change significantly with different readings, two methods were used to suppress this higher frequency noise. They were data averaging and electronic filtering. Data averaging utilized the datum with its adjacent data to represent the best possible value of the determination. The amplitude of oscillating noise could be reduced according to the number of adjacent data being averaged and retained underlying rate changes. A nine-sample averaging procedure provided an optimal degree of noise reduction without excessive degree of averaging. However, a 10 sample averaging procedure was selected for routine use and a BASIC program was written to handle the data averaging. A 1-Hz low pass electronic filter was used for suppressing higher frequency noise. The 1-Hz electronic filtering method was essentially as effective in noise suppression as the post-acquisition 10 sample averaging procedure with the exception of a 0.7 s time shift of the trace. Therefore, the 1-Hz low pass electronic filter was used for on-line data acquisition and 10 sample averaging procedure was used for post-acquisition noise suppression of the filtered data.

A computerized multiple regression procedure using commercial software (SigmaPlot) was developed to determine the pre-exposure, exposure and post-exposure rate of temperature change (in °C s^{-1}) and to calculate the SAR (in W/kg) by:

$$SAR = C(A_{exp} - ((A_{post} + A_{pre})/2)$$

Where C is the specific heat capacity for the tissue, 0.83 kcal kg^{-1} $°C^{-1}$ or 3474 J kg^{-1} $°C^{-1}$. The temperature trace was divided into three different segments. The data were fitted until intercept points of any two segments (i.e., pre-exposure-exposure and exposure-post-exposure) coincided. The multiple regression procedure was to provide a visual aid to eliminate decision and calculation biases caused by an inconsistent curve fitting routine. Thus, a microwave dosimetry method was developed from theoretical and

practical considerations. The current procedure can be performed in a dynamic system and does not require a time consuming equilibration of the sample to the environment.

Sixty-eight exposures (8 files per exposure, 544 files) in rabbits were performed and analyzed. The sensitivity of the current microwave dosimetric method was on the order of 0.02 °C/s or SAR of 69 W/kg for a 10 s exposure. The 0.2 °C (0.02 °C/s x 10 s) reflected the instrumentation error (Luxtron) which could not be remedied by filtering, post-acquisition noise suppression, or data fitting. The error of the determination (based on the coefficient of variance from 4 replicates) was inversely proportional to the specific absorption rate. To maintain an average coefficient of variance in 4 replicates less than 10 %, the SAR needed to be higher than 300 W/kg or the heating rate needed to be higher than 0.086 °C/s or 0.86 °C in 10 s exposure time, and 0.43 °C in 20 s exposure time. However, the average dosimetric data 4 replicates was highly accurate since the difference between dosimetry performed with 500 W and 1000 W was less than 1 %. In short, a reliable and replicable dosimetric procedure was developed.

Microwave absorption in the rabbit eyes was inhomogeneous. The highest microwave absorption at retina was 1.02 W/kg per watt incident power at 0.5 cm from the incident interphase into the eye. The microwave absorption decreased exponentially from a distance of 1 cm (0.73 W/kg per watt incident power) into the eye facing incident field and down to the point of exit path of the microwave at the eye facing away from the incident wave (0.082 W/kg per watt incident power). The ratio between maximum and minimum ocular specific absorption rate was 12.4. The average specific absorption rate for the eye at the incident site of the microwave was 0.755 ± 0.038 W/kg (Mean ± S.E., n=40) per watt incident power and for the eye at the exit path of microwave was 0.245 ± 0.027 W/kg. Therefore, the average ratio between eyes at the incident site and exit path was 3.08. Two microwave specific absorption rates can thus be simultaneously delivered to the the rabbit if the microwave transmitting path is parallel to the horizontal axis formed by the eyes.

The hyperthermic potency of localized 1.25 GHz microwave exposure was evaluated in anesthetized rabbits using 8.6 watt average incident power that pulse

parameters were 1 MW peak power, 10 μs pulse width and 0.86 Hz pulse repetition rate. Hyperthermia (increase in body temperature other than due to fever) was observed in rabbits subjected to above exposure for four hours. Hyperthermia was noted at 7 sites at both eyes and in the rectum. In contrast to inhomogeneous SAR (maximum SAR/minimum SAR = 12.4), the ocular temperature changes were relatively homogeneous. The temperature increments from the datum immediately before exposure were between 2.59 ± 0.28 °C (Mean ± S.E., n=5) and 3.35 ± 0.26 °C. The ratio of maximum and minimum temperature increments was 1.29. The difference among these 7 ocular temperature increments was not statistically significant. The ocular temperature increments were barely dependent on local SARs but highly dependent upon the depth from the ocular surface in each eye. Thus, radiating, conductive and convective heat losses at corneal and eyelids could play a modifying and interacting role on temperature increments in the eye. Within the eye, the primary mechanism of heat diffusion is by conduction since blood circulation for convective heat dissipation is primary at the iris and retina. Therefore, very complex thermodynamics could be anticipated.

Because of the exposure configuration, the power density decreased by more than 30 dB (one thousandth) of the highest power density in 10 cm from the center of the microwave field where the eye facing the waveguide was placed. In essence, lower chest, abdomen including rectum and hind quarters of the rabbit were exposed to a negligible microwave fields. Therefore, an increase in rectal temperature was not anticipated if a heat-sink effect was not in effect. Present results indicated that rectal temperature increased by 2.74 ± 0.30 °C, which was not different from the ocular temperature increments. Dynamically, the rectal temperature increase was delayed in relation to ocular temperatures. In other words, the convective heat-loss mechanism in the eye and its surrounding tissues redistributed the absorbed microwave energy in the form of heat to the rest of body, the heat sink effect.

Rectal-ocular temperature gradients were noted. The rectal-ocular temperature gradients were positive and decreased with the depth into the orbit. These gradients represented the dynamic end-results of local tissue metabolic heat production (including

energy deposition from microwave absorption, other heat source such as ultrasound, infrared, etc.), conduction and convective heat distribution, and radiant, conductive and convective heat exchange between the body surface and the environment. Temperature gradients were too complex to model reliably. The preferred method of identification of change in tissue temperature change is by experiment. The rectal-ocular temperature gradient was usually reduced by the end of these 4 hour localized microwave exposures. The maximum magnitude of reduction in 7 rectal-ocular temperature gradients was -1.08 ± 0.19 °C (Mean ± S.E., n= 5) and none was lower than -1.6 °C. These results indicated that magnitude of ocular temperatures could rise higher than that of rectal temperature. Because of individual differences, precise prediction of ocular temperature from rectal temperature could not be achieved. However, the upper limit of ocular temperature could be deduced from the rectal temperature.

Relatively uniform ocular temperature changes from variable local ocular SARs can assist in elucidating the planned research on mechanism of microwave-induced retinal injury. The experimental design for microwave induced retinal injury would benefit by subjecting one eye of the rabbit to microwave exposure above the "threshold" SAR and the other eye below. Under this experimental condition, a thermal-dependent injury may be deduced if retinal injury occurred in both eyes and an SAR-dependent injury may be deduced if ocular injury occurs in the eye with higher SAR only [Lu, DeAngelis and Gambrill 1992; 1993].

Induction of Ocular Injuries in Rabbits by High Peak Power but Low Average Power Pulsed Microwaves (Protocol N-01-92) (in progress)

Recent advances in studying retinal injuries induced by high peak power pulsed microwaves indicates the need to confirm the enhancement and/or specificity of peak power in causing retinal injuries. The threshold for pulsed microwave induced retinal injuries was reputed to be lower than an average ocular SAR of 4 W/kg for 4 hours daily, 3 exposures per week for 3 to 11 exposures in monkeys. This threshold for retinal injury represented the only known injury at or lower than 4 W/kg, a SAR which was considered to be incapable for inducing any type of injury by several national and international committees which reviewed and recommended protection guidelines for personnel protection against the harmful effects of radiofrequency radiations including microwaves. However, several confounding factors were apparent in the experimental design and execution. They were

- long distance transport (30 miles each way) between the laboratory and the exposure facility weekly,
- repetitive use of chemical restraint before and after each exposure,
- repetitive use of chemical restraint and drugs for electroretinogram determination and other ocular examinations,
- varied number of exposures to induce retinal injuries, and
- inadequate number of control (sham-exposed) subjects.

That the retinal injury required repetitive exposures points to a possible accumulation of injury over time. Due to the profound importance and implication of microwave-induced retinal injuries in a soldier's ocular health, a series of experiments was designed to study this effect in absence of confounding factors.

Male Dutch belted rabbits were selected as an experimental model based on their pigmented eyes, a SAR for each eye from each microwave exposure and a good toxicological practice for extrapolation of a known effect from more than one species to

human. Experimental protocol was followed meticulously to avoid introducing undesirable confounding factors into the experiment. Anesthesia and chemical restraint was not used. Electroretinograph and other eye examines were not incorporated except daily eye examination under regular laboratory lighting to exclude the presence of corneal injury and conjunctivitis. Rabbits were adapted to a restrainer for 4 hours daily the week before being subjected to the sham-exposure, pulsed microwave exposure or continuous wave microwave exposures. Each rabbit was exposed to one of those three different exposures. The sequence of these three exposures was rotated in sequence such that equal number of animals would be subjected to any one of the three exposures. Ears were taped together with a labeling tape to prevent ear movements which could cause accidental exposure to higher field intensity. They were exposed for 4 hours daily without anesthesia or chemical restraint in three consecutive days per week. The rabbit was placed at 7.6 cm from the opening of the Teflon-loaded waveguide with the right eye in the geometric center of the opening and the axis of eyes in line with the direction of propagation. The microwave used was 1.25 GHz with horizontal electric polarization and vertical magnetic polarization to limit the amount of tissue being exposed. At the designated time after the designated number of exposures, they were anesthetized with intramuscular katamine-xylazine injection followed by lethal dose of intracardiac sodium pentobarbital injection and pneumothorax. Both eyes were extirpated and fixed in a formaldehyde-glutaraldehyde fixative after separating the eye into cornea, lens and retina-sclera by circumcision at the limbus. Each eye was coded with a rabbit number and its position (left or right) for identification. Twenty-four hours later, the eye was transferred to a buffered formaldehyde fixative until it could be processed into paraffin block and sectioned into 4μm sections, mounted on glass slides and stained with Hematoxylin and Eosin for histological evaluation. Histological evaluation was done blind without revealing the treatments to the pathologist.

A microwave transparent temperature probe (Luxtron MAM-05 probe) was used to monitor rectal temperature 5 cm in depth continuously. During the exposure (sham, pulsed or CW), the rabbit could become restless which required interruption of the exposure to realign the animal to original exposure position. The number of restless episodes and changes in rectal temperature were recorded for assessing the extent of

behavioral arousal and thermal stress. Initially, rabbits were exposed for three daily exposures as following:

- sham exposure, a simulation of microwave exposure conditions without applying the microwave power,
- pulse exposure, 1.25 GHz, 1 MW peak power, 10 μs pulse width, 0.8 Hz pulse repetition rate, 8.6 watts average incident power, and
- CW exposure, 1.25 GHz, 8.6 watts incident power.

