Research and Development

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# **ŞEPA** Project Summary

# Immunological and Hematological Effects of Microwave Power Transmission from a Satellite Power System

Part I. Systems for Exposing Mice to 2450 MHz Electromagnetic Fields

C. K. Chou and A. W. Guy

For the engineering aspect of this study, two systems for exposing mice to 2450 MHz electromagnetic fields were developed. The first system was used to expose mice dorsally to circularly polarized electromagnetic fields. Four mice were placed in a styrofoam cage and exposed in a vertically positioned circular waveguide [Guy et al. 1979]. The temperature and humidity in the mouse holder were kept constant by forced ventilation. The uniformity of energy absorption in the four mice was found to be most optimal when the mice were exposed at a location of 5/8 of the length of the waveguide measured from the top of the waveguide where the energy had been fed. For one watt input power to the waveguide, the average specific absorption rate (SAR) was determined by twin-well calorimetry to be 3.60  $\pm$  0.11 (SEM) W/kg in mice at a body mass of 26.94  $\pm$  0.27 g. The maximum surface SAR determined thermographically was 8.36 W/kg in the head of the mouse. The second system was a miniature anechoic chamber modified from the original design by Guy [1979]. Six mice were exposed dorsally to far field plane waves in the chamber. Copper shielding and high temperature absorbing material were

lined inside the chamber to accommodate the high input power. The air ventilation at the location of the mice was separately controlled so that any heating in the absorber would not affect the animals. For one watt input power, the average SAR was  $0.17 \pm 0.01$  W/kg and the maximum surface SAR was 0.41 W/kg in the animal when exposed with body axis parallel to the E field; the SARs were  $0.11 \pm 0.01$  W/kg and 0.64 W/kg respectively when exposed perpendicular to the E field.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

#### Introduction

In studying the biological effects of microwave radiation, exposure of groups of animals to radio-frequency electromagnetic fields has been a common practice. Under normal laboratory conditions, the animals are housed in a group with water and food ad lib. These conditions can cause serious dosimetry problems during exposure to electromagnetic fields since

intensification occurs within the animals and scattering of fields occurs among the animals and the water bottle. The exposure of single subjects under properly controlled conditions in a large anechoic chamber can be prohibitively expensive. Guy and Chou [1975] and Guy et al. [1979], have described two circularly-polarized waveguide systems for exposing single small laboratory animals to 918 or 2450 MHz electromagnetic fields. To simulate free field exposure, Guy [1979] also developed a miniature anechoic chamber for exposing laboratory animals.

In this study, the authors modified the above two systems for the exposure of mice to 2450 MHz electromagnetic fields.

### **Exposure System Modifications**

#### Circularly Polarized Waveguide

The horizontally-positioned circular waveguides, as described by Guy et al. [1979], were positioned vertically for the exposure from the top of four mice in each waveguide (see Figure 1). Four 250cc polypropylene beakers provided space for housing four mice comfortably in the waveguide. The holes at the bottom of the polypropylene beaker and the top acrylic cover allowed air to flow through the space occupied by the animals. The mouse cage was placed at a distance of 5/8 of the length of the waveguide from the top of the waveguide where the transmitting transducer was located. At this position, the uniformity of the energy absorption in the animals is optimal. The lower part of the waveguide was wrapped with a plastic sheet, and a muffin fan was attached to the bottom of the waveguide for air ventilation. The fan voltage was controlled by a variable transformer to adjust the air velocity through the mouse cage.

