within an object can be mapped by moving a probe along a selected path. Implantable E-field probes provide the most sensitive and direct means of local SAR measurement. Probes with a dipole sensor length of less than 5 mm can detect SARs as low as 0.2 W/kg [Bassen et al., 1977; Stuchly et al., 1984; Bassen and Babij, 1990]. The sensitivity of an E-field probe is proportional to the length of the dipole (antenna) elements; the spatial resolution is inversely proportional to the length. Guy et al. [1987] and Chou et al. [1987] used a diode sensor, a microwave source modulated at 100% with a 1000 Hz square wave, and a standing wave ratio meter used as a voltage detector to increase the measurement sensitivity. SARs as low as 0.2 mW/kg were measured.

To measure the small E-field induced by a low-power RF source, such as a hand-held cellular telephone, sensitivity can be increased by the use of synchronous-detection or signal-averaging techniques. When the frequency decreases, spurious RF pickup in high-resistance leads significantly degrades resolution, which limits the use of currently available probes to frequencies above 150 MHz. The upper frequency is limited by the ratio of the RF wavelength in the tissue-equivalent material to the length of the dipole. The wavelength of an RF field in dielectric materials is approximately equal to the wavelength in air divided by the square root of the relative dielectric constant. Therefore, the internal wavelength is much shorter than the wavelength in free space. When the ratio of the wavelength in tissue material to the dipole length becomes approximately less than ten, the electric field is no longer sufficiently uniform over the length of the dipole to provide accurate results. This limits the use of commercially available probes when they are implanted in tissue-equivalent material to a few gigahertz (GHz). Practical E-field probes use three small orthogonal dipole antennas to provide isotropic measurements. Isotropic response of a probe is important whenever the polarization of an internal E-field is unknown (e.g., in curved or irregularly shaped biological objects). An implantable isotropic E-field probe is commercially available (Loral Microwave-Narda, Hauppauge, NY).

To determine SAR accurately using implantable E-field probes, the probes must be calibrated. The overall goal of any SAR calibration technique is to define SAR accurately within a specified tissue-equivalent model. For example, a slab or a spherical model can be used, provided that the SAR distribution is known and has been determined from classical theory. For calibration, the model should be large compared to the length of the probe sensors (dipoles), and the SAR should be uniform throughout its volume. Spherical or block models filled with tissue-equivalent material have been used by several researchers to calibrate implantable E-field probes [Bassen et al., 1977; Stuchly et al., 1984; Guy et al., 1987]. These models are irradiated by uniform microwave fields of a known strength and polarization. The voltage output from each of the probe's three orthogonal sensors is recorded during irradiation. Calibrations can also be performed in waveguides (a hollow rectangular or circular tube used for EM energy transmission at microwave frequencies) filled with tissue-equivalent liquids [Hill, 1982]. These calibration techniques are described in detail in the ANSI/IEEE C95.3-1992 measurement standard [ANSI/IEEE 1992b].

Cleveland and Athey [1989] used an implantable electric field probe with three orthogonal dipoles to study the SAR in a model of a human head consisting of simulated bone, muscle, eye, and brain tissue. The model was exposed to 810–820 MHz and 850–860 MHz fields emitted from 1.0 and 1.8 W hand-held radio transceivers. When the transceivers were held within 1 cm of the head, the maximal local SAR occurred in the eye or in other parts of the head, depending on the transceiver and the position of its antenna with respect to the head. The two highest SARs measured were 3.2 W/kg, which occurred at the surface of the eye at 810–820 MHz, and 3.5 W/kg, which occurred in the temple area at 850–860 MHz. Both values were normalized to a transmitter radiating 1.0 W. SAR peaks, as expected, were highly dependent on the relative position of the radios with respect to the head and the distance between the head and radiating structures.

Balzano et al. [1995] mapped the SAR in a model human head exposed to a 0.6 W cellular telephone operated at 835 MHz. They used a robot arm holding an implantable electric-field probe to scan the interior of a fiberglass skull filled with liquid material that had the dielectric properties of human brain. At 0.4 cm from the inside surface of the skull, the maximum SAR was 1.1 W/kg for a flip phone with the antenna extended, as shown in Figure 2. When the antenna was collapsed, the maximum SAR increased to 1.8 W/kg. In both cases, the maximum occurred near the lower portion of the ear, and the 1 gram averaged SAR was less than 1.6 W/kg, which is the peak value of the ANSI/IEEE C95.1-1992 SAR exclusion for the uncontrolled environment [ANSI/IEEE, 1992a]. Kuster et al. [1993] measured local SARs induced by 1.0 W European cellular phones operating at 890–915 MHz. When the telephone was held away from the face and the antenna was touching the occipital area of the head (worst-case exposure), the maximal SAR located within the model head was about 6.0 W/kg averaged over a volume of 1 cm^3 in brain-equivalent material.