The average ocular SAR was 6.49 ± 0.33 W/kg (Mean ± S.E.) for the eye (right eye) facing the waveguide applicator and 2.11 ± 0.23 W/kg for the eye (left eye) facing away the waveguide applicator. These SARs bracketed the reputed threshold for microwave induced retinal injury (4 W/kg). Five rabbits were exposed to each type of exposures. A total of 15 rabbits were used. These fifteen rabbits were sacrificed 24 hours after the third exposure. One case of sub-retinal exudation was noted in one of the left eyes (2.11 W/kg x 4 hours x 3) exposed to pulsed microwave. A retina break was found in one of the sham-exposed rabbits. No other retinal changes were noted in the rest of the rabbits.

Because of this low probability of histopathological changes, three rabbits were exposed to the same ocular SARs (6.49 W/kg in the right eye and 2.11 W/kg in the left eye) either in pulsed, CW or sham exposure only and their retinal histopathology was studied one week after the completion of 3 daily exposures to allow an adequate of time for injury to manifest if such injury existed. Retinal histopathologic change was absent in these three rabbits. Three additional rabbits were exposed to the same ocular SARs either in pulsed, CW or sham exposure only but the number of exposures was increased to 9 in 3 daily exposures per week. The retinal histopathology was studied 24 hours after the completion of exposures. Again, no apparent retinal histopathologic changes were noted among these three rabbits.

Baseline rectal temperatures immediately before exposures in these 21 rabbits (7 in each exposure regimen) were 39.07 ± 0.07 °C (mean ± S.E., n= 21, sham-exposed

group), 38.91 ± 0.05 °C (pulsed group), and 38.94 ± 0.05 °C (CW group). The difference among these groups of rabbits was statistically insignificant ($F_{(2,60)}$= 2.20; p> 0.05) and the largest difference was 0.16 °C indicating an uniform pre-exposure conditions among these three groups of rabbits. The time average rectal temperature change from its own baseline rectal temperature was used to quantify the change of rectal temperature in these rabbits during the entire 4 hour exposure period. Sham exposed rabbits were characterized by cooling or a decrease in rectal temperature (-0.46 ± 0.06 °C, mean ± S.E., n=21). Both hyperthermia (increased rectal temperature) and cooling could be noted in rabbits exposed to pulsed or CW microwaves. The average rectal temperature change from baseline values was 0.19 ± 0.07 °C (n= 21) in pulsed group and -0.08 ± 0.08 °C (n=21) in CW group. The difference among these three groups was statistically significant ($F_{(2,60)}$= 7.86, p< 0.001). Therefore, hyperthermia in exposed rabbits was clearly evident and the magnitude of hyperthermia was higher in pulsed than CW exposed groups.

Rabbits could become restless in the restrainer within a period of 4 hours. In the sham exposed group, the number of restless episodes ranged from 0 to 12 with an average of 5.8 ± 0.9 episodes (mean ± S.E., n= 21 in 7 rabbits). In the pulsed exposed group, the number of restless episodes was slightly less than that of sham exposed group and ranged between 1 to 12 episodes. The average was 4.6 ± 0.7 episodes (n= 21 in 7 rabbits). The restless behaviors appeared to be enhanced in rabbits exposed to CW microwaves. The number of restless episodes ranged between 0 and 36 in these rabbits. The average restless episodes was 12.2 ± 2.1 episodes (n= 21 in 7 rabbits). The restless enhancement in the CW exposed group was statistically different from sham exposed and pulse exposed groups ($F_{(2,60)}$= 8.77, p < 0.001, Duncan's New Multiple Range Test). Rabbits showed signs of enhanced restlessless (more than 12 restless episodes in 4 hours) in 7 of the 21 CW exposures. However, correlation of the occurrence of enhanced restless to which of the three individual exposures within each individual rabbit was not fruitful.

Apparently, localized microwave exposure at these SARs (6.49 W/kg in the right eye and 2.11 W/kg in the left eye) was incapable of inducing retinal injury in rabbits even

in the presence of discernable hyperthermia. The latency of injury and the ccumulative nature of the microwave injury (theoretical basis for repetitive exposures) could not be evaluated due to the absence of retinal injury. These rabbits appeared to be able to tolerate the exposures without other ill effects except an restless enhancement during CW exposures. It was decided to increase the number of exposures and SARs to increase the probability of retinal injury. Six 4 hour exposures (3 daily exposures per week for 2 weeks) and one week latent period were incorporated into a new study. These exposures were:

- sham exposure, a simulated exposure without turning on the power to the transmitter,
- pulse exposure, 1.25 GHz, 1 MW peak power, 10 μs pulse width, 1.05 Hz pulse repetition rate, 10.5 W average incident power, and
- CW exposure, 1.25 GHz, 10.5 W incident power.

The average ocular SAR was 7.93 ± 0.40 W/kg (mean ± S.E.) for the eye facing waveguide applicator (right eye) and 2.57 ± 0.28 W/kg for the eye facing away from waveguide applicator(left eye). The endpoints of the study are retinal histopathology, hyperthermia potency, and behavioral arousal.

Four rabbits have been exposed to pulsed microwave, three to CW and two to sham exposures. Evaluation of endpoints of this study is still in progress.

Inability to induce retinal injury in rabbits is a very likely outcome of the current study. It can be interpreted that the susceptibility to the microwave induced retinal injury is different among different species of animals. A contributing factor for this genetic dependency is the difference in anatomy of eyes between monkeys and rabbits. Three different histopathological changes were noted in the monkey subjected to microwave exposure, i.e., pyknotic degeneration of nuclei at pigmented epithelium, pin-head retinal detachment and karyolysis in the cone photoreceptor at the fovea and macula. Pyknotic degeneration of the pigmented epithelium was not noted in rabbits.

Two cases of retinal detachments, in the form of subretinal exudation and retinal break, have been found in the present study. However, these two cases of retinal detachment occurred evenly between sham exposed and pulse exposed rabbits. Association of retinal detachment to microwave exposures cannot be concluded due to a lack of statistical power to substantiate such a claim. Because of low probability of occurrence, large treated and control populations have to incorporate to provide adequate statistical power for associating this retinal injury (retinal detachment) to microwave exposure. It is clear that such association cannot be accomplished because the limited number of rabbits used in the present study. The rabbit retina does not have a fovea and only a few cone cells are present in the functional macula area. A working hypothesis, that microwave radiation only targets the cone photoreceptors but leaves rod photoreceptors intact, has been forwarded from observation of the microwave induced retinal injury in monkeys. Absence of discernable cone photoreceptor karyolytic degeneration associated with microwaves tends to support the working hypothesis that microwave radiation did not injure the rod photoreceptors. On the other hand, absence of more advanced histopathological changes in cone photoreceptors remained to be demonstrated.

Confounding factors in monkey experiments performed in the other laboratory can be the source of variation in species susceptibility to microwave induced retinal injury. Synergistic interaction between microwave and drug in inducing microwave associated ocular injuries has been demonstrated in monkeys. Therefore, synergistic interaction by the confonding factors may contribute to the species difference in susceptibility to microwave induced retinal injury. This hypothesis can be studied by incorporating confounding factors in monkey experiments. Most important factors are repetitive use of chemical restraint, and chemical restraint, drug and strong light in electroretinographic evaluation and other ocular examinations [Lu, Gambrill and Elson 1993; Gambrill, Elson and Lu 1994].

Microwave Dosimetry of the Rat Lens In Vitro

(Protocol N-02-93)

Injuries to the eye caused by intense microwave radiation have been known for many years. It has been suggested that lenticular temperature elevation above a critical level may be sufficient to cause microwave induced cataracts. However, results of the past research (Protocol N-05-86) in this laboratory demonstrated that the microwave-induced cataract did not require the cited critical lenticular temperature for induction of cataracts in rat lens *in vitro*. By present technical standards, lenticular dosimetry in that protocol (N-05-86) was found to be inadequate when an in depth review of the past research was made. We have recently developed a reliable thermometric dosimetry method for an *in vivo* determination of the microwave specific absorption rate (SAR) in specimens accurately (Protocol N-14-90). Because of the health implication of lenticular injuries in humans, it is necessary to determine a more accurate dose in the rat lens than the previous values used in Protocol N-05-86. These dosimetric determinations were also used to test the validity of the thermometric procedure.

The 915 MHz, 1250 MHz CW, and pulsed exposure systems were reassembled and recalibrated for lenticular dosimetry. Lens exposure cells for these exposure systems, perfusion apparatus and a precision water bath were also reassembled and tested.

Fresh lenses were obtained from rats for dosimetry. Sixty-four day old rats were euthanized individually in a polycarbonate cage with carbon dioxide from a 100 % carbon dioxide cylinder. Eyes were removed immediately after death. Each lens was obtained by dissecting the eye following the outline of the Limbus. A microwave transparent temperature probe (Luxtron MPM probe, < 1 mm in diameter) was inserted into the lens through the lenticular with a dissecting/positioning guide fabricated from an 18 G hypodermic needle. The tip of the microwave transparent temperature probe was positioned in lenticular cortex to avoid the penetration of the lenticular nucleus. Three additional probes were placed above the lenticular probe with 5 mm spacing to characterize the SAR distribution within exposure cell. These dosimetries were

performed at 37 °C intra-lenticular temperature. The perfusate (physiologic saline, 0.9%) was warmed continuously in a 37.5 °C precision water bath to compensate for the heat loss in the perfusion loop. The perfusion rate was 60 to 80 ml/min. The flow rate was adjusted until all 4 temperatures spaced 1.0 cm apart in the exposure stabilized and equalized for at least one minute. The perfusion was stopped at the beginning of each dosimetry run to prevent convective heat loss from the perfusate. After each dosimetry trial, the lens was perfused until all 4 temperatures stabilized at 37 °C. Each lens was exposed four times to CW and pulsed microwaves. The exposure duration was 20 s for each dosimetry run. The dosimetry procedure was described earlier, namely, a segment analysis method to compensate the exposure rate of temperature changes with weighed pre-exposure and post-exposure rate of temperature changes. The following microwave exposures were evaluated:

- 915 MHz pulsed microwave, 15 kW peak power, 20 μs pulse width, 50 Hz pulse repetition rate, 15 W average incident power,
- 915 CW microwave, 13 W average incident power,
- 1250 MHz pulsed microwave, 10 kW peak power, 20 μs pulse width, 100 Hz pulse repetition, 20 W average incident power, and
- 1250 MHz CW microwave, 16.5 W average incident power.

Twelve lenses were used to obtain 384 temperature files in 96 exposures.

The intra-lenticular SAR was 6.46 ± 0.98 W/kg per watt (mean ± S.D., n=24, 4 replicates of 6 lens) of the pulsed 1250 MHz microwave and 6.26 ± 1.10 W/kg of the CW 1250 MHz microwave. The difference between these two values was ±1.6 % of the average value. The intra-lenticular SAR was 10.01 ± 1.50 W/kg per watt of the 915 MHz pulsed microwave and 9.81 ± 2.10 W/kg per watt of the CW 915 MHz microwave. The difference was ±1.0 %. The differences in SARs determined in the perfusate between CW and pulsed microwave was ±0.08 %, ±0.1 %, ±0.4 %, ±0.7 %, ±1.3 %, and ±2.0 %. From these results, it can be concluded that the result of SAR determination is dependent on average power and independent on the modulation.