#### Miniature Anechoic Chamber

The original design of this chamber was described by Guy [1979] (see Figure 2). The inside of the chamber was lined with a copper sheet to prevent leakage of microwaves at high intensity exposure. At the ventilation holes, copper screen mesh pads were soldered to the copper sheet. Near the wide side of the horn (Narda 644), where E fields are maximum, a section (2.54 x 30.5 x 30.5 cm) of high temperature open cell absorbing material (Eccosorb RM)\* was placed at each side of

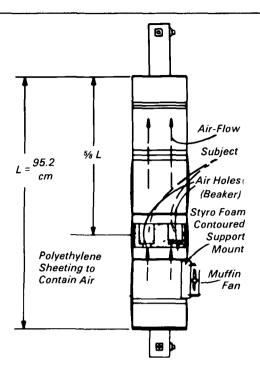


Figure 1. Modified circularly polarized waveguide for exposing mice to 2,450-MHz electromagnetic fields in environmentally controlled condition.

the horn in place of the original Eccosorb AN-77 absorber. Cooling of these absorbers was provided through the sixteen ventilation holes on each side of the chamber by two suction fans at the top. A three mil plastic sheet separated the tapered section and the bottom of the chamber so that the upper section was ventilated independently from the lower section. There are three ventilation holes (6.35 cm diameter) on all four sides of the chamber above and below the plastic sheet separation. The holes were cut at a 45° angle downward on the AN-77 absorber to minimize microwave leakage through these holes. The light bulbs used in the original design were removed since room light passes through the ventilation holes giving a more uniform lighting than obtained with the light bulbs. A muffin fan was located at the outside of the lower section to provide airflow.

#### Dosimetry

The average power density inside the circular waveguide can be estimated by averaging the input power over the cross sectional area of the waveguide. Therefore, for each watt of input power to the 20.3 cm diameter waveguide, the average power density in the waveguide is 3.1

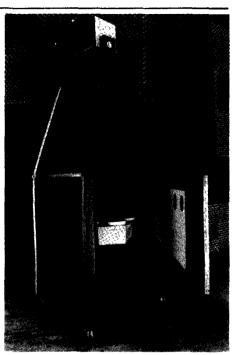


Figure 2. Front view of the modified miniature anechoic chamber for exposing mice to high intensity microwave radiation (door open to show the mouse holder).

mW/cm². In the anechoic chamber, the power density at the location of the mice (1.375 m from the horn) was mapped every cm² using an energy density meter (NBS EDMI-C4). The results are very similar to those obtained previously [Guy 1979], indicating that the modification did not alter the power distribution characteristics. For 1 W input power, the power density at the center of the chamber was 0.175 mW/cm² and 0.15 mW/cm² at the locations of the mice.

The SAR in the mice was determined using two methods. The whole body average SAR was measured with a twinwell calorimetry system. For the circular waveguide system, five dead mice were used in each calorimetry run; one control and four exposed. One of the exposed mice and the control mouse were placed in a twin-well calorimeter. It was found that when the mice were positioned radially with head toward or away from the center of the waveguide, the normalized average and standard error of SAR to 1 watt input power to the waveguide was  $3.60 \pm 0.11$  W/kg in a group of mice of  $26.94 \pm 0.27$  g in body weight. If the exposed mice were positioned perpendicular to the radial direction, the normalized SAR was 2.40  $\pm$  0.09 W/kg. For the miniature chamber, seven mice were

<sup>\*</sup>Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S Environmental Protection Agency.

used in each calorimetry run: one control and six exposed. The six exposed mice were positioned either parallel or perpendicular to the E field. Then one of the exposed mice was compared with the control mouse in the twin-well calorimeter. When the mice were exposed parallel to the E field, the normalized SAR to one watt input power to the horn was  $0.17 \pm 0.01$  W/kg. The SAR decreased to  $0.11 \pm 0.01$  W/kg when the exposed mice were oriented perpendicularly to the F field.

The two-dimensional SAR pattern was determined thermographically. Since mice are very small in mass, the method of bisection used before can cause large errors due to the heat dissipation during the data acquisition process. It was decided, therefore, to measure the surface SAR of mice. In order to avoid the messy process of removing hair from mice for better skin emissitivity, nude BALB/c mice were used. The SARs were higher in regions of mice near the center of the waveguide. The maximum normalized SAR was 8.36 W/kg in the head of the mouse exposed facing the center of the waveguide. In the anechoic chamber, the SAR was quite uniform in the mouse exposed with body parallel to the E field. The highest SAR was 0.38 W/kg at the base of the tail.