Temperature probes. Because tissue temperature increases linearly during brief exposure to high-intensity RF radiation, the SAR can be obtained from temperature measurements as well. The maximal

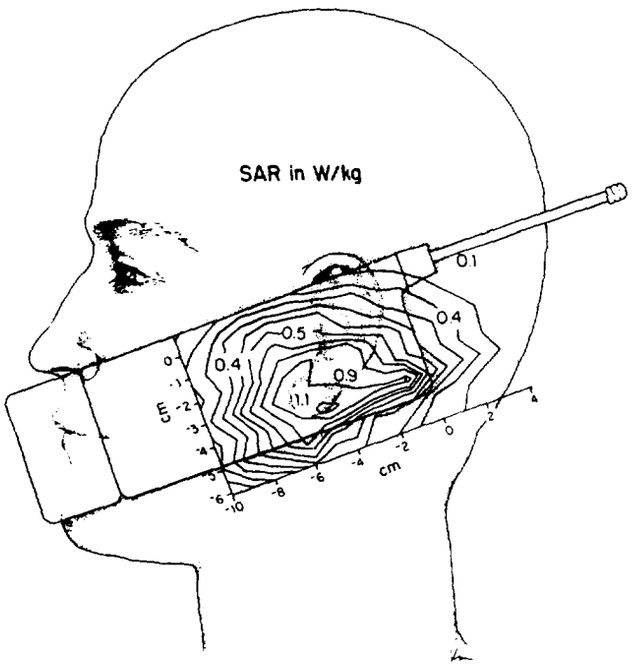

Fig. 2. Peak standard absorption rates (SARs) inside a human head exposed to a "flip" cellular telephone with the antenna extended [from Balzano et al., 1995].

temperature rise is usually kept below 10 °C to prevent increased tissue conductivity (approximately 2% per °C) from causing "thermal runaway." The runaway effect causes the hot area to absorb more RF energy than the cooler areas, and the hot area gets even hotter during the course of the exposure. In addition, even without thermal runaway, the changes in dielectric properties also alter the absorption patterns. Therefore, the high intensity exposure must be of short duration to minimize heat diffusion and to keep the maximal temperature rise at less than 10 °C.

Temperature measurement is the technique that is used most commonly for determining SAR in tissues and in in vitro culture systems. This does not imply that the mechanism for any observed biological effect is thermal in origin. Mittleman et al. [1941] were the first to quantify RF energy absorption by using temperature measurements. Initially, the unit W per liter was used, which was changed subsequently to W/kg by Cogan et al. [1958]. The relationship between SAR and temperature rise is

$$SAR \cong 4186 \frac{c_H \Delta T}{t} \quad (W/kg), \qquad (5)$$

where c_H is the specific heat capacity of the tissues (kcal/kg· °C), 4186 is the conversion factor from kcal to joule, ΔT in °C is the temperature rise, and t is the exposure duration in seconds. The approximation symbol over the equal sign implies that accuracy is based on negligible energy loss by the irradiated body. Irradiation of the tissue-equivalent model must be performed at a high exposure level and short exposure duration to produce a measurable linear temperature rise. This must be done to allow accurate quantification of the rate of temperature rise in the absence of any significant thermal conductive loss. If these conditions are not fulfilled, then the SAR will be significantly underestimated.

A normalized SAR (W/kg per W input) is a convenient quantity. To calculate the actual SAR for low-power exposure, one simply multiplies the normalized SAR with the applied input power. For example, in one dosimetry study, an SAR in the brain of a rat carcass exposed at 300 W net input power to the exposure system was measured to be 60 W/kg. For a 0.5 W net power used in a biological study, the corresponding SAR in the rat brain is extrapolated to be $(60/300) \times 0.5 = 0.1$ W/kg. The temporal peak SAR in pulse-modulated RF exposures also can be calculated by dividing the average SAR by the duty factor. The duty factor is the ratio of pulse duration to the pulse period.

Mistakes have been made by investigators attempting to measure SAR in objects exposed to low power-density levels over long time periods. Removal of thermal energy via diffusion reduces the RF-induced temperature rise and greatly distorts the actual rate of energy absorption. In the case of living animals, the thermoregulatory effects of blood flow further reduce the accuracy of SAR measurements. Determination of the initial rate of a temperature rise is necessary to determine accurately the SAR.

Metallic temperature sensors, such as thermocouples and thermistors with metallic leads, cannot be used casually for temperature measurements in RF fields. The perturbation of the fields being measured by metallic sensors and wires prevents them from providing accurate data [Cetas, 1990]. EM fields also can induce interference in the electronic readout devices that are connected to temperature sensors via metallic wires. In addition, RF heating of metallic sensors and leads can produce erroneous SAR measurements. Shielding thermocouples or thermistors may minimize the interference problem, but it cannot solve the perturbation problem. Several microwave-transparent temperature probes have been available commercially for single-point measurement in biological bodies and exposure systems, but only two remain on the market. One is the Vitek-101 high-resistance-lead thermistor probe [Bowman, 1976], which is available from the BSD Company (Salt Lake City, UT). The carbon-loaded Teflon, high-resistance leads have electrical conductivity similar to that of biological tissues. Therefore, these leads do not disturb the field within the object. However, it must be noted that, when the leads

are exposed in air parallel to the electric field, induced current may heat the leads. The other probe is the Luxtron (Mountain View, CA) Model 3000 multi-channel fiber-optic thermometer [Wickersheim and Sun, 1987]. The sensors are made of temperature-sensitive phosphors, and the leads are plastic optical fibers. Up to 12 single- or multiple-array Luxtron sensors allow simultaneous SAR measurements at a number of locations in an exposed object. Multiple Vitek probes may also be used for this purpose.