These results are consistent with the theory for SAR determination. Therefore, the current local dosimetry developed in this laboratory is a procedure with high degree of precision and repeatability.

There was a fundamental difference in the SAR pattern surrounding the lens in these two different microwave exposure systems operated at 915 and 1250 MHz. In the 1250 MHz exposure system, the SAR was 6.36 W/kg per watt transmitted in the lens, 10.08 W/kg per watt transmitted at 1 cm above the lens, 12.92 W/kg per watt transmitted at 2 cm above the lens and 17.36 W/kg per watt transmitted at 3 cm above the lens. In the 915 MHz exposure system, these values were 9.91 W/kg per watt transmitted in the lens, and 14.08, 13.80, and 7.54 W/kg per watt transmitted at 1, 2, and 3 cm above the lens. Apparently, neither of these two exposure systems provided a uniform SAR within the exposure cell and lenticular exposures performed in the past had not utilize the relative uniform area of the field within exposure cell [Lu, Gambrill and Brown 1994]. These dosimetry results have been forward to the Principal Investigator who is responsible for Protocol N-05-86.

Whole-Body Calorimetric Dosimetry

(In Support of the Protocol N-10-88, Effects of Continuous-Wave and Extremely High Peak Power Pulsed Microwave on Rats Responding on High and Low Rates)

In studying the biological effects of radiofrequency radiation including microwaves, the investigator needs to understand and characterize the amount of energy being coupled to a biological specimen. Dosimetry is used to determine the amount of energy coupled into a biological specimen which includes local specific absorption rate (SAR) and whole-body average specific absorption rate. Whole-body dosimetry measures the integrated or volume averaged SAR, whereas local dosimetry deals with SARs in a specific site of the biological specimen. Both of these SARs are used to describe the energy coupling in biological specimen and as a basis of any rational personnel protection guideline. This section deals with a cost-effective and accurate whole-body dosimetry method developed in this laboratory.

Based on the assumption that all the radiofrequency energy absorbed by a biological specimen will eventually be converted to heat, a calorimetric dosimetry method has been developed in this laboratory. The concept was not new but details in methodology were frequently missing, inadequate, cumbersome or containing uncertainties. Calorimetric methods used in the past utilize a Dewar-flask calorimeter, a twin-well calorimeter, or dual-gradient-layer calorimeter to measure the amount of heat energy in the specimen. The Dewar-Flask calorimeter is based on the final temperature equilibrium between specimen and transfer medium (usually water), between transfer medium and calorimeter, and between specimen and calorimeter. The method depends entirely on the accuracy of the specific heat capacity values of the specimen, transfer medium and calorimeter. Control specimens are required to obtain the difference between exposed and control specimens. Although the cost of equipment is relatively low, calibration of the calorimeter and determination of the precise specific heat capacity of the specimen and calorimeter is difficult. In addition, the heat loss in a Dewar-flask calorimeter during actual operation cannot be accounted for easily. Thus, Dewar-flask

dosimetry tends to yield a lower value than other dosimetric methods.

The twin-well calorimeter is a piece of custom-made equipment which is available commercially as a gradient layer calorimeter. The gradient layer calorimeter measures the rate at which heat passes from the specimen through the walls of the calorimeter to a room environment. The heat flow is sensed by multi-junction thermopile. The heat flow rate can be calibrated easily with a high degree of precision and accuracy and it does not depend on the knowledge of the specific heat capacity of the specimen, transfer medium or calorimeter. Integration of heat flow rate over time yields the total amount of energy dissipated from the specimen to the calorimeter and to the surrounding environment. A thin layer of constant temperature high specific heat capacity fluid is usually used to prevent the room environment from influencing the determination of the heat flow rate. Therefore, the gradient layer calorimeter overcomes all the operational deficits and shortcomings of the Dewar-flask calorimeter. Nevertheless, a control specimen is still required to obtain the amount of energy deposition in the exposed specimen. Twin-well and dual-gradient-layer calorimetry determines the difference between exposed and control specimens following a prolonged period necessary for the establishment of an identical heat content between specimens. The equilibrium period takes 4-11 hours to achieve. The procedure can only be performed by introducing repetitively the same identical pairs of specimens, one as control and the other as exposed, into the calorimeter and removing one specimen for exposure while maintaining an identical environment for the control specimen. Matching of the exposed and control specimens is a pre-requisite for a successful operation. Errors can be introduced easily due to inadequate equilibrium, difference between specimens and errors introduced by repetitive introduction of the specimens. These procedures become more wasteful with the increasing number of determinations and can be rather time-consuming.

To overcome wasting specimens and to avoid uncertainties in dosimetry by a twin-well calorimeter, a calorimetric dosimetry procedure using a single gradient-layer calorimeter was designed. The design features included:

- standardization of a specimen equilibration procedure and criterion such that a

uniform and replicable thermal content of a specimen can be obtained,

- equilibration of the specimen temperature (26 °C) slightly above laboratory environment (24 °C) so that error caused by introduction of the specimen is a negligible amount and characteristics of the output curve will be distinguishable from background noise of the calorimeter output,
- use of a Styrofoam box to minimize heat loss during microwave exposure,
- exact replication of the equilibration and exposure procedures, and
- digitization of the calorimeter output and computerized data acquisition, data storage and data analysis so that soft and hard copies of the data can be easily reproduced.

A gradient-layer Seeback Envelope Calorimeter (Thermonetics Model SEC-A-1202) with internal dimensions of 30 x 30 x 30 cm was used. The calorimeter output constant was calibrated by an electrical heater, distilled water samples at a fixed temperature but variable masses, and distilled water samples at fixed mass but variable temperature. The output of the calorimeter was monitored by a nanovoltmeter (Keithley Model 181). The digitized output of the nanovoltmeter was recorded every 15 s by a personal computer equipped with a data-acquisition hardware using a data acquisition program written in QBASIC. The calorimeter was equilibrated for a minimum of 30 minutes before introduction of the specimen.

Twenty-nine fresh Sprague-Dawley rat carcasses with an average body mass of 327 grams were used. Rats were euthanized with carbon dioxide. A thermistor based temperature probe (Yellow Spring Instruments, YSI 423) was inserted 5 cm into the colon of the carcass and then the carcass was placed in a plastic bag and submerged with a counter-buoyancy weight into a precision water bath (Neslab RTE 110, set at 26 °C) for equilibration to predetermined temperature (26 °C). Carcass temperature was assumed to be uniform when the colonic temperature equaled the bath temperature for at least 3-5 minutes. The equilibrated carcass was quickly transferred into a Styrofoam box and placed at the exposure position in the anechoic chamber for a 40 s sham or microwave exposure.

The microwave exposure was a 1.25 GHz pulsed microwave in an anechoic chamber equipped with a corner reflector. Exposures were sham (0 W) and pulsed microwaves at 500, 1,000 or 1,500 W (1 MW peak power, 10 μs pulses at 50, 100, 150 Hz pulse repetition rates). Each exposed carcass was transferred immediately into the calorimeter. Calorimeter output was recorded at least 30 minutes prior to the exposure and continued until the calorimeter output returned to the baseline value. The duration of data acquisition lasted approximately 4 h. Eight carcasses were used in sham exposures and seven carcasses were used for each power density level (0.75, 1.50 and 2.25 W/cm^2 corresponding to a 500, 1,000 and 1,500 W incident power).

The energy content of the sham exposed carcass was 11.2 J/g under the current procedure. The energy content of the carcass increased with the power density of the pulse microwave exposure. Linear regression of all the data yielded a slope of 6.44 J/kg per mW/cm^2 ($r= 0.997$; $df= 27$, $p<0.005$). The correlation coefficient (0.997) indicated a very small deviation from linearity, i.e., 0.6 %. For 40 s exposure time, the normalized whole body specific absorption rate was 0.16 W/kg/mW/cm^2. The free field whole-body average absorption rate was calculated to around 0.37 W/kg/mW/cm^2 without the corner reflector. It would appear that absorption cross-section in presence of the corner reflector reduced to 43% of the free field radiation of the same field intensity. On the other hand, a 10 dB field enhancement was achieved by the presence of the corner reflector. Therefore, the overall absorption enhancement ratio of the corner reflector was 4.35 or 6.36 dB. A highly reliable and cost-effective whole-body calorimetric dosimetry method is developed. The procedure used can also be extended to determine the complex specific heat capacity of the animals [Mathur, Akyel and Lu 1991; 1992].

Subcutaneous And Skin Surface Temperature Increments in Rats Exposed to One-second Gated CW Microwaves

(In Support of the Protocol N-04-91, Modification of Acoustic and Tactile Startle by Microwave Pulses)

Microwave-evoked body movements were initially considered to be a representative example of "non-thermal" biological effects and/or direct neural effects of radiofrequency radiation including microwaves. A non-thermal mechanism was alleged on two observations:

- microwave-evoked body movements occured in the absence of increased rectal temperature, and
- the effective amount of microwave energy, when averaged over an entire body, was equal to less than the amount of thermal energy to cause a 0.1 °C increase which is considered to be insignificant biological noise.

However, recent studies of the microwave evoked body movements in mice (Protocol N-11-88, Reflexive Responses Evoked by High Peak/Low Average Power Microwaves Pulses) have indicated that the "non-thermal" concept of the microwave-evoked body movements was inappropriate because the microwave-evoked body movements occurred in absence of a change in rectal temperature but in the presence of significant subcutaneous temperature change.

Microwave evoked body movements were similar to "startle". A second species of laboratory animal, the rat, was selected for use in studying the microwave-evoked body movements for two reasons. These are that previous "startle" studies have used rats and that it represents good proctice to extrapolate to man based on research on more than one species of experimental animals. In addition, there exists substantial published knowledge and data-bases on startle and neural circuits for comparison. A 1.25 GHz L-band waveguide exposure system (Protocol A-01-90, Pilot Study for Modification of Startle by Microwave Pulses) was designed for this purpose. Due to similarities in incident fields and head structures between mice and rats, it was considered prudent to evaluate subcutaneous temperatures of the rat exposed to microwaves.

Each rat was anesthetized with sodium pentobarbital intraperitoneal injection (60 mg/kg). The hair was clipped from the head and neck regions. An 18 G thin-wall hypodermic needle was inserted from the neck region to the tip of the nose following the midline of the head. A microwave transparent temperature probe (Luxtron MAM-05) was inserted through the bore of the hypodermic needle. The hypodermic needle was then withdrawn to the back of the Luxtron probe (12") connector. The temperature probe was advanced carefully to the tip of the nose which provided sensing points at 0.5, 1.0, 1.5, and 2 cm from the tip of the nose. A second microwave transparent temperature probe (Luxtron MAM-05) was attached to the dorsal skin surface in parallel to the first probe with a piece of labeling tape. The incident powers were 40, 80 and 160 W in the L-band waveguide exposure system for 1 s. Four replicate exposures were studied.