#### **Discussion**

The authors have described two systems for exposing mice to 2450 MHz

electromagnetic fields. The circular waveguide system can be used to expose four mice simultaneously to circularly polarized electromagnetic fields. The miniature anechoic chamber system can be used to expose six mice at a time to plane wave electromagnetic fields. In the anechoic chamber system, the animals are separated from each other at a greater distance than that in the circular waveguide. Therefore, the multiple scattering among the animals in the circular waveguide is larger than in the anechoic chamber. However, the SARs in mice are well documented using the techniques of twin-well calorimetry and thermography.

There are several advantages of the circular waveguide system over the anechoic chamber system. The advantages make the circular waveguide system a better choice for exposing a large population of mice to electromagnetic fields for the study either of biological effects or hyperthermia for cancer therapy. The advantages are:

 More energy efficiency: The average SAR in mice was 21 times higher when exposed in circular waveguide than in the anechoic chamber for the same input power. This efficiency is important if a large number of animals are to be exposed, since smaller and less expensive power sources will be sufficient to feed multiple waveguides.

- Less orientation dependence: The difference in SAR between mice positioned in orientations was somewhat smaller in the circular polarized waveguide (i.e., 3.6 and 2.7 W/kg in circular waveguide versus 0.17 and 0.11 W/kg in anechoic chamber).
- Less space requirement: The waveguide required 324 cm² for four mice and the chamber occupies 5519 cm² for six mice. Therefore, 11 times more space is needed for exposing one single mouse in the chamber than in the waveguide.

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# Part II. Failure to Detect an Effect of 2450 MHz Microwave Irradiation on a Variety of Immunological and Hematological Parameters

K. E. Hellstrom, I. Hellstrom, C. C. Jones, H. J. Garrigues, C. K. Chou, and A. W. Guy

Following two reports by Wiktor-Jedrzejczak et al., (1977a, b) that microwave irradiation of CBA/J mice affects their immune system, as assayed in vitro, CBA/J male mice were subjected to 2450 MHz microwave irradiation using both circular waveguides and anechoic chambers, giving both single and multiple exposures at 14 or 28 W/kg. The effects of microwave

irradiation on the immune system were studied utilizing in vitro assays to detect possible changes in cell populations of the spleen. These assays included complement-receptor assays, plaque assays, T and B cell assays, and mitogen assays. No consistent effects of microwaves could be detected when irradiated mice were compared with shamexposed mice which had been treated in

an identical fashion and no consistent effects on various hematological parameters could be observed either.

#### Introduction

The increased interest in using microwave irradiation for various purposes (including microwave ovens, telecommunication, and solar power satellite as a

means to transfer energy), has led to concern whether exposure to microwaves can have any biological effects.

Wiktor-Jedrzejczak *et al.*, reported (1977 a, b) that a single exposure of CBA/J mice to 2450 MHz microwaves (specific absorption rate of 12 to 15 W/kg body weight), in an environmentally controlled waveguide facility, induced a significant increase in the proportion of complement-receptor positive (CR+) spleen lymphocytes. This effect was enhanced by repeated exposure, which, in addition, produced a significant increase in the number of cells with immunoglobulin at the cell membrane. Similar effects were observed by Sulek et al. (1980) and found to be highest 6 days after irradiation. Smialowicz et al. (1978), on the other hand, failed to induce any significant immunological effects with an analogous protocol. However, these authors used BALB/c mice, which, according to Schlagel et al. (1980) are not sensitive to any microwave effects on CR1 cells, the responsive mice all having the same genetic makeup at the H-Z locus which is different from CBA/J, (H-2k) and BALB/c mice (H-2<sup>d</sup>).

In this study, attempts were made to confirm the findings of Wiktor-Jedrzejczak et al. (1977 a, b), using CBA/J mice, and employing both circular waveguides and anechoic chambers for irradiation. As controls, sham-treated mice were used, which except for irradiation, were treated in an identical manner to the mice exposed to microwaves. Some tests also included control mice which were of the same age and sex but housed in our ordinary animal facility ("cage controls").