Temperature probes may be used to determine the local SAR in a model of the head or body of a human only if sufficiently high RF power is radiated from the source. A spatial peak SAR of approximately 20 W/kg should be produced in a body or model to allow accurate measurements with temperature probes. Therefore, this sensitivity is far less than the 0.2 W/kg of commercial E-field probes. It is extremely difficult to use temperature to measure accurately the SAR delivered to a model of the human head by a low-power source, such as a hand-held radio [Balzano et al., 1978]. Guy and Chou [1986] constructed a high-power antenna and generated a field pattern similar to that of the original low-power mobile antenna and were able to provide enough RF energy to heat a model. Table 1 gives an example of the SARs measured by four Vitek 101 probes implanted in the model of a woman exposed to an RF field when it stood 9.5 cm away from a high-power, trunk-mounted, mobile antenna. The peak measured SAR of 0.147 W/kg per W input to the antenna occurred in the stomach region, 0.5 cm inside the model.

Planar (Two-Dimensional) SAR Measurements

The SAR distributions in biological objects or models are complex. Therefore, the single-point SAR measurement technique can be very tedious and time consuming for mapping the spatial distribution of local SAR throughout an object. Because dielectric properties vary with temperature, models or biological tissues must be cooled between exposures. Localized probing or mapping cannot guarantee that hot spots in the exposed subject have been located. Detailed mapping is necessary. To study the SAR pattern in a planar area within a three-dimensional volume, several methods have been explored. One simple method is the use of a temperature-sensitive liquid-crystal sheet. This method allows qualitative visualization of the temperature distribution on a surface. Leaving the liquid-crystal sheet sandwiched in a precut object during RF exposure can reveal an internal SAR pattern. However, the qualitative nature of this method limits its usefulness. Recently, Cristoforetti et al. [1993] reported the use of a CCD camera to convert the hue of liquid-crystal film color during heating to quantitative temperature data. Depending on the polarization of the E-field within the model, the presence of film can significantly affect the RF-induced current. This can result in an altered heating pattern, particularly when the film is perpendicular to the E-field within the model. The effect is also dependent on the frequency of the field.

Guy et al. [1968] developed a thermographic technique for the rapid measurement of SAR in an internal plane. A bisected mammal cadaver or model must be utilized. During RF exposure, the bisected halves are joined. After a brief high-power exposure, the halves are quickly separated, and the internal surface of one of the halves is immediately scanned by an infrared thermographic camera. Temperatures before and after the RF exposure are compared to determine the temperature elevations on the plane of interest. Depending on the resolution of the system, several hundred thousand points can be measured within seconds. The accuracy of this technique depends on the size of the animal or model, because heat loss between the time of exposure and the thermogram becomes relatively large for smaller objects.

Thermographic techniques have been used to determine the SAR distributions in human models standing near trunk- and roof-mounted mobile antennas similar to those used with cellular telephones [Guy and Chou, 1986]. Full-sized human models were filled with a high-water-content, simulated-muscle material and were exposed to an 835 MHz field from a mobile antenna (Fig. 3). Figures 4 and 5 show the SAR patterns in a woman model (sagittal plane) and in a child model (horizontal plane at the eye level), respectively.

Models may also be scaled [Guy et al., 1976]. With these models, far less RF energy is needed for SAR determination than for full-sized models. Consequently, the use of size-reduced (scaled) models is technically easier and is more economical. When a full-sized model is scaled down by a factor, the conductivity of the tissue-equivalent material and the RF

TABLE 1. Specific Absorption Rates (SARs) of a Standing Woman Exposed to a Trunk-Mounted Antenna (835 MHz) at 9.5 cm Distance*

	SAR (W/kg per W)			
Depth (cm)	Heart	Kidney	Liver	Stomach
0.5	.0207	.0746	.1170	.1470
1.0	.0260	.0642	.1100	.1350
2.0	.0121	.0307	.0540	.0727
3.0	.0092	.0171	.0289	.0381
4.0	.0042	.0074	.0148	.0189
5.0	.0026	.0034	.0076	.0084
6.0	.0014	.0025	.0039	.0040

*From Guy and Chou [1986].