The same animal was also used for studying the subcutaneous and rectal temperature changes in the neck region and in the colon. The MAM-05 probe was again inserted through the bore of an 18 G hypodermic needle which was inserted under dorsal skin between scapulae and following the midline of the neck and the head to 1.7 cm from the tip of the nose. The 4 sensors of the MAM-05 probe were at 2.2, 2.7, 3.2 and 3.7 cm from the tip of the nose. The position of 2.2 cm from the tip of the nose was approximately at the outside of the waveguide. An additional probe was inserted into the rectum to a depth of 5.5 cm which provided 5.0, 4.5, 4.0 and 3.5 cm depth from the rectal orifice. The incident powers were 40, 80 and 160 W. Four replicates were used for each power level.

Both raw data and 0.1 Hz low-pass filtered data from Luxtron 3000 were collected with a computer acquisition hardware and software system (ASYSTENT+) at a 10 Hz acquisition rate. The data acquisition periods were 10 seconds before exposure, 1 second of exposure and 14.6 seconds after exposure. The 10 seconds pre-exposure timing was maintained precisely for marking the beginning of the exposure in order to capture the 1 s temperature rise due to the microwave absorption. A total of 192 files (4 replicates of 16 different tissue sites subjected to 3 power levels) were analyzed.

None of the 4 rectal temperatures showed a discernable change. On the other hand, 0.11 ± 0.03 °C (mean ± S.E., n=4) to 0.23 ± 0.01 °C could be noted in the cranial subcutaneous tissue of the rat exposed to a 40 W incident power. The temperature increment due to a 1 s 80 W pulse ranged between 0.28 ± 0.01 °C and 0.33 ± 0.04 °C in the exposure area, peaking anteriorly at 0.5 cm from the tip of the nose. The temperature increment dropped significantly posteriorly away from the exposure to 0.19 ± 0.02 °C at 2.2 cm from the tip of the nose and blended with the noise of the instrument, 0.09 ± 0.01 °C. At 160 W incident power, the range of temperature increments were between 0.55 ± 0.03 and 0.65 ± 0.04 °C in the exposure area and between 0.35 ± 0.02 and 0.15 ± 0.01 °C. Therefore, the microwave induced subcutaneous temperature increase was clearly evident and different from the temperature profile in the mouse exposed in a different waveguide exposure setups. The magnitude of the temperature increase in the exposed area of the rat was more uniform than the mice.

The incidence rates of microwave-evoked body movements were 86 % at 160 W, 41 % at 80 W, and 16 % at 40 W. The rate of spontaneous body movements in sham exposure was 8 %. The incidence rate of the microwave evoked body movements was proportional to the incident power and to the average subcutaneous temperature increment. Extrapolation of the linear curve to the incidence rate of the spontaneous body movements (absolute threshold) yielded a 22 W incident power or a 0.12 °C subcutaneous temperature increase in the cranial region of the rat. The whole-body average absorption rate was 73 W/kg (22 W / 0.3 kg) at the extrapolated threshold. The effective absolute threshold converted from a 0.12 °C/s was 417 W/kg (4.18 J/cal x 830 cal/°C g x 0.12 °C/s). In the mouse, the corresponding extrapolated whole-body average threshold was 300 W/kg and the subcutaneous threshold was 1,000 W/kg. It would appear that the rat was even more prone to exhibit evoked body movements by microwave exposure than the mouse. The extrapolated whole-body threshold for microwave-evoked body movements in the mouse and rat were lower than the current IEEE/ANSI personnel protection guideline for a controlled environment if it only occurs as one pulse in every 6 minutes. The extrapolated local SAR for evoked body movements in the mouse (2.8 W/kg) was less than the allowed local SAR (8 W/kg) in a

control environment and in the rat (1.2 W/kg) less than the allowed local SAR in an uncontrolled environment (1.6 W/kg) if it occurs as one pulse in every 6 minutes. The progress of this research can have a profound impact on the setting of standard [Raslear, Akyel, Lu, Swearengen, Varle, DeAngelis and Seaman 1992].

Tempo Dosimetry

(In Support of Protocol N-21-88, Nervous System Effects of TEMPO Radiation I, and Protocol N-06-90, Time Perception in Rats: The Effect of High Power Pulsed Microwave Irradiation)

The Transformer Energized Megavolt Pulsed Output (TEMPO) transmitter is a 3 GHz power source with extremely high peak power (700 MW), narrow pulse width (80 ns), slow pulse repetition rate (0.125 Hz) and low duty cycle (10^{-8}). The transmitted power varies, a characteristics inherent to the virtual cathode oscillators, from less than 100 MW to 700 MW within the pulse. In terms of energy transmitted ($\approx$ 16 J), the TEMPO pulse may be represented by a rectangular pulse of 200 MW for 80 ns (16 J / 80 ns). The average transmitted power is 2 W (200 MW x 10^{-8}). A longitudinally slotted circular waveguide is used as an antenna which radiates a fan-shape (narrow horizontal, wide vertical) horizontally polarized beam. Subjects were exposed in a dual corner reflector assembly, 2.25 m from the antenna. The corner reflectors provide a 10 dB enhancement of the free field power density. Therefore, the free-field power density was 0.1 mW/cm^2 per watt transmitted power and 1 mW/cm^2 with the corner reflector. Thus, at the peak transmitted power (700 MW), TEMPO's exposure system achieves a power density of 700 kW/cm^2 or an E-field strength of 1.6 MV/m with the corner reflector. These pulse characteristics are quite different from those in the Cober exposure system which provided 1.25 GHz, 1 MW (variable peak power), 10 μs rectangular pulses up to 150 Hz. Direct application of the Cober dosimetry data is hindered by the difference in carrier frequency.

Microwave dosimetric methods (whole-body average SAR by calorimetric method and local SARs by thermometric method) developed for the Cober exposure system were applied to quantify the energy coupling into the experimental subjects, in this case, rats. For this dosimetry, the TEMPO transmitter was replaced by a CW power source operated at 1 kW to provide adequate energy coupling for calorimetry and thermometry.

Carbon dioxide euthanized rat carcasses equilibrated in 25 °C water bath were

used for the whole-body average SAR determination by a calorimetric method. They were TEMPO-exposed or sham-exposed in a Styrofoam box to prevent heat loss. The difference between normalized heat contents of TEMPO-exposed and sham-exposed carcasses divided by the duration of exposure was converted to whole-body average SAR. The whole-body average SAR was 0.036 W/kg per watt transmitted or 0.072 W/kg at full power (2 W) of the TEMPO exposure system. The peak whole-body average SAR was 25.2 MW/kg or 7.2 MW/kg averaged over the 80 ns pulse duration.

Twenty-four different locations, including brain, skin and colon were selected for the thermometric dosimetry. Deep anatomic locations were rhinencephalon, anterior cerebrum, mid cerebrum, cerebellum, and rectum/colon locations 3.0, 3.5, 4.0, and 4.5 cm into the anus. Sixteen subcutaneous locations were selected to represent the anatomical cross section of the rat since absorption patter of a 3 GHz carrier frequency would not be uniform and the air-tissue interphase and small anatomical cross section would both enhance absorption. Therefore, 8 dorsal cranial/facial locations were selected: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 cm from the tip of the nose. Four dorsal tail locations (0.0, 0.5, 1.0 and 1.5 cm from the base of the tail) were used to represent another sudden decrease in anatomical cross section in the rat. To represent a larger anatomical cross section, 4 dorsal subcutaneous locations at the back (5.5, 6.0, 6.5 and 7.0 cm from the head, corresponding to lower chest and upper abdominal cavities) were used. Four replicates of 20 s exposure at 1,000 W incident power were used. A total of 96 temperature files were evaluated.

The rat absorbed 3 GHz microwave in the TEMPO configuration non-uniformly. Two subcutaneous SAR hot spots were noted at 0.5 cm from the tip of the nose (0.276 W/kg per watt transmitted) and 1.5 cm into the tail (0.257 W/kg per watt transmitted). However, the SAR at the base of the tail was not measurable and increased gradually from the base of the tail to the tail tip. A Standing wave pattern was noted at the facial and colonic/rectal regions. In fact, two of the deeper locations in the colon proved unmeasurable while the remaining two absorbed at less than 10 % of the SAR hot spot. The SARs in the brain ranged between 0.057 and 0.078 W/kg per watt transmitted power indicating a relatively uniform absorption region. The brains absorbed about 25 % of

the energy absrobed by the SAR hot spot. Subcutaneously, the dorsal skin absorbed 16-26 % of the amount abosrbed by the SAR hot spot. The subcutaneous hot spots produced by this longetunidinally slotted circular antenna are interesting. These hot spots were localized where vibrissae and bristle hairs were concentrated. Each of these structures are considered organs of touch in this species of animal as they have high concentrations of mechanical receptors. Microwave evoked body movements may have a lower threshold under this exposure configuration than under other whole body exposure configurations that provide a more uniform SAR profile or produce a SAR hot spot elsewhere [Raslear, Akyel, Bates, Lu, Elson, Akyel and Mathur 1993; Raslear, Akyel, Bates, Belt, and Lu 1993].

Effect of Interaction Between Ambient Temperature and SAR on Microwave-Induced Hyperthermia In Rats

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The most investigated and documented effects of radiofrequency radiation including microwaves on biological tissue is the transformation of absorbed energy into kinetic energy of the absorbing molecules, which produces a general heating in the absorbing medium. This process creates an increase in exogenous heat load to the normal metabolic heat load. A homeotherm is capable of maintaining the core body temperature in a very narrow range, a condition known as euthermia, when it is exposed to various ambient temperatures. Homeotherms maintain this euthermic state by two major mechanisms, behavioral and autonomic thermoregulation. Behavioral means of controlling or selecting a micro-environment may not always be available, or a homeotherm may intentionally override behavioral thermoregulation during engagement in other more urgent activities. It also requires energy expenditure to perform behavioral thermoregulation, therefore, additional metabolic heat load. Autonomic thermoregulation maintains a constant core body temperature by activating heat dissipation and/or heat conservation mechanisms without the awareness of the host. Hyperthermia, in contrast to fever, is from an exogenous heat source and a state of elevated core body temperature without changes in the thermoregulatory set point. The body temperature is being controlled by a controller and it is also a signal source providing inputs for the controller (hypothalamus) to integrate and to initiate the effector. It is hard to imagine a change in thermoregulation without a change in body temperature whether the change is detected by the investigator or not. Thermosensors are located in the peripheral superficial tissue (skin) and central (hypothalamus and extrahypothalamus)/deep body tissues. While the peripheral superficial tissue is influenced by ambient condition, the central/deep body tissue remains relatively constant in various ambient conditions. The skin also serves as an important thermoregulatory effector wherein the majority of the radiant, conductive, convective, and evaporative heat

loss occur.

