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An increasing number of studies are being conducted to determine the biological effects of radiofrequency radiation in which small laboratory animals such as rats and mice are utilized as the experimental subjects. Many of these experiments are conducted either with plane wave radiation incident on an array of experimental animals, with the animals exposed in a high scatter field, or with animals exposed in restraining devices which are so small as to preclude use of conventional field monitoring probes to define the radiation exposures. In

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these cases, calorimetric techniques offer a method for determining the radiation insult by measuring the specific absorbed radiation (SAR). Two types of multiple cell calorimeter systems were constructed, one for mice and one for rats. The data readout system is small, relatively inexpensive, and provides automated thermistor calibration, precise temperature measurements and a printout of total body SAR for each animal. This system was used to determine exposure parameters for an 18-animal array of mice exposed to 2.6 GHz radiation.

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Calorimetric measurements of microwave energy absorption by mice after simultaneous exposure of 18 animals

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A multiple-cell calorimetric system was constructed, and the experimental procedure for its use was developed. An associated data-readout system is small, relatively inexpensive, and provides automated thermistor calibration, precise temperature measurements, and a printout of whole-body SARs. One system was used to determine the SAR of each of 18 mice that were simultaneously exposed to 2.6-GHz radiation.

1. INTRODUCTION

The task of defining RF exposure conditions is relatively simple when a single animal is exposed in a far-field, low-scatter equipment. Definition of the RF field becomes tedious and extremely expensive if not impossible when long-term exposure of many animals is desired. One solution is to expose animals simultaneously in an array. This solution presents another problem in that scatter from adjacent animals creates perturbations of the field that severely limit the use of conventional field-monitoring techniques for high frequency (> 1 GHz) exposures [*Gandhi et al.*, 1979]. For any multiple-animal, or high-scatter exposure, calorimetric techniques for defining absorption of RF energy by the animals may be used. One application of these techniques is determination of specific absorption rates (SARs) in W kg^{-1} for animal cadavers that are exposed in the same array as the live animals. The SAR of a single animal in a low-scatter environment can be determined and from the observed value, an equivalent power density of incident plane-wave radiation can be calculated [*Durney et al.*, 1978]. This paper describes construction and use of a calorimetric system by which SARs were obtained for mice in an 18-animal array after exposure to microwave irradiation.

2. MATERIALS AND EQUIPMENT

Calorimeters were constructed for mice and for rats from pint and quart wide-mouth vacuum bottles. Added insulation was accomplished by molding the vacuum bottle into Eccofoam FP, in a 1-to-15 mixture with catalyst. Cans (16.5-cm height by 130-cm diameter) were used as forms and were left on the outside of the mouse calorimeters (pint-sized vacumm bottles) for mechanical protection. The quart vacuum bottles (rat calorimeters) were molded using plastic forms with a paste-wax mold release. Six mouse and eight rat calorimeters were constructed. Stoppers and lids were constructed from foamed polystyrene (Styrofoam). A small hole was punched into each lid to provide access for a thermistor. Six Yellow Springs thermistors were connected to a Monitor Labs Model-1200 scanner, which was controlled by a Hewlett Packard (HP) Model -9830A programmable calculator. Analog-to-digital conversion was accomplished with a Data Precision Model-3500 digital multimeter. The calorimeters and instrumentation are shown in Figure 1. Water was used as the heat-transfer medium.

3. EXPERIMENTAL PROCEDURE

Thermistors were calibrated by immersing them with a bulb thermometer in a constant-temperature water bath. The temperature of the water bath was increased from 17 to 26 °C over a six- to eight-hour period, and the resistance of each thermistor was monitored at 0.5-°C increments. A fit with a polynomial regression curve was performed to obtain an equation of temperature in terms of each thermistor's resistance. These parameters were stored in the programmable calculator for thermistor readout in °C.