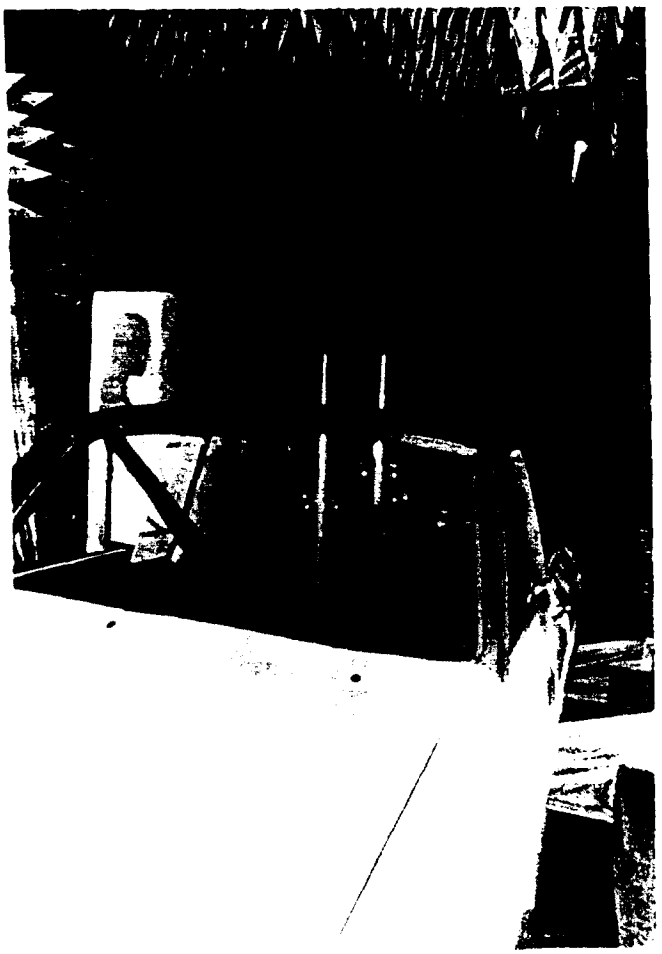

Fig. 3. Photograph showing a full-sized woman model (sagittal one-half) exposed to high-power (10 kW) mobile antenna fields in an anechoic chamber for the quantification of energy absorption.

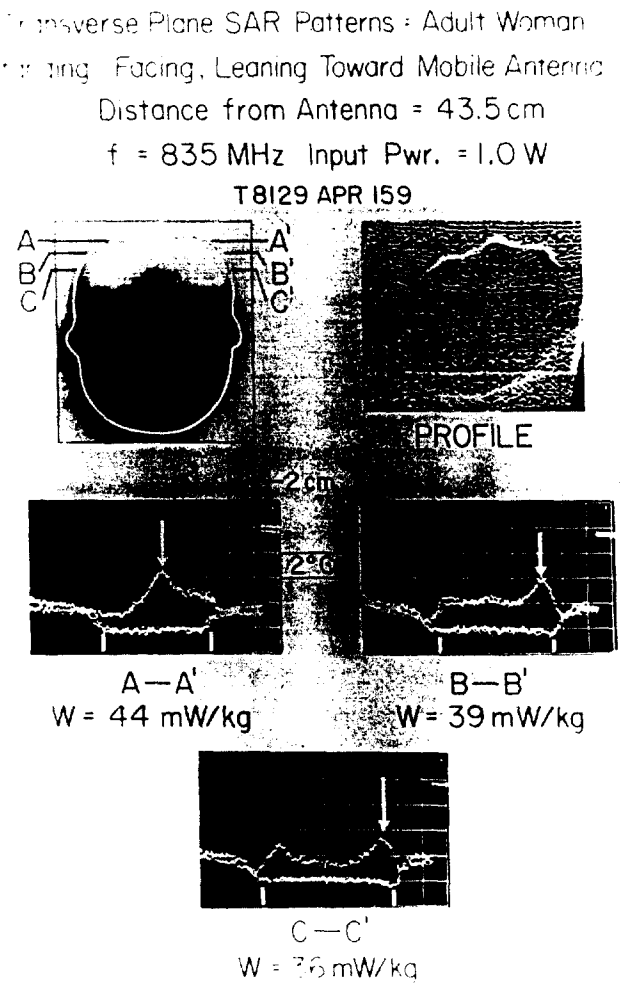

Fig. 4. Horizontal-plane SAR pattern at the eye level of a woman model exposed to 835 MHz UHF fields from a mobile antenna (43.5 cm distance, normalized to 1 W).

frequency must be increased by the same factor. These adjustments are made to preserve the same wavelength to body-size ratio. The final SAR measured in the scaled model also must be reduced by the same factor to represent the SAR that would be induced in a full-sized model. Scaling also allows a single-frequency RF generator to be used with different sizes of models to determine the SAR at a number of frequencies. This method has been used to obtain induced current densities in pig and man models exposed to 60 Hz fields. The results based on scaled models have been reported by Guy et al. [1976, 1982, 1984].

A new alternative to the thermographic method uses magnetic resonance imaging to obtain the heating pattern inside a model [Samulski et al., 1992]. This method would be desirable for noninvasive thermometry during hyperthermia treatment. However, the present cost of the procedure is too high to be practical for most applications. Recently, a luminescence imaging technique has been developed to map SAR distributions in optically transparent models [Bruno and Kiel, 1994]. The technique uses a chemiluminescent compound, diazoluminomelanin, and a quantitative luminescence imaging system to derive maps of the SAR distribution. This technique uses the phenomenon of changes in light intensity emitted by the chemical during RF exposure, and it is now under commercial development (Beam Tech Corporation, San Antonio, TX).