It has been known in dogs that the magnitude of microwave-induced hyperthermia, defined as an elevation in core body temperature, was affected by ambient temperature. The majority of studies on the biological effects of radiofrequency/microwave radiation utilized rodents exposed to such radiations, however, these exposures were under various ambient conditions. The potential interaction between radiofrequency/microwave energy and ambient condition on hyperthermic potency have not been elucidated completely. Since the majority of reliable and replicable biological effects of radiofrequency/microwave radiation are usually accompanied by an increase in core temperature, it is important to assay the hyperthermic potency of radiofrequency/microwave radiation under various ambient conditions. In addition, the tail is a major organ for heat loss and thermosensor organ in the rat. Therefore, it was also included in this study.

Sixty-four to 65-day-old male Long-Evans hooded rats were used. Their body weights were 310 ± 18 g (mean ± S.D., n=184) ranging between 260 and 378 g. They were acclimated and gentled to adapt them to experimental procedures and the unfamiliar stimuli which could contaminate the experimental results. Rats were subjected to 23 different treatments in foamed polystyrene cages (20x20x30 cm) with polystyrene grid bottoms and fiberglass screen tops. They were unanesthetized and unrestrained during these experiments. Rats were subjected to these experiments four at a time in an anechoic chamber (1.2x1.2 m) which was housed in a customized environmental chamber (1.8x3.0x1.8 m). The average air flow inside these cages was 1.6 m/s. The treatments included various ambient temperatures (18, 24, 28, 32, and 36 °C) and various microwave exposures (0.2, 2, 4, 6, 8, 10, and 10 W/kg whole body average specific absorption, SAR) each at three different ambient temperatures (18, 24, and 28 °C). The ambient temperatures were maintained within ±0.15 °C and relative humidity was maintained between 45 and 50 % at each ambient temperature. The animal was equilibrated at 24 °C for 2.5 h and remained undisturbed for an additional 0.5 h during the switching to a new ambient temperature. The duration of exposure was 2 h to allow animals to fully readjust to the exposure regardless whether changes in ambient

temperature or microwave exposure/ambient temperature were made. Colonic temperature (5 cm into rectal orifice, YSI 423) and tail skin temperature (0.5 cm from the base of the tail, YSI 427) were taken immediately after the exposure at 1400 hours, the mid-light point of 12L/12D cycle (light on: 0800 to 2000 hours). The 2.45 GHz continuous wave microwaves were used. Statistical analysis included analysis of variance, a *post hoc* Dunnett's test, student t-test, and regression analysis. The null hypothesis that no change existed was rejected at the $\alpha = 0.05$ level.

Rats were able to maintain a constant colonic temperature in ambient temperature ranging between 18 and 28 °C. However, hyperthermia was noted in rats subjected to 32 and 36 °C. Microwave exposure could cause an increase in colonic temperature in rats. The minimal hyperthermic microwave SAR (threshold SAR) was inversely proportional to the ambient temperature. They were 8 W/kg at 18 °C, 6 W/kg at 24 °C, and 2 W/kg at 28 °C. The colonic temperature increments was roughly proportional to SAR once the threshold SAR was exceeded. The microwave hyperthermic potencies were 0.31 °C/W/kg at 18 °C, 0.37 °C/W/kg at 24 °C, and 0.46 °C/W/kg at 28 °C. Therefore for a microwave SAR, it will be less likely to observe a colonic temperature change as the ambient temperature becomes lower. The hyperthermic potency of a given SAR increased proportionally with the ambient temperature.

The tail skin temperature increased proportionally with ambient temperature. The potency of ambient-air-induced tail temperature increment was 0.39 °C tail skin temperature increase for every 1 °C increase in ambient temperature. The minimal threshold SAR that could induce a tail skin temperature increase was 2 W/kg at 18 °C, 4 W/kg at 24 °C, and 2 W/kg at 28 °C. Tail skin temperature was significantly correlated with SAR at each ambient temperature (correlation coefficient, $r = 0.97$ to 0.99, $p < 0.005$). The potencies of microwave exposure on tail skin temperature were 0.49 °C/W/kg at 18 °C, 0.49 °C/W/kg at 24 °C, and 0.48 °C/W/kg at 28 °C. They were not different from each other. Therefore, microwave absorption could be equated to change in ambient temperature without a complex formula, i.e., each 1 W/kg of microwave absorption in rats could be equated with a 1.26 °C increase in ambient temperature.

The relation between colonic temperature and tail skin temperature assumed a "hockey stick" relation. A demarcation tail skin temperature (31.8 °C) clearly divided the euthermic (constant core body temperature and variable skin temperature) and hyperthermia (core body temperature above normal) of the homeotherm. Microwave exposures appeared to be similar to changing ambient temperature. Therefore, the threshold of the radiofrequency/microwave thermal effect can be modified by ambient temperature. The influence of the ambient temperature on manifestation of the biological effect of radiofrequency/microwave radiation should be considered in protection guideline setting [Lu and Michaelson 1992; 1993].

Pulsed Microwave Ocular Exposure of the Monkey

(In Support of the Protocol O13-88 and Protocol N-O16-92, High-Peak-Power Microwaves: A Health Hazard. PI: Henry A. Kues, Johns Hopkins University)

Research in biomedicine is of interest to the Department of Defense, particularly in providing insight into the processes by which disease, trauma, or environmental stresses affect living systems. In the framework of this interest, there is a growing concern over potentially hazardous biological effects due to extended low-level or "unique" exposures to non-ionizing radiation (radiofrequency/microwaves). In recent years the number of devices, both civilian and military, which produce this type of radiation, has proliferated. In the performance of their duties some military personnel are exposed to microwave radiation on a daily basis. In most cases these daily exposures, from radars and various communication equipment, are below levels thought to be of biological significance. However, with the move toward radars of higher peak power and the development of new high-peak-energy devices, present human safety guidelines may not be adequate. This is especially apparent when considering the fact that the present scientific base for understanding the results or implications of repeated low-level or single or multiple high-peak pulse exposures is virtually non-existent or controversial at best.

It has been demonstrated that low-level non-ionizing radiation may present a hazard to human ocular health. Exposure to low-level pulsed microwaves can cause corneal endothelium cell death and disruption, iris vascular leakage, choroidal leakage, photoreceptor (both cone and rod) damage, and changes in electroretinography. The primary objective of this study was to establish whether high-peak pulsed microwaves constitute more or less of a hazard to human eyes that may be exposed than previously known. To reach this objective, the subhuman primate (Macaca fascicularis and the Macaca mulatta) is an excellent model in that the anatomical structure and physiology of these primate eyes are almost identical to that of human eye. This study is relevant to soldier's health in defining the potential for ocular trauma caused by the radiofrequency/microwave radiation in a controlled laboratory environment.

Male or female Rhesus monkeys (Macca mulata or depending on availability Cynomolgus monkeys, Macca fascicularis) were used at 3 years of age. Prior to exposure by the microwave irradiation, the primates were subjected to the following diagnostic procedures at the Wilmer Institute to establish baseline normality. For handling purposes the animals were given an intramuscular injection of ketamine hydrochloride, 100 mg/kg. Each animal's throat was sprayed with 2 % lidocaine to prevent laryngeal spasm, intubated, and the animal maintained on halothane gas anesthesia, using a Fraser Harlke small animal anesthesia machine. The following ocular diagnostic procedures for baseline were performed under halothane anesthesia:

- Baseline Ocular Fluorophotometry
  Each primate was given a 0.2 ml IV injection of sodium fluorescein for fluorophotometry. This procedure was repeated on three separate occasions to establish normal curves.
- Electroretinographic Responses (ERG)
  a) White light stimulus
  b) Photopic
  c) Scotopic
  d) Flicker,
- Anterior chamber examination and photodocumentation
  Slit-lamp examination of the cornea, lens, iris, aqueous,
- Corneal endothelial examination and photodocumentation
  a) Visual examination with specular microscope
  b) Contact specular photomicrograph of the corneal endothelial cells,
- Retinal examination and photodocumentation
  a) Visual funduscopic examinations
  b) Color fundus photographs (8 stereo fields)
  c) Cross polarization fundus photographs.

Prior to initiation of the microwave exposures, all experimental subjects were placed in restraining chairs and allowed to acclimate to the handling and restraint

procedure. For microwave exposures, these animals were transported from the Wilmer Institute to the Walter Reed Army Institute of Research, Department of Microwave Research in an appropriate transport cage in an environmentally-controlled vehicle provided by Johns Hopkins Applied Physics Laboratory.

Upon arrival at the Walter Reed facility each primate was given an intramuscular injection of ketamine hydrochloride (10 mg/kg) and positioned in a custom-made restraining chair. The animal was allowed to recover from the ketamine, then positioned in front of the microwave source for a period of four hours over three consecutive days each week. Throughout the exposure, subjects were visually monitored on closed-circuit television for signs of undue stress or other problems such as movements of the subject. Significant misalignments between the monkey and the antenna were corrected by interrupting the exposure for realignment.

After the daily exposure, each animal was given another IM injection of ketamine and returned to its cage at Walter Reed facility. Following the third day's exposure, each monkey was returned to the Wilmer Institute for diagnostic fluorophotometry. After three weeks of exposure, a complete set of ocular diagnostic procedures was performed for comparison. Each animal was then sacrificed with an IV overdose of sodium pentobarbital (> 100 mg/kg) and the eye enucleated for histologic examination.

The personnel at the Department of Microwave Research, Division of Neuropsychiatry, Walter Reed Army Institute of Research, namely, the Ogden BioServices personnel were to provide technical support in constructing an appropriate monkey restraining chair, microwave exposure and monitoring equipment, and provide routine maintenance of the monkeys at the Walter Reed facility. The microwave exposure equipment used was a Cober transmitter fitted with power monitoring devices, and a open end L-band waveguide as an antenna. These exposures were performed in an anechoic chamber under the Standard Operating Procedure for Cober transmitter operation, maintenance, and RF equipment calibration. The Cober transmitter was operated at 1.25 GHz in pulsed mode. The peak power was set at 1 MW while the pulse width and pulse repetition rate varied according to request made by the principal

investigator in advance.

Because the contractor was not privy to the results of these investigations, the following results were extracted from a series of abstracts published by the principal investigator [Kues, McLeod, D'Anna, Lutty, Gambrill and Elson 1989; Kues, McLeod, D'Anna, Johnson, Perry and Monahan 1991; Kues and Monahan 1992; Kues, D'Anna, Johnson, Lutty, and Monahan 1993]. Anesthetized adult Cynomogus monieys were exposed for four hours per day on two consecutive days to 1.25 GHz microwaves (1 MW peak power, 10 μs pulse width, 0.225 Hz pulse repetition rate) at 12.5 mW/cm^2. The average ocular SAR was 3.6 W/kg at the center of the eye. Fluorescein iris angiography was performed following the last daily exposure. Wide field specular microscopy and slit-lamp examinations were conducted 24 hours later. Several animals were sacrificed for histologic examination after three 2-day exposure sessions (6 exposures total) spaced at 2-week intervals. Results indicated that ocular changes were similar to those produced by 2.45 GHz radiation, including corneal endothelial changes, iris vascular leakage, formation of macro-melanosomes and melanosome complexes in the ciliary body pigment epithelium, vacuolation of the iris posterior pigment epithelium and disruption of photoreceptor outer segments.