The study was unable to confirm the reported increase in lymphocytes expressing complement receptors. Also, there were no affects on the plaque-forming response of spleen cells to sheep red blood cells (SRBC) or dinitrophenol (DNP), the relative proportions of T and B cells in the spleen, the response of spleen cells to mitogens, or various hematological parameters.

#### Materials and Methods

### Mice

CBA/J males, with an average weight of  $24.8 \pm 1$  g and an age of 8-12 weeks, were used for all experiments. The mice were bought from the Jackson Laboratories (Bar Harbor, ME).

## Exposure Systems and Dosimetry

The circular waveguides and miniature anechoic chamber, as described in Part I,

were used for exposing mice to 2450 MHz microwaves at an SAR of 14 and 28 W/kg. The mean and standard error of the rectal temperature of the mice before and after a 30-minute exposure to 14 or 28 W/kg in both circular waveguides and anechoic chambers were measured. The only statistically significant difference (t test, p <0.01) was found between the control group and the group exposed for 30 min to 28 W/kg in the circular waveguides.

#### Complement-Receptor Assays

Mice were exposed to microwave radiation that produced an average SAR of 14 W/kg, with separate groups of animals being exposed either once ("single") or three times ("multiple"). Another group of animals were given multiple exposure at double power or an average SAR of 28 W/kg. Six days following exposure, spleen cells from control and irradiated animals were tested to determine if exposure to microwaves affected the number of complement receptor positive spleen cells.

#### Assays of Plaque Forming Cells

Mice were immunized with either DNP or SRBC, as done by Wiktor-Jedrzejczak et al. (1977b) and exposed to microwave radiation (SAR of 14 W/kg) for three consecutive days. On the sixth day after immunization, their spleen cells were tested for production of antibody to DNP or SRBC, using the Jerne plaque assay.

#### Tests for Mitogen Response

Mice were either irradiated one time ("single") or on day 0, 3, 6 ("multiple") at a specific absorption rate of 14 W/kg. Three, six, and nine days following the last exposure of mice to irradiation, their spleens were assayed for responses to PHA, Con A, LPS, PWM, PPD, poly IC (PIC) and dextran sulfate (DS). The response of irradiated animals was compared to that of sham-exposed animals and, in some experiments, entirely untreated and referred to as "cage controls."

#### T and B Cell Enumeration

Mice were either irradiated one time ("single") or on day 0, 3, 6 ("multiple") at a SAR of 14 W/kg. Six days following the last exposure their spleen cells were treated with anti-lgG antibody (to detect B cells) or anti-Thy 1.2 antibody (to detect T cells in the presence of guinea pig complement); this was to determine the relative numbers of T and B cells. Data from six irradiated animals were compared to that from six sham-exposed animals.

The mean percentage lysis (± SE) for both exposed and sham-treated groups was recorded, and the differences between these means were analyzed by the Student t test or by analysis of variance (ANOVA).

Mice were anesthetized with ether and bled from the retro-orbital cavity. Smears were prepared for differential counts and whole blood was diluted in Isoton II (Coulter Electronic, Inc., Tukwila, WA) and evaluated on a Model ZBI Coulter Counter and an S. Coulter Counter (Coulter Electronic, Inc.) for erythrocyte count, leukocyte count, hematocrit and hemoglobin concentration. The means and standard errors of exposed sham and control groups were calculated and the differences among these means were analyzed by the discriminant analysis.

#### Results

Mice were exposed to microwaves at one or repeated occasions with the exposure being done in either circular waveguides or anechoic chambers, after which several immunological parameters were measured, as presented below under separate headings for each of the different parameters. Data from a hematological study are given last.