Whole-Body Averaged SAR

The whole-body averaged SAR in animals can be determined by twin-well calorimetry [Phillips et al., 1975; Blackman and Black, 1977; Allen and Hunt, 1979; Chou et al., 1984a]. Two animals of similar body mass are euthanatized and brought to temperature equilibrium. Then, one of the animals is exposed to an RF field.

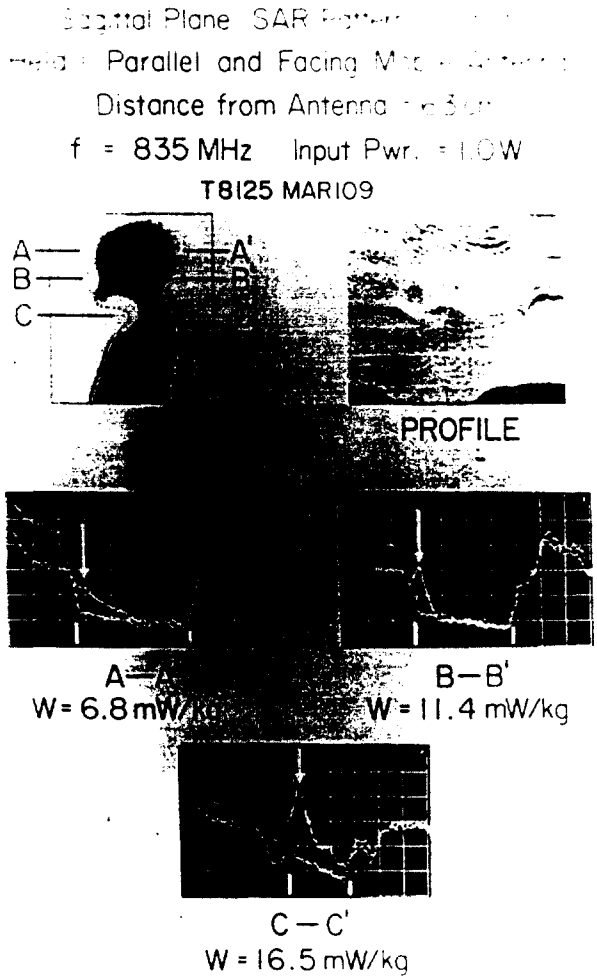

Fig. 5. Sagittal-plane SAR pattern of a child model exposed to 835 MHz UHF fields from a mobile antenna (63 cm distance, normalized to 1 W).

Immediately after exposure, the two carcasses are placed in a twin-well calorimeter. The whole-body averaged SAR in the exposed animal can be calculated from the heat differential between the two carcasses. In contrast to the thermographic technique that works well for large objects, twin-well calorimetry is a better method for small animals. The long heat diffusion time for large animals creates problems in twin-well calorimetry, because the animals lose heat so slowly that it may be several days before they reach an equilibrium temperature. During this time, decomposition of the cadaver generates additional heat that introduces errors in SAR measurements. Olsen and Griner [1989] have built a large calorimeter that can accommodate a full-sized human model. Scaled or full-sized models filled with tissue-equivalent material can be used to measure the whole-body averaged SAR. After exposure, the liquid is stirred to equalize the temperature throughout the medium, and the temperature rise measurement is obtained. The average SAR can be calculated by using Equation 5.

LIMITATIONS AND UNRESOLVED PROBLEMS OF SAR

Although SAR has been accepted world wide as a dosimetry unit and is the basis for national and international RF safety standards [NCRP, 1986; ANSI/IEEE, 1992a; IRPA, 1993; NRPB, 1993], there are limitations of its application in standard setting. The ANSI/IEEE C95.1-1992 standard [ANSI/IEEE, 1992a] specifies that the criteria for maximal permissible SARs for human exposure are applicable between 0.1 MHz and 6.0 GHz, i.e., a whole-body averaged SAR of 0.08 or 0.4 W/kg, depending on whether or not the person is aware of the exposure. At frequencies below 0.1 MHz, surface effects (shocks and burns) become issues. Research is needed on the dosimetric thresholds for both electrostimulation and energy deposition in the range of 0.01 to 1.0 MHz [Reilly, 1992]. The information is needed to shift from exposure limits based on electrostimulation at frequencies below 0.1 MHz to an energy-deposition criterion (SAR) above 0.1 MHz. At frequencies above 6 GHz, superficial power deposition (hence, external power densities) are more useful than the SAR produced within exposed tissues. Research is needed on heating of the human body above 6 GHz to verify both the transition from deeper deposition to surface absorption and the validity of the resulting short thermal time constants. Some mathematical analyses for planar surface heating yield thermal time constants of minutes. However, these results are questionable in view of the literature on IR surface heating [Foster et al., 1978]. Furthermore, the redistribution of thermal energy due to blood flow (convective) or conductive cooling must be taken into account.