Several methods for determination of heat capacity of the calorimeters were assessed. All methods involving transfer of the water were found to be extremely inaccurate because of heat loss in the water during the transfer process. Several methods of transfer, including pouring of water from one Dewar flask to another, and transfer with a plastic hypodermic syringe, were attempted, but temperature losses on the order of 0.1 to 0.2 °C were noted. The heat capacity of the calorimeters was ultimately determined with a small resistor immersed in 140 g of distilled water that closely approximated the combined mass of water and animal to be used in formal assays. The heat capacity was computed from the temperature changes

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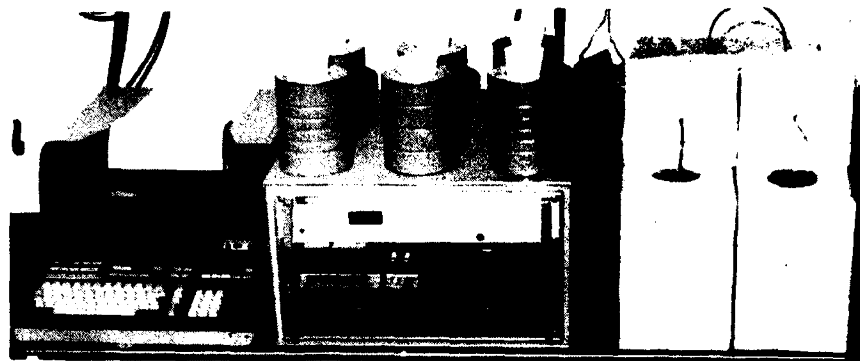

Fig. 1. Calorimeters with associated instrumentation.

observed when a potential of 25 V was applied across a 1000-ohm resistor, which resulted in ohmic heating at 0.625 W. Voltage and current were measured and were found to be constant over a 30-minute period.

One of the difficulties of calorimetric techniques is determination of heat loss to the environment. It was found that the best procedure is to adjust the initial temperature of the water from 0.5 to 1.0 °C below the ambient temperature. This maneuver resulted in less than 0.02 °C drift of temperature per hour. Irradiation of an animal was kept to a duration of 10 minutes or less, and intensity of irradiation was adjusted to produce a ΔT below 5 °C. These procedures result in negligible heat loss from the animal during exposure and during transfer to the calorimeter. This was proven experimentally by waiting after termination of a 10-minute exposure for a time equal to the exposure time; the same SAR values were determined as for animals transferred immediately. When the cadaver of an irradiated animal was added to the calorimeter, its temperature rapidly increased toward that of the ambient and resultant heat losses in the calorimeter were less than .01 °C h^{-1}.

For determination of SARs in mice exposed in an 18-animal array (Figure 2), selected animals were first euthanized by rapid cervical dislocation the day before irradiation to allow adequate time for carcasses to equilibrate to the ambient temperature. On the day of the experiment, 100 g of water approximately 1 °C below the ambient temperature was placed in the calorimeter. While the calorimeter attained thermal equilibrium, the mice were weighed. Five cadavers were placed in the exposure cage, and the remaining 13 positions were occupied by live mice to simulate the actual exposure geometry. The sixth cadaver, the control, was placed in the anechoic chamber but away from the RF field.

The array of animals was placed 150 cm from the front edge of a standard-gain horn and was exposed to a 2.6-GHz CW field with the transmitter's output stabilized at 280 watts. The mice were exposed with the long axis of the body parallel to the vector of the *E* field. Immediately following a ten-minute exposure, the initial temperature (T_i) of each calorimeter was measured and one mouse carcass was placed in each of five calorimeters. Temperatures were recorded every five minutes. Manual stirring, by gently moving the calorimeters in a circular motion with the bottoms flat on the table, was performed for approximately 20 s of each 5-minute period. When thermal equilibrium was reached (i.e., when ΔT was less than 0.01 °C during a 15-minute period), the final temperature (T_F) was measured and used to evaluate T_e for each cadaver according to:

$$T_e = [(Z_D + M_w \cdot C_w)(T_F - T_i)]/(M_s \cdot C_s) + T_F \quad (1)$$

where T_e = Rationalized temperature of the mouse upon insertion in the calorimeter
M_s = Mass of the mouse in kg
C_s = Specific heat of the mouse in J kg^{-1} K^{-1}
M_w = Mass of water in calorimter in kg
C_w = Specific heat of water in J kg^{-1} K^{-1}
T_i = Temperature of calorimeter just before insertion of the mouse (in °C)
T_F = Final temperature of calorimeter (in °C)
Z_D = Heat capacity of calorimeter in J K^{-1}

The SAR in W kg^{-1} was determined by:

$$SAR = C_s[T_e(\text{exposed}) - T_e(\text{control})]/\text{Exposure Time in Seconds}$$

For these experiments C_s = 3448 [*Hart*, 1951], C_w = 4185, and Z_D = 26.78 J K^{-1}.