### Assays for Spleen Cells Bearing Complement Receptors (CR<sup>+</sup>)

## Irradiation in Circular Waveguides

Mice were exposed to microwave irradiation at an average SAR of 14 W/kg. Separate groups of animals were exposed to this dose either once or three times. Another group of animals was given multiple exposures at an average SAR of 28 W/kg. Six days following exposure, spleen cells from sham-treated, and irradiated animals were tested to determine if exposure to microwaves had an effect on the frequency and/or total number of CR+ spleen cells.

There were no significant differences in CR $^+$  cells in mice exposed once or repeatedly to 14 W/kg. However, the group exposed to 28 W/kg, showed a weak but significant increase (p <0.05) in the total number of CR $^+$  cells.

A repeat experiment in which mice were exposed to 28 W/kg also showed a significant increase (p <0.02) in the total number of CR $^+$  cells in the irradiated mice when compared to either sham-exposed mice or the cage-controls. There was also a weak but significant increase in the % CR $^+$  cells in irradiated mice when compared to sham-exposed mice.

One more experiment was performed in which the mice were exposed in a circular waveguide with 3 treatments being given at a dose of 14 W/kg. No differences between sham-treated and irradiated animals were then seen.

### Irradiation in Miniature Anechoic Chambers

Mice exposed in anechoic chambers were tested in a way similar to the mice exposed in the circular waveguides. No significant differences were observed in the percentage of CR<sup>+</sup> cells or in the total number of CR<sup>+</sup> cells between shamtreated and irradiated groups, independently of whether the mice were irradiated once (14 W/kg) or repeatedly (28 W/kg).

#### Plaque Assays

Three groups exposed in circular waveguides were first tested, namely cage controls, sham-exposed and exposed mice. Without immunization, there was a significant difference between the cage controls and the other groups tested with more anti-DNP plaques formed by cells from the cage controls. Also irradiated animals showed slightly (p <0.05) elevated total PFC/spleen to DNP antigen as compared to the sham-treated animals.

Exposure to microwaves in the anechoic system failed to show any significant differences in the DNP response of irradiated and sham-treated mice. A decreased response to the T-dependent antigen SRBC was seen in both sham-treated and irradiated groups, as compared to "cage control" mice.

#### T and B Cell Enumeration

Assays of the numbers of splenic T and B cells failed to show any significant differences between animals exposed to microwaves and the parallel sham-treated controls. No effects of microwaves were noted on T and B populations following either single or multiple exposures in circular waveguides. Neither were any effects seen when the mice were exposed, once or repeatedly, in anechoic chambers.

#### Response to Mitogens

Animals exposed to a 14 W/kg single dose of microwaves demonstrated no significant differences compared to shamtreated animals in their response to either T or B cell mitogens. Similar exposure to multiple doses demonstrated reduced responses to Con A, in one experiment if the mice were tested 9 days after exposure

When mice were exposed in anechoic chambers to a single dose of microwaves,

an increased response to LPS was seen at 3 days after exposure, and the response to PWM was increased significantly at 6 days after exposure. At 9 days, the response to PPD was significantly decreased and the response was increased to PHA. In addition, both exposed and sham-tested groups demonstrated a decreased response (not significant) to LPS at 6 and 9 days. These results suggest an initial increase in the response of B cells followed by a decrease at a time when the T cell response showed a slight increase.

With multiple exposures, an increased response to T cells was indicated by higher counts for Con A at 6 days after the last exposure or 12 days after the first exposure. This finding was supported by a second experiment. In addition, the second experiment showed increased response to PWM at 6 days.

#### Hematological Studies

#### Circular Waveguides

For single exposure to 14 W/kg, the blood was tested 3, 6 and 9 days after the exposure. There was no difference between sham and exposed groups at p <0.05 level. When the cage control data was compared with the 6 days after sham-exposed data, there was a significant difference (p <0.03) on the RBC count. There were several differences after 3 and 9 day multiple exposure. Due to the small sample size (N = 3), these differences are probably due to biological variations, since there is no consistent change on any of the 9 parameters.

When the power was increased to 28 W/kg in the circular waveguide, there were no differences in WBC, RGB, HGB and HCT.