The SAR limits in the ANSI/IEEE C95.1-1992 standard are based on reproducible SAR thresholds for disruption of learned behavior of laboratory animals accompanying whole-body RF exposure [de Lorge and Ezell, 1980: de Lorge 1984; ANSI/IEEE, 1992a]. Although there are few data corresponding to partial-body animal exposures, the present standard relaxes the SAR criterion for partial-body exposures of humans. In addition, the resulting energy induced in eyes and testes is limited more stringently than in other regions of the body. The application of C95.1 SAR limits to small volumes is an issue that needs more attention. For example, SAR measurements in 1 cm^3 volumes in heads have been performed in conjunction with cellular telephone dosimetric studies based on models. However, the biological effects of moderate duration exposures (e.g.,

30 min per day) of small volumes of brain tissues have not been documented adequately in terms of acute and chronic biological effects. Further dosimetric and biological effect studies on partial body exposures are needed, especially on the head. In medical applications of partial body exposure to diathermy or cancer hyperthermia, the SAR can be as high as 100 W/kg [Lehmann 1994].

It is important to continue performing experimental and theoretical research to improve the accuracy of data on the properties of biological tissues in RF fields. Recent reports by Gabriel [1995] indicate that live bone may have dielectric parameters that are closer to those of muscle, i.e., higher dielectric constant and conductivity, than have been assumed previously. These unresolved new data would suggest that an overestimation has occurred in brain SAR data in past dosimetric studies that used tissue-equivalent or computer models.

Unresolved issues in experimental dosimetry include the complexity and difficulty of performing SAR measurements. Higher uncertainties exist in experimental data obtained when the SAR values are small (less than 10 W/kg). This occurs when using low-power RF transmitters, such as hand-held wireless telephones. There is a lack of availability of complete calibrated, internal E-field measurement systems and sensitive (0.01 °C resolution), RF-transparent, temperature-probe systems. The internal E-field and temperature measurement systems that have been produced to date are relatively expensive and require a great deal of expertise and ancillary equipment. In addition, standard methods for calibrating E-field or temperature probes to an SAR reference standard do not exist. Finally, there is no commercial source for obtaining standardized models and tissue-equivalent materials, except on a costly, customized basis. This combination of factors points to a lack of consistent data from experimental SAR measurement systems and a tremendous dependence on the personal expertise of the dosimetric specialist.

The SAR is a scalar quantity. If the direction of the induced field or current is of importance to a biological effect, then the SAR cannot provide this information. In such a case, knowledge of the SAR alone would be inadequate, because it would not provide directional information. Furthermore, if an effect on biological cells or tissues is related to a direct interaction with the magnetic field (e.g., an interaction with magnetite), then the SAR concept is insufficient [Stuchly and Stuchly, 1986], and the exposure and dosimetric measurements would have to involve the internal magnetic field as well as the electric field. However, because the amount of magnetite in tissue is very small, the minuscule SAR component due to any magnetic field absorption would be difficult to measure.

It should be pointed out that the accuracy of SAR measurement by either an E-field probe or the temperature method is ±2-3 dB [Bassen and Babij, 1990]. The uncertainty is due to the steep gradients in induced field distribution and the thermodynamics of an RF-exposed biological body. The accuracy of the whole-body averaged SAR based on calorimetry is better, because it is within a few percentage points [Gandhi et al., 1979]. However, whole-body SAR is not suitable for dose-response evaluations of RF effects due to localized exposures of specific organ systems and regions of the body. Table 2 provides a list of some characteristics of RF dosimetry and its limitations.

DISCUSSION

Most of the research on RF exposure of animals has the ultimate goal of applying the results to risk assessment of human beings exposed to RF fields. Therefore, when effects are observed in experimental animals, there should be a way to extrapolate the effects to human beings [Michaelson and Lin, 1987]. Without quantification of EM field interaction with biological

TABLE 2. Characteristics of Radio Frequency (RF) Dosimetry

Whole-body averaged standard absorption rate (SAR)
Biological objects absorb RF energy at a higher whole-body averaged rate when they are exposed to a uniform E-field with the E vector aligned parallel to the long axis of the object
When a biological object moves with respect to an incident field, the whole-body average SAR can change by a factor of ten or more
Biological objects absorb maximal RF energy when they are exposed at their *resonant* frequencies; at the resonant frequency, the object length is 25–50% of the wavelength of the external exposure field
Local SAR
The highest local SAR is usually at or near the surface of an object exposed to an RF field
For curved surfaces and resonant objects, high SARs ("hot spots") may exist at various depths
Local SARs exceed the whole-body averaged SAR by 10–100 times. This ratio depends on the size of the local area over which the local SAR is averaged
The local SARs at various points in an immobilized biological object can be measured with an uncertainty of 10–30%
When the source of RF radiation is close to the biological object, the magnetic field may induce a higher maximal local SAR than the E-field
When a biological object changes its alignment with respect to an incident field, the local SAR at each point can change by a factor of two or more

tissues, it is impossible to make such an extrapolation and, thus, to predict safe exposure levels for human beings. Garn and Gabriel [1995] recently compiled results of 12 relevant publications concerning the relationship between external field strengths and the SAR inside the human body. The physical parameter termed SAR is now widely accepted by researchers in this field as a common unit for comparing biological effects of RF exposure. However, the techniques for measuring SARs are complicated and are not always reliable. Whole-body averaged SARs provide a first step in quantification of dose. However, planar or three-dimensional analysis of SAR and the spatial distribution of SAR in different tissues and body locations are also needed to make meaningful comparisons across animal species and to human beings. For example, the local SAR in the brain and spinal cord might be a more appropriate parameter for risk assessment than the whole-body SAR for relating animal studies of combined microwave and drug effects on behavior to human beings.