Electroretinograph, a non-invasive diagnostic technique was used to evaluate the microwave induced retinal injury in adult rhesus monkeys. Baseline electroretinogram (ERG) measurements were obtained before microwave exposure and after 7 four-hour exposures to pulsed microwaves (1.25 GHz, 1 MW peak power, 0.5 μs pulse width, 16 Hz pulse repetition rate) at an average ocular SAR of 3.5-4.0 W/kg. During exposure, all subjects were nonanesthetized but restrained. A comparison between pre- and post-exposure ERGs indicated a 50% reduction in the scotopic single flash response and a 90% reduction in the 30-Hz flicker response following microwave exposure. The single flash photopic response was completely extinguished in the post-exposure ERG. A significant time delay in the electrophysiological response following light stimuli was noted. Result of ERG measurements made 1 week after the last microwave exposure showed a return of all ERG components to normal. The ERG changes pointed to a high probability of cone photoreceptor damage. Subsequent histological examination of

6the exposed eyes confirmed the degeneration (karyolysis) of cone photoreceptors in the fovea and macula of the eye where a high concentration of cone photoreceptors are found. These results indicated that a specific population of cones were being selectively damaged. In addition, corneal endothelial lesions and increased permeability of the iris vasculature were also demonstrated in these monkeys exposed to 1.25 GHz pulsed microwave without anesthesia. These ocular injuries were not observed in monkeys exposed to a 2.85 GHz pulsed microwaves at a similar SAR (3.5 W/kg, 1 μs pulse width, 20 Hz pulse repetition rate).

Modification of Startle by Microwave Pulses in the Rat

(Protocol A-01-90, Pilot Study for Modification of Startle by Microwave Pulses and Protocol N-04-91, Modification of Acoustic and Tactile Startle by Microwave Pulses)

One type of electromagnetic energy in the environment of Army personnel consists of high power microwave pulses from radar transmitters and other generators. The pulses are too brief and infrequent to cause a general heating of the whole person. On the other hand, pulses of sufficient intensity are known to result in animal and human auditory sensations and in modification of the reflexes of mice. As higher and higher peak powers are used, it becomes increasingly necessary to study biological effects over a range of pulse intensities to understand the potential impact of exposure on Army personnel.

The startle response consists of a sequence of reflexive muscular contractions which are elicited by sudden, intense stimuli. The response begins within a few milliseconds of a startling stimulus and runs its course within a hundred milliseconds or so. Startle has been observed in many mammals, including humans, and is thought to be related to rapid escape responses. Startle in the rat, the most commonly used experimental animal, is elicited by certain acoustic and tactile stimuli. The startle response evoked by intense acoustic pulses, or "acoustic startle," is most often studied. In the rat, pre-pulses (stimulus preceding the startle stimulus but incapable of causing startle by itself) of photic, tactile, and acoustic stimuli have been shown to modify startle amplitude and latency when presented in respective effective time windows preceding the startle pulse.

The pulse energy that could elicit the microwave-evoked body movements (a form of startle) was higher than the threshold for the microwave acoustic effect. The dependence of the microwave-evoked body movements on microwave hearing needs to be evaluated. Therefore, two hypotheses were proposed as the main thrust of the proposed research. They were:

- Microwave pulses cause the same modification of acoustic startle as do sensory stimuli, and
- A functional auditory periphery is necessary for microwave pulse modification of startle.

In order to perform these tasks, a new microwave exposure apparatus was designed and constructed, and a surgical procedure for deafening the animal was developed. The development of deafening procedure was in corporation with the Division of Veterinary Medicine, Walter Reed Army Institute of Research.

For startle measurement, a ventilated plastic cylinder which restricts extraneous motion and contains a piezoelectric motion detector was designed and fabricated. The microwave exposure device was a downward pointing WR650 waveguide with an endplane short and a hole in one broad wall to accept the cylindrical animal holder. The ventilated cylinder was placed on a support made from a semicircular metal tube mounted to the waveguide and moved through the waveguide hole to position the head of the animal inside the waveguide. A speaker was located on the opposite broad wall of the waveguide to deliver startling acoustic stimuli and acoustic pre-stimuli through a metallic screen. The startling acoustic stimulus was gated white noise with a duration of 50 ms, onset and offset times of less than 1 ms, and intensity of 100-120 dB SPL (sound pressure level) in the animal holder. The acoustic pre-stimuli were either a gated tone or a click. The frequency range of the tone was 5-10 kHz with an intensity of 50-80 dB SPL. The pre-stimulus tone preceded the startling stimulus by 0-200 ms, lasted 100-200 ms with onset and offset times of 5 ms. The click was generated by a 80-120 μs voltage pulse applied to the speaker producing a 50−80 dB SPL sound, and preceded the startling stimulus by 0-200 ms.

Pulses of Air (puffs) were applied to the back of the animal through a hole in the cylindrical animal holder for startling tactile stimuli. The hole accepted a plastic tube which was connected to a compressed air source. An electrically activated valve is briefly opened under computer control to apply a short burst of air to the animal. The air

pressure was 48-52 psi (330-350 kPa).

The microwave exposure apparatus with speaker/air puff attachments was mounted inside a ventilated sound attenuating chamber. The background noise level was 67 dBA. Microwave pulses at 1.25 GHz could be transmitted through WR650 waveguide equipped with directional couplers and crystal detectors for microwave power measurements. The brain SAR was determined to be 18.8–21.5 W/kg per watt transmitted. For precision and/or accuracy, the presentation of the acoustic, tactile and microwave pre-stimuli and stimuli was controlled by the computer.

The amplitude of the piezoelectric film output was used to measure the amplitude of acoustic startle response and latency from termination of the acoustic startling stimulus (100 dB SPL, 50 ms) to response (startle) were used. Acoustic pre-pulses (tone, 60 dB SPL, 100 ms before stimulus) were effective in prolonging the latency of response and suppressing the startle amplitude. This result indicated the appropriateness of the exposure apparatus and animal model selected.

Initially (Seaman and Beblo 1992), the modification of acoustic startle by microwave pulses was tested in 4 Long-Evans rats. A 1.25 GHz microwave pre-pulse (100 ms before acoustic startling pulse) of 1.1 kW peak power (0.8-1.0 μs pulse width, 23-48 kW/kg brain absorption rate, 22-43 mJ/kg brain specific absorption per pulse) could not suppress the amplitude of the acoustic startle (100 dB SPL, 50 ms) but prolonged the latency of the acoustic startle. A microwave pre-pulse (100 ms before acoustic startling pulse) at 5.2 kW peak power (0.8-1.0 μs, 63-111 W/kg brain absorption rate, 59-107 mJ/kg brain specific absorption per pulse) failed to modify either latency or the amplitude of the response to the acoustic startling stimulus (100 dB SPL, 50 ms).

Modification of the acoustic startle by microwave pulses was further tested in a larger group of male Long Evans rats (twelve), each subjected to 10 presentations of 8 experimental conditions over 5 testing sessions [Seaman, Beblo and Raslear 1992a; 1993a; 1994]. Each experimental condition was presented in random order. Microwave pre-pulses were 1 μs pulse of the 1.25 GHz microwave operated at 1.5 and 5 kW peak

powers which delivered 30 and 100 kW/kg average brain specific absorption rates or 30 and 100 mJ/kg brain specific absorption. The acoustic startling pulses were 50 ms broad-band noise at 106 dB SPL. The 8 experimental conditions were microwave pre-pulse only, acoustic startle only, and microwave pre-pulses delivered at 201, 101, 51, 3 and 1 ms before, and 1 ms after the acoustic startling pulse. Startle amplitudes were measured from strip chart records.

The 1.5 kW microwave pre-pulse failed to modify the amplitude of the acoustic startle. In contrast, the 5 kW microwave pre-pulse significantly suppressed the amplitude of the acoustic startle with a lead time between 51 and 201 ms. Therefore, microwave pulses of adequate intensity could modify (suppress) startle if it was applied in a short period before the startling stimulus. These results appeared to be inconsistent with the absence of acoustic startle modification by a similar microwave pre-pulse in the initial experiment using a different group of animals. The apparent inconsistency could have resulted from a larger group of animals and better control of the microwave pre-pulses in the later experiment than in the initial experiment. It could be concluded from these experiments that there exists a threshold for microwave pre-pulses to influence the acoustic startle.

The modification of the tactile startle was evaluated in 14 animals [Seaman, Beblo and Raslear 1992b; 1993b; 1994]. The startling tactile stimulus was a 50 ms burst of compressed air delivered to the back of the neck at 48-52 psi (330-350 kPa). An acoustic click (94 dB SPL peak, 80-120 μs) was used as a positive control to identify the presence of the modification of the tactile startle by pre-pulse. The microwave pre-pulse used was a single 8 μs pulse generated by an Epsco PH40K at 5 kW peak power which provided a 100 kW/kg brain specific absorption rate and 800 mJ/kg brain specific absorption. The tactile startle modification by acoustic-click and microwave pre-pulses was tested in separate testing sessions. During each session, 6 experimental conditions were presented in random order. These experimental conditions were tactile startle only and pre-stimulus delivered at lead times of 150, 100, 50 and 0 ms relative to, and 50 ms after the onset of the startling air burst. Each animal experienced 4 presentations of each experimental condition in each testing session. Seven animals were tested with acoustic

clicks first; the other seven, with microwave pre-pulses first. The animal motion detector output was monitored on a digital storage oscilloscope and on a Gould TA2000 strip chart recorder.

For acoustic-click pre-pulse, the mean amplitude of the startle response was lower, but statistically insignificant, in 150 and 100 ms conditions than in the condition in which the tactile startling stimulus was administered alone. The startle amplitude suppression could be noted statistically in the 50 ms condition only. The result was consistent with the notion that acoustic pre-stimulus reduced startle amplitude at lead times of 50-100 ms.

The microwave pre-pulse could not modify the tactile startle if it was administered before the tactile startling stimulus. A slight but insignificant enhancement of the startle amplitude was noted when microwave "pre-stimulus" coincided with the tactile startling stimulus. The tactile startle amplitude was significantly enhanced if the microwave pre-pulse was delivered 50 ms (-50 ms condition) after the onset of the tactile startling stimulus which lasted 50 ms. This enhancement indicated that the animal could have perceived the strength of the startling stimulus by summing the tactile startling stimulus and the microwave "pre-stimulus" which occurred immediately after the tactile stimulus.

One of the hypotheses that explains microwave-evoked body movements ("startle") was microwave hearing via thermoelastic expansion waves sensed by the cochlea. The movement of the cochlea fluid is essential in generating the auditory sensation through the auditory pathway. A deaf rat model with a bilateral patent cochlea is essential to evaluate the accuracy of this hypothesis. Deaf rats do not occur in any great frequency and ototoxicity of antimicrobials cannot produce consistent deafness by cochlea dysfunction. Therefore, it is necessary to develop a surgical procedure for deafening rats with patent cochlea to obliterate the primary site of microwave hearing.