#### Anaechoic Chamber

No consistent significant effects were seen between the sham and exposed groups. Although there were differences in WBC, SEG and lymphocyte counts in mice exposed to 28 W/kg, the WBC of exposed mice was well in the normal range and the normal SEG and lymphocytes also varied over a large range. Mice were also studied which had been immunized with PBS, DNP or SRBC and exposed in circular waveguides at 14 W/kg. The results show differences in WBC counts when the mice have been immunized with DNP or SRBC.

### Discussion

The study attempted to confirm the results (Wiktor-Jedrzejczak et al., 1977a;

1977b; Sulek et al., 1980) that microwaves can induce immunological effects, detectable in vitro, most notably an increase in the numbers of spleen lymphocytes expressing receptors for complement at their surface (CR+ cells). For this reason, the authors tried to duplicate, to the best of their abilities, the experimental set-ups used in the studies reporting an effect of the radiation, including the use of mice of the same strain (CBA/J), the same sex (males), the same relative ages (8-12 weeks), weight (24.8  $\pm$  1 g), and the same doses of radiation (14 W/kg and, for some experiments, 28 W/kg). Mice were irradiated using either circular waveguides or anechoic chambers. Shamtreated controls, which had been handled identically to the irradiated animals were always included; in some experiments the authors also used "cage controls" which were mice of the same age and weights, which had been housed in our regular animal facility until they were tested. Care was taken to keep constant both the temperature and humidity in the room in which the exposed and the shamtreated mice were maintained, the values being 25  $\pm$  1°C and 50  $\pm$  10%, respectively.

No consistent and significant effects of microwave irradiation were observed. Most important, the authors were unable to confirm the reported findings that CR+ spleen cells are more frequent in mice subjected to microwave irradiation. Although an increase in such cells was observed in two of our experiments, when circular waveguides were used for irradiation, it was not consistent, and no increase was found when mice were irradiated in anechoic chambers. The only reproducible effects of radiation observed were effects on the response to spleen cells to mitogens, indicating an initial increase of B cell response followed by a decrease, at a time when the T cell response increased (6-9 days). However, these changes were slight and were seen only in mice treated in anechoic chambers (as compared to circular waveguides). In the experiments of Wiktor-Jedrzejczak et al. (1977b), weak increases of reactivity to B cell mitogets (particularly LPS) were seen in mice exposed once or repeatedly, using rectangular waveguides. Likewise, temporary but statistically significant and reproducible fluctuations in the response of lymphocytes to mitogens were reported by Huang and Mold (1980). In the current study, the authors failed to confirm the reported decrease in the response of irradiated mice to SRBC (Wiktor-Jedrzejczak et al. 1977b).

The reason for the discrepancy between the current findings on CR+ cells and those reported by Wiktor-Jedrzejczak et al. (1977a) and by Sulek et al. (1980) is not clear. It cannot be attributed to differences in mice, dose of irradiation, or time points of observation. However, the fact that the authors of the current study occasionally saw increases in the number of CR<sup>+</sup> cells in mice which were repeatedly irradiated using circular waveguides makes them believe that occasional, slight increases of such cells sometimes occur (as in Wiktor-Jedrzejczak's experiments). The explanation for these increases, and the failure of irradiation in anechoic chambers to produce it, remains unclear.

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Huang, A.T.F. and Mold, N.G. (1980): Immunologic and hematopoetic alteration by 2450-MHz electromagnetic radiation. Bioelectromagnetics, 1:77. C. K. Chou and A. W. Guy are with the University of Washington, Seattle, WA 98195; K. E. Hellstrom, I. Hellstrom, C. C. Jones, and H. J. Garrigues are with the Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Ralph J. Smialowicz is the EPA Project Officer (see below).

The complete report, entitled "Immunological and Hematological Effects of Microwave Power Transmission from a Satellite Power System," (Order No. PB 83-226 480; Cost: \$10.00, subject to change) will be available only from:

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