Although the SAR is not a thermal unit, exposure to RF fields transfers energy to the exposed object at a rate proportional to the exposure level. Therefore, it is important that the absolute temperature values and their distribution are thoroughly documented. This should be done during biological effects studies before, during, and after exposure of in vivo and in vitro subjects. During exposure of experimental animals or human beings, temperature distributions may equilibrate by means of physiological regulatory mechanisms. In in vitro exposures, thermal gradients and temperature distributions are especially critical. For example, temperatures in vitro might be controlled by a constant temperature bath. However, a monolayer of cells at the bottom of a culture dish may not be at the same temperature as that measured in the center of the culture medium.

When considering modulated, applied EM waves, the SAR should also be modified to reflect the time-varying nature of the RF field. For example, the perception of sound accompanying pulsed microwave exposure can occur for a single pulse [Lin, 1978; Chou et al., 1982]. It is improper to quantify this effect by using the SAR averaged over time. Instead, the calculation of SA for each pulse is a more suitable quantification. Another example of a modulation effect is the increase or decrease in Ca^{2+} efflux accompanying exposure to microwave radiation, which was reported by Bawin et al. [1975] and Blackman et al. [1985]. They found that the effect occurs only when the EM field is amplitude modulated, especially at 16 Hz. For this case, the 16 Hz modulation frequency also should be specified. Therefore, when specifying exposure parameters involving modulation, detailed information about the modulation (pulse width and repetition rate of a pulsed wave) should be provided. This is particularly important, because increasing numbers of pulse-modulated systems are being used (e.g., systems for the new generation of digital cellular telephones).

The relationship between the duration of exposure and any resulting biological effects in animals and the extrapolation of these data to humans is not trivial. Extrapolation of time requires knowledge of the mechanism of the biological effects. For example, the microwave auditory effect mentioned above has been shown to be caused by thermoelastic expansion of tissues for microwave pulses of less than 50 μs duration. The same exposure time can cause auditory perception in both rats and humans (different pitch due to the different cranium size), but the threshold intensities are different. The SAR does not imply a mechanism of effect; therefore, questions of thermal time constant, animal response time, and scaling of thermal effects are irrelevant.

CONCLUSIONS

Dosimetry is an important part of any scientific effort to assess the effects of RF fields on biological systems and species. During assessment, it is far better to use the SAR to quantify the RF fields within the object than to use only external field exposure data. Accurate determination of SAR is a complicated matter, even at a single frequency. Specialized physical measurements and computation can be used to determine approximate whole-body average and spatially localized SARs in a biological object. A significant amount of work must be performed to assess the SAR distribution in an object over a wide range of exposure frequencies. There are limitations on the rigor and completeness of SAR to define dose rate. However, despite the complexity, this should not be a deterrent to using SAR to quantify the biological effects of RF exposure and to develop RF guidelines. It is only with continued RF dosimetry research that the development of EM technology can be fostered safely for the benefit of mankind while avoiding unreasonable restrictions.

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REFERENCES

Allen SJ, Hunt WD (1979): Calorimetric measurements of microwave energy absorption by mice after simultaneous exposure of 18 animals. Radio Sci 14(6S):1–4.

ANSI (1982): ANSI safety levels with respect to human exposure to radio frequency electromagnetic fields, 300 kHz to 100 GHz (ANSI C95-1982). New York: American National Standards Institute.

ANSI/IEEE (1992a): IEEE standard for safety levels with respect to human exposure to radio frequency electromagnetic fields, 3 kHz to 300 GHz (ANSI/IEEE C95.1-1992; revision of ANSI C95.1-1982). Piscataway, NJ: Institute of Electrical and Electronics Engineers, Inc.

ANSI/IEEE (1992b): IEEE recommended practice for the measurement of potentially hazardous electromagnetic fields—RF and microwave (ANSI/IEEE C95.3-1992). Piscataway, NJ: Institute of Electrical and Electronics Engineers, Inc.

Balzano Q, Garay O, Steel FR (1978): Energy deposition in simulated human operators of 800-MHz portable transmitters. IEEE Trans Vehicular Technol VT 27:174–181.

Balzano Q, Garay O, Manning T (1995): Electromagnetic energy exposure of the users of portable cellular telephones. IEEE Trans Vehicular Technol 44:390–403.

Bassen HI, Babij TM (1990): Experimental techniques and instrumentation. In Gandhi OP (ed): "Biological Effects and Medical Applications of Electromagnetic Energy." Englewood Cliffs, NJ: Prentice Hall, pp 141–173.