A surgical procedure for bilateral cochleotomy in rats was developed [Swearengen, Kittell, Davis, Raslear, Beblo and Colleton 1993]. This procedure began with anesthetizing the rat with a intramuscular injection of 5 mg/kg xylazine and 45 mg/kg

ketamine hydrochloride. When a surgical plane of anesthesia was attained, which was determined by the lack of digital and corneal reflexes, the rat was given 0.027 mg/kg butorphanol tartrate subcutaneously and 2200 IU/100 g procaine/benzathine intramuscularly. The anesthetized rat was placed in a stereotaxic device which allowed vertical and long axis rotational positioning of the head. The surgical field was disinfected by applying 5 % povidone-iodine solution to pinna, external auditory canal, and surrounding areas. A 4-5 mm cutaneous incision was made starting at the intertragic notch extending ventrally down the surface of the skin surrounding the opening to the external auditory canal. The incision was extended down the ventral aspect of the external auditory canal to the external auditory meatus with a pair of iris scissors. The tympanic membrane was ruptured as the malleus was grasped with a pair of splinter forceps. The malleus (hammer bone) was withdrawn and the remnants of the tympanic membrane removed. The incus bone was removed if visible. The promontory was located in the middle ear and the lateral wall of the cochlea. The promotory was used as the drilling landmark. A 0.024" wire gauge drill bit mounted on a brass handle was used to perforate the cochlea at promotory. A blunted 26-gauge 0.5" needle attached to a tuberculin syringe fitted with a rubber bulb was used to penetrate into the cochlea and to remove endolymph and perilymph by suction. When all signs of fluid drainage were absent, a 4-5 mm piece of sterile 3-O silk suture was wedged into the cochlea through the cochlear opening with approximately 2 mm extending into the middle ear. A small piece of sterile absorbable gelatin sponge was placed in the middle ear to absorb any residual endolymph or perilymph and help to stabilize silk wick. No skin closure was performed. The rat was turned over and the same procedure was performed on the opposite ear. Sham cochleotomy included the same medications and preparations and the initial incision of the intertragic notch. The sham cochleotomy prevented any visual bias.

Postoperative cares included administration of Butorphanol tartrate (0.027 mg/kg) at 8-h interval for 72-h to alleviate pain associated with the surgery and administration of the procaine/benzathin penicillin 72-h after the surgery. Startle tests were performed between 7 and 14 days after surgery.

The startle tests included a tactile (50 ms air burst) and an acoustic (100 ms of the 117 dB SPL noise) stimuli. The threshold for auditory stimulus to elicit a startle response is approximately 90 dB SPL for rats, therefore, the acoustic stimulus (117 dB) was at least two orders of magnitude higher (more than 100 times) in intensity than the acoustic startling threshold. The air burst was used to positively identify that the startle response in deafened rats was not obliterated by the surgical procedures and to serve as the baseline for identifying the suppression of the acoustic startle in deafened rats. Twelve bilateral cochleotomized rats and two sham bilateral cochleotomized rats were tested. Tactile startle were positively identified in all these rats regardless of the type of the surgery. Acoustic startles were also present in sham-cochleotomized rats. In cochleotomized rats, the amplitude of the acoustic startle response was lower than the amplitude of the tactile startle response. In fact, the majority of the cochleotomized rats failed to shown any startle by this intense acoustic stimulus even under repetitive testing. Due to the low rate and low amplitude of the acoustic startle responses in cochleotomized rats, it could be concluded that the surgical procedure was appropriate for the purpose of this research.

Histological examination were performed at the conclusion of the development stage of the cochleotomy procedure. No evidence of secondary bacterial inflammation was observed within the semicular or spiral canals of any of cochleotomized rats. In addition, none of the test group displayed any clinical signs of vestibular dysfunction such as head tilt, circling and loss of balance. Where wick placement was found, a very localized granulomatous reaction (scar tissue) was seen immediately surrounding the wick within the cochlea.

Microwave-evoked body movements were suspected to be an end result of the microwave hearing which required an intact cochlear function. With the success of developing a cochleotomy procedure, hypothesis testing on the dependence of the microwave startle (microwave-evoked body movements) on hearing can be performed. This hypothesis was tested in 14 rats, 7 were deafened by cochleotomy and the remaining rats received sham cochleotomy. A 1.25 GHz microwave generated by a Epsco PH40 K transmitter was used as a test stimulus. Gated CWs were used instead of high peak

power pulsed microwave stimuli. Microwave stimuli of 40, 80 and 160 W for 1 s corresponding to 0.8, 1.6 and 3.2 kW/kg brain specific absorption rates were used. The corresponding brain specific absorptions were 0.8, 1.6 and 3.2 kJ/kg. These peak brain specific absorption rates were at least 1-2 orders of magnitude lower than the effective microwave pre-pulse (100 kW/kg) used for acoustic startle modification but the brain specific absorptions were at least 3 orders of magnitude higher than the effective microwave pre-pulse (0.8 J/kg). The rationale for these differences between microwave stimuli and microwave pre-pulse was to elicit startle by an intense stimulus, not to modify startle, caused by an intense stimulus, a pre-pulse which in itself could not elicit a startle. Each rat received randomly 10 microwave and 2 sham trials at each power level on two different days. Interval between trials averaged about 90 s. Outputs of the motion detector were recorded on a Gould TA2000 strip chart recorder. The progress of the experiment was monitored on a digital storage oscilloscope.

The incidence rate of the spontaneous movements were 8 and 12 % respectively in hearing (sham cochleotomized) and in deafened (cochleotomized) rats. The startle incidence rate increased with the microwave doses regardless of the surgical treatments. The startle incidence rates were 23, 56 and 72 % in the deafened rats and they were 16, 43 and 85 % in the hearing rats subjected to 40, 80 and 160 W microwave stimuli. The response curves were not different between each other as indicated by an insignificant difference between least-square fitted slopes [Raslear, Akyel, Lu, Swearengen, Varle, DeAngelis and Seaman 1992].

It can be concluded from these results that auditory sensations secondary to microwave exposure cannot account for the microwave evoked whole-body movements or startle. Since heating potential of these microwave stimuli (0.23-0.92 °C) were of biological significance, the direct effect on central nervous system could not be eliminated entirely.

CONCLUSIONS

OGDEN is determined to continue to provide, under the general guidance of the COR and appropriate WRAIR program managers, high-quality, peer-reviewed scientific journal publishable research support. Specific research protocols will focus primarily on the behavioral and biological effects of the electromagnetic radiation in the microwave region of the spectrum. OGDEN researchers will continue to investigate potential hazards of the RF Directed Energy Sources to personnel operating in a tactical environment using the existing TEMPO, COBER, UWB and EMP sources supplied by the Government.

OGDEN will continue to give priority to behavioral and CNS experiments with a commitment to highest scientific standards resulting in high quality peer-reviewed publications. We understand that the DMR research efforts in high power microwave fields have little precedent and are considered innovative and pioneering.

In recent years, scientists and epidemiologists found growing, well-publicized but inconclusive evidence that electromagnetic fields might increase the risk of certain cancers and brain tumors. Many physicists doubted any RF-cancer link because there were no plausible mechanisms by which electromagnetic fields could affect biological tissue. However, in the last two years, microscopic magnets made of crystals of the iron mineral magnetite were found in human brain. Homing pigeons, whales, salmon and some bacteria were known as having similar structures. Increasing emphasis given to avian responses after exposure to nonionizing radiation, some evidence on human visual abnormalities associated with high-energy microwave exposure, and our own finding of disturbed circadian rhythmicity in rats after exposure to TEMPO pulses provide a new ground to start a new line of research.

To determine safe exposure levels for exposure to electromagnetic radiation characteristic of military equipment and systems, we propose to study behavioral and biological effects of long term exposure to low level microwave fields. Most of the

experiments designed to date study acute effects of RF fields. However, in real life, complaints of the military personnel range from short-term incapacitation to long term health consequences. The length of the future contract will allow our scientists to design experiments that will investigate long term effects of repeated low level exposure to continuous, pulsed microwave fields, electromagnetic pulses and ultra-wide band electromagnetic radiation.

Department's new UWB exposure system will allow our investigators to study the effects of this new system in a controlled laboratory setting. Dr. Akyel already has approved protocol to study behavioral effects of UWB exposures. OGDEN staff is in the process of designing an operant chamber that will be suitable for the behavioral analysis of the rats exposed to such fields. With the approval of the COR. for acute exposure designs, OGDEN scientists would like to give emphasize on in-field experiments rather than after-effects experiments.

REFERENCES–PUBLICATION LIST

- Akyel, B., and Y. Akyel [1991]: Altruistic behavior and prosocial moral reasoning among Turkish high school students. In: Abstracts of the Third Annual Convention of the American Psychological Society, Washington, D.C.
- Akyel, Y. [1990]: Post-exposure effects of high peak power microwave pulses on operant behavior. In: Abstracts of the Second Annual Convention of the American Psychological Society, Dallas, Texas.
- Akyel, Y. [1991]. Behavioral sequelae of high power microwave fields. Current Psychology: Research and Reviews 10: 211-220.
- Akyel, Y. [1992]: Behavioral effects of high power microwaves. Invited presentation. BHS Seminar. Armed Forces Radiobiology Research Institute, Bethesda, MD.
- Akyel, Y., and M. Belt [1992]: A microwave transparent operant chamber for rats. Physiology and Behavior 51: 647-650.
- Akyel, Y., M. Belt, T.G. Raslear, and R.M. Hammer [1993]: The effects of high-peak power pulsed microwaves on treadmill performance in the rat. In: Electricity and Magnetism in Biology and Medicine, M. Blank (Ed.), San Francisco: San Francisco Press, pp. 668-670.
- Akyel, Y., M. Blair, R. Serafini, B. Akyel, and D. Varle, [1991]: Effects of high-power pulsed microwave fields on schedules of reinforcement. Proceedings of the 13th Annual International Conference of the IEEE Engineering in Medicine & Biology Society 13: 956-957.
- Akyel, Y., E.L. Hunt, F. Bates, and C. Gambrill [1988]: Effects of high powered pulsed microwave irradiation on rats responding on high and low rates. Report presented to U.S. Army Medical Research and Development Command under the contract # DAMD17-85-C-5083.
- Akyel, Y., E.L. Hunt, C. Gambrill, and C. Vargas [1991]: Immediate post-exposure effects of high peak microwave pulses on operant behavior of Wistar rats. Bioelectromagnetics 12: 183-195.