Six mice and six thermal controls were individually exposed to low-scatter 2.6-GHz fields while located on a Styrofoam table. The incident field was uniform within ± 0.5 dB and was adjusted to 20 mW cm^{-2} for three of the ex-

Fig. 2. 18-mouse exposure array.

posed animals. The exposure time was ten minutes. The other three animals were exposed at 40 mW cm^{-2} for five minutes. The SAR values determined in each of these experiments were normalized to the average power density of incident radiation.

Field maps were made utilizing the National Bureau of Standards (NBS) Model EDM-1B electric-energy-density meter and were determined for the free field as well as for the field across the exposure array with all the mice removed. Measurements were also completed with all mice in the array with the exception of the space at which the measurement was being made.

4. RESULTS

The experimental results of this study are presented in Table 1. The position of the holder is given in the first column, and the power density at the empty holder is presented in the second column. (All power-density values are based on the incident field.) It was noted that levels of power density at positions near the center of the array were higher than those near the corners, which is the normal pattern for fields radiating from a standard-gain horn. The average power density was 12.6 (± 1.6 SD) mW cm^{-2}. However, when mice were inserted in all but the position at which the incident field was measured, a very erratic field pattern resulted. These data are shown in column 3. The average power density as determined by this technique was 9.6 (± 3.9 SD) mW cm^{-2}. These measurements were erratic and not reproducible because of the complex nature of the standing waves that were generated when mice were placed in the exposure array. The SARs as measured by the calorimetric technqiue are shown in column 4. Each of these values is the mean of two or three measurements.

The mean normalized SAR for mice exposed at 20 mW cm^{-2} for ten minutes was 1.11 (± 0.08 SD) W kg^{-1} per mW cm^{-2}. The mice exposed at 40 mW cm^{-2} for five minutes had a mean normalized SAR of 1.19 (± 0.14 SD) W kg^{-1} per mW cm^{-2}. The mean SAR for all free-field mouse exposures was 1.15 (± 0.11 SD) W kg^{-1} per mW cm^{-2}. Applying this factor to the data on SARs in column 4 results in the equivalent free-field power densities shown in column 5. The average effective power density was determined to

TABLE 1. Measurements of power density and SARs at each of 18 positions of a multiple-animal array. Means and standard deviations are rounded to the first decimal.

Holder Position #	Average Power Density All Holders Empty (mW/cm^2)	Average Power Density Holders Occupied[1] (mW/cm^2)	Specific Absorption Rate (W/kg)	Effective Power Density (mW/cm^2)
1	11	10	22	19
2	14	5	23	20
3	14	5	23	20
4	10	7	16	14
5	12	7	14	12
6	14	15	28	24
7	12	12	22	19
8	14	13	23	20
9	10	19	17	14
10	12	8	19	17
11	14	8	22	19
12	14	8	25	22
13	11	7	14	12
14	11	7	19	17
15	13	6	24	21
16	15	15	20	17
17	14	8	22	19
18	12	12	15	13
$\overline{X}$ (S.D.) =	12.6 (±1.6)	9.6 (±3.9)	20.4 (±4.0)	17.7 (±3.5)

[1]Except at position where power density was measured.

be 17.7 (± 3.5 SD) mW cm^{-2}. The average of seven power densities of the central positions (6, 7, 8, 10, 11, 12, and 13) for free-field measurements, i.e., with no animals in the holders, was 5% higher than the average of the 11 peripheral measurements. The SARs associated with the seven inner positions averaged 12% higher than those of the periphery when all but one of the holders were occupied by animals. This increase is thought to be due to enhancement of field intensity that results from scatter, which is more predominant near the center than near the edges of the array.

5. DISCUSSION

Standard calorimetric techniques can be used to determine SARs of rodents exposed in an array. Equivalent free-field power densities can be assigned to each position of the array. Special care must be taken to determine the heat capacity of the calorimeter flask and to keep temperatures of the calorimeters within 1 °C of the ambient temperature. By use of the techniques described, reliable and accurate dosimetry can be accomplished in a high-scatter geometry. When multiple-animal exposure techniques are used, it is sometimes necessary to fill the entire array with animals, but only the positions that provide equivalent exposure of experimental animals are used. For example, in the array we tested, if positions 1, 2, 3, 7, 8, 11, 14, 16, and 17 were used for experimental animals, an equivalent exposure of 19 mW cm^{-2} would result (SD ~ 1 mW cm^{-2}).

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council.

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