- Akyel, Y., E.L. Hunt, and C.A. Vargas, Jr. [1989]: Behavioral recovery from hyperthermia induced by high-peak power microwave pulses. Proceedings of the 11th Annual International Conference of the IEEE Engineering in Medicine & Biology Society 11: 1138-1139.
- Akyel, Y., and T.G. Raslear [1992]. Bilaterally cochleotomized rats do not startle to tactile stimulus. Abstracts of the Fourth Annual Convention of the American Psychological Society, San Diego, CA.
- Akyel, Y., and T.G. Raslear [1993]: The effects of acute EMP fields on the behavior of rats. Proceedings of the EMP Human Health Effects Science Review Panel, Williamsburg, VA, pp. 45-57.
- Akyel, B., T.G. Raslear, Y. Akyel, and R.L. Seaman [1993]: Automated measurements of the microwave-evoked whole-body movement. In: Abstracts of the 14th Annual Meeting of the Bioelectromagnetics Society.
- Akyel, B., T.G. Raslear, Y. Akyel, R.L. Seaman, S.-T. Lu, M. DeAngelis, S. Pournagash, and M. Belt [1993]: Automated measurements of the microwave-evoked whole-body movement. In: Abstract of the Fifteenth Annual Meeting of the Bioelectromagnetics Society.
- Akyel, Y., T.G. Raslear, F. Bates, and M. DeAngelis [1992]: Behavioral responses of acute EMP fields. Proceedings of the 14th Annual International Conference of the IEEE Engineering in Medicine and Biology Society 14: 287-288.
- Akyel, Y., T.G. Raslear, R.M. Hammer, and M. Belt [1992]: The effects of high-peak power pulsed microwaves on treadmill performance in the rat. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine. Lake Buena Vista, FL.
- Akyel, Y., T. Raslear, S. Mathur, R. Serafini, and F. Bates [1990]: Effects of EMP exposures on memory functions and general motor activity of Wistar rats. Report presented to U. S. Army Medical Research and Development Command under the contract DAMD17-89-C-9021.

- Akyel, Y., T. Raslear, and R. Seradini [1990]: Exposure of rodents in a parallel plate EMP simulator; behavioral effects. Proceedings of the 12th Annual International Conference of the IEEE Engineering in Medicine & Biology Society 12: 1551-1552.
- Brown, D.O., S.-T. Lu, and E.C. Elson [1994]: Characteristics of microwave evoked body movements in mice. Bioelectromagnetics 15: 143-161.
- Brown, D.O., S.-T. Lu, S. Mathur, and H. Wachtell [1991]: Randomized testing of microwave evoked body movements. Proceedings of the IEEE EMBS Conf. 13: 964-965.
- Gambrill, C.S., E.C. Elson, and S.-T. Lu [1994]: Discrepancy in rectal temperature responses in rabbits subjected to cranial pulsed and CW exposures. In: Abstracts of the sixteenth Annual Meeting of the Bioelectromagnetics Society.
- Gambrill, C.S., and S.-T. Lu [1992]: Error analysis of a thermometric microwave dosimetry procedure. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Gambrill, C.S., and S.-T. Lu [1993]: Error analysis of a thermometric microwave dosimetry procedure. In: Electricity and Magnetism in Biology and Medicine, Ed.: M. Blank, San Francisco, CA: San Francisco Press, Inc., 1993, pp 593-595.
- [1] Kues, H.A., S.A. D'Anna, M.A. Johnson, G.A. Lutty, and J.C. Monahan [1993]: Absence of comparable ocular effects in animals exposed to pulsed microwave radiation from two separate systems. In: Abstracts of the Fifteenth Annual Meeting of the Bioelectromagnetics Society.
- [1] Kues, H.A., D.S. McLeod, S.A. D'Anna, M.A. Johnson, C.R. Perry, and J.C. Monahan[1991]: Microwave-induced electroretinographic changes in the primate. In: Abstracts of the Thirteenth Annual Meeting of the Bioelectromagnetics Society.

[1] Publications that OBC personnel is not a co-author.

- Kues, H.A., D.S. McLeod, S.A. D'Anna, G.A. Lutty, C. Gambrill, and E.C. Elson [1989]: Ocular changes following exposure to high-peak pulsed 1.25 GHz microwaves. In: Abstracts of the Eleventh Annual Meeting of the Bioelectromagnetics Society.
- Kues, H.A., and J.C. Monahan [1992]: Pulsed microwave-induced ocular changes in the restrained non-human primate. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Lu, S.-T., D.O. Brown, C.E. Johnson, S.P. Mathur, and E.C. Elson [1991a]: Cardiovascular deficits induced by localized high power microwave exposure. In: Abstracts of the Thirteenth Annual Meeting of the Bioelectromagnetics Society.
- Lu, S.-T., D.O. Brown, C.E. Johnson, S.P. Mathur, and E.C. Elson [1991b]: Bradycardia and decreased pulse pressure in rats. Proceedings of the IEEE EMBS Conf. 13: 906-961.
- Lu, S.-T., D.O. Brown, C.E. Johnson, S.P. Mathur, and E.C. Elson [1992]: Abnormal cardiovascular responses induced by localized high power microwave exposure. IEEE Trans. BME 39: 484-492.
- Lu, S.-T., D. Brown, S. Mathur, C. Gambrill, R. Serafini, and H. Wachtel [1991]: Thermal nature of microwave evoked body movements. Proceedings of the IEEE EMBS Conf. 13: 997-998.
- Lu, S.-T., M.L. DeAngelis, and C.S. Gambrill [1992]: Ocular microwave thermometric dosimetry and thermometry in the rabbit. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Lu, S.-T., M.L. DeAngelis, and C.S. Gambrill [1993]: Ocular microwave thermometric dosimetry and thermometry in rabbit. In: Electricity and Magnetism in Biology and Medicine. Ed.: M. Blank, San Francisco, CA: San Francisco Press, Inc., 1993, pp 675-678.
- Lu, S.-T., C.S. Gambrill, and D.O. Brown [1994]: Validation of a thermometric microwave-dosimetry procedure *in vivo* and *in vitro*. In: Abstracts of the Sixteenth Annual Meeting of the Bioelectromagnetics Society.

[1] Publication that OBC personnel is not a co-author.

- Lu, S.-T., C.S. Gambrill, and E.C. Elson [1993]: Absence of behavioral arousal in rabbit subjected to a cranial pulsed microwave treatment. In: Abstracts of the Fifteenth Annual Meeting of the Bioelectromagnetics Society.
- Lu, S.-T., and S.M. Michaelson [1992]: Interaction between ambient temperature and SAR on microwave-induced hyperthermia in rats. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Lu, S.-T., and S.M. Michaelson [1993]: Effect of interaction between ambient temperature and SAR on microwave-induced hyperthermia in rats. In: Electricity and Magnetism in Biology and Medicine. Ed.: M. Blank. San Francisco, CA: San Francisco Press, Inc., 1993, pp. 679-682.
- Mathur, S.P., Y. Akyel, and S.-T. Lu [1991]: Whole-body dosimetry in a gradient layer calorimeter. In: Abstracts of the Thirteenth Annual Meeting of the Bioelectromagnetics Society.
- Mathur, S.P., Y. Akyel, and S.-T. Lu [1992]: Whole-body dosimetry using a single gradient layer calorimeter. Bioelectromagnetics 13: 435-438.
- Raslear, T.G., and Y. Akyel [1992]: Null responses, decision confidence, and temporal bisection in rats: The effect of high-power pulsed microwaves. *In:* Abstracts of the 63th Eastern Psychological Association Annual Meeting, Boston, MA.
- Raslear, T. G., Y. Akyel, and F. Bates [1992]: The behavioral effects of high-peak power pulsed microwave irradiation in rats. In: Abstracts of the Sixth National Conference on High Power Microwave Technology, Lackland AFB, Texas.
- Raslear, T. G., Y. Akyel, F. Bates, and M. Belt [1993]: The effects of high-peak power pulsed microwave irradiation on time bisection in rats. In: Electricity and Magnetism in Biology and Medicine, M. Blank (Ed.), San Francisco: San Francisco Press, pp. 671-674.
- Raslear, T.G., Y. Akyel, F. Bates, M. Belt, and S.-T. Lu [1993]: Temporal Bisection in Rats: the effects of high-peak-power pulsed microwave irradiation. Bioelectromagnetics 14: 459-478.

- Raslear, T.G., Y. Akyel, F. Bates, S.-T. Lu, E.C. Elson, B. Akyel, and S.P. Mathur [1993]: The behavioral toxicology of high-peak, low average power, pulsed microwave irradiation. Technical Report 001-92, Washington, D.C.: Department of Microwave Research, Walter Reed Army Institute of Research.
- Raslear, T.G., Y. Akyel, R. Serafini, F. Bates, and M. Belt [1991]: Food demand and circadian rhythmicity following high-peak power microwave Irradiation. Proceedings of the 13th Annual International Conference of the IEEE Engineering in Medicine & Biology Society 13: 962-963.
- Raslear, T.G., Y. Akyel, R. Serafini, F. Bates, and M. Belt [1991]: Memory consolidation in the rat following high-power microwave irradiation. Proceedings of the 13th Annual International Conference of the IEEE Engineering in Medicine & Biology Society 13: 958-959.
- Raslear, T.G., Y. Akyel, S.-T. Lu, J.R. Swearengen, D. Varle. M.L. DeAngelis. and R.L. Seaman [1992]: CW microwave fields evoked body movements in bilaterally cochleotomized rats. Proc. Ann. Int. Conf. IEEE EMBS 14: 295-296.
- Seaman, R.L., and D.A. Beblo [1992]: Modification of acoustic startle by microwave pulses in the rat: a preliminary report. *Bioelectromagnetics* 13: 323-328.
- Seaman, R.L., D.A. Beblo, and T.G. Raslear [1992a]: Modification of acoustic startle amplitude by microwave pulses. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Seaman, R.L., D.A. Beblo, and T.G. Raslear [1992b]: Modification of tactile startle amplitude by microwave pulses. In: Abstracts of the First World Congress for Electricity and Magnetism in Biology and Medicine.
- Seaman, R.L., D.A. Beblo, and T.G. Raslear [1993a]: Modification of acoustic startle amplitude by microwave pulses. In: M. Blank (ed.), Electricity and Magnetism in Biology and Medicine. San Francisco: San Francisco Press, 1993, pp 662-664.
- Seaman, R.L., D.A. Beblo, and T.G. Raslear [1993b]: Modification of tactile amplitude by microwave pulses. In: M. Blank (ed.), Electricity and Magnetism in Biology and Medicine. San Francisco: San Francisco Press, 1993, pp 665-667.

- Seaman, R.L., D.A. Beblo, and T.G. Raslear [1994]: Modification of acoustic and tactile startle by single microwave pulses. Physiol. Beh. 55: 587-595, 1994.
- Swearengen, J.R., C.L. Kittell, J. A. Davis, T.G. Raslear, D.A. Beblo, and C.A. Colleton [1993]: A surgical technique for bilateral cochleotomy in the Long-Evans rat. J. Invest. Surg. 6: 431-437.
- Wachtel, H., Y. Akyel, and F. Bates [1987]: Hypokinesis produced by exposure of the mouse brain to microwave pulses and pulse trains. Report presented to U.S. Army Medical Research and Development Command under the contract # DAMD17-85-C-5083.
- Wachtel, H., D. Beblo, C. Vargas, H. Bassen and D. Brown [1989]: Comparison of the efficiency of pulsed versus CW microwave fields in evoking body movements. Proc. Ann. Int. Conf. IEEE EMBS 11: 1136-1137.