Bassen HI, Herchenroeder P, Cheung A, Neuder SM (1977): Evaluation of an implantable electric field within finite simulated tissues. Radio Sci 12(6S):15–25.

Bawin SM, Kaczmarek LK, Adey WR (1975): Effects of modulated VHF fields on the central nervous system. Ann NY Acad Sci 247:74–81.

Bini MG, Ignesti A, Millanta L, Olmi R, Rubino N, Vanni R (1984): The polyacrylamide as a phantom material for electromagnetic hyperthermia studies. IEEE Trans Biomed Eng 31:317–322.

Blackman CF, Black JA (1977): Measurement of microwave radiation absorbed by biological systems. 2. Analysis by Dewar-flask calorimetry. Radio Sci 12(6S):9–14.

Blackman CF, Benane SG, Rabinowitz JR, House DE, Joines WT (1985): A role for magnetic field in the radiation-induced efflux of calcium ions from brain tissue in vitro. Bioelectromagnetics 6:327–337.

Bowman RR (1976): A probe for measuring temperature in radio frequency heated material. IEEE Trans Microwave Theor Tech 24:43–45.

Bruno JG, Kiel JL (1994): Synthesis of Diazoluminomelanin (DALM) in HL-60 cells for possible use as cellular-level microwave dosimeter. Bioelectromagnetics 15:315–328.

Cetas TC (1990): Temperature. In Lehmann JF (ed): "Therapeutic Heat and Cold." Baltimore, MD: Williams and Wilkins, pp 1–61.

Chou CK (1990): Use of heating rate (HR) and specific absorption rate (SAR) in the hyperthermia clinic. Int J Hyperthermia 6:367–370.

Chou CK (1992): Evaluation of microwave hyperthermia applicators. Bioelectromagnetics 13:581–597.

Chou CK, Guy AW (1977): Microwave and RF dosimetry. In Taylor LS, Cheung AY (eds): Workshop on the physical basis of electromagnetic interactions with biological systems. Proceedings of the workshop held at the University of Maryland, College Park, MD, June 15–17, 1977, pp 165–216.

Chou CK, Guy AW (1985): Research on nonionizing radiation: Physical aspects in extrapolating infra human data to man. In Monahan JC, D'Andrea JA (eds): "Behavioral Effects of Microwave Radiation Absorption." Washington, DC: HHS Publication FDA 85-8238, August, 1985, pp 135–149.

Chou CK, Guy AW, Galambos R (1982): Auditory perception of radiofrequency electromagnetic field. 80th review and tutorial paper. J Acoust Soc Am 71:1321–1334.

Chou CK, Guy AW, Johnson RB (1984a): SAR in rats exposed in 2450-MHz circularly polarized waveguide. Bioelectromagnetics 5:389-398.

Chou CK, Chen GW, Guy AW, Luk KH (1984b): Formulas for preparing phantom muscle tissue at various radiofrequencies. Bioelectromagnetics 5:435–441.

Chou CK, Guy AW, McDougall JA (1987): Shielding effectiveness of improved microwave protective suit. IEEE Trans Microwave Theor Tech 35:995–1001.

Chou CK, Guy AW, Kunz LL, Johnson RB, Crowley J, Krupp JH (1992): Long-term, low-level, microwave irradiation of rats. Bioelectromagnetics 13:469-496.

Cleveland RF Jr, Athey TW (1989): Specific absorption rate (SAR) in models of the human head exposed to hand-held UHF portable radios. Bioelectromagnetics 10:173–186.

Cogan DG, Fricker SJ, Lubin M, Donaldson DD, Hardy H (1958): Cataracts and UHF radiation. AMA Arch Indust Med 18:299–302.

Cristoforetti L, Pontalti R, Cescatti L, Antolini R (1993): Quantitative colorimetric analysis of liquid crystal films (LCF) for phantom dosimetry in microwave hyperthermia. IEEE Trans Biomed Eng 40:1159–1164.

de Lorge JO (1984): Operant behavior and colonic temperature of *Macaca mulatta* exposed to radio frequency fields at and above resonant frequencies. Bioelectromagnetics 5:233–246.

de Lorge JO, Ezell CS (1980): Observing-responses of rats exposed to 1.28- and 5.62-GHz microwaves. Bioelectromagnetics 1:183–198.

Durney CH, Johnson CC, Barber PW, Massoudi H, Iskander MF, Lords JL, Ryser DK, Allen SJ, Mitchell JC (1978): "Radiofrequency Radiation Dosimetry Handbook, Second Edition (Report SAR-TR-78-22)." Brooks Air Force Base, TX: USAF School of Aerospace Medicine.

Durney CH, Massoudi H, Iskander MF (1986): "Radiofrequency Radiation Dosimetry Handbook, 4th Edition (Report TR-85-73)." Brooks Air Force Base, TX: USAF School of Aerospace Medicine.

Foster KR, Schwan HP (1986): Dielectric permittivity and electrical conductivity of biological materials. In Polk C, Postow E (eds): "Handbook of Biological Effects of Electromagnetic Fields." Boca Raton, FL: CRC Press, pp 27–96.

Foster KR, Kritikos HN, Schwan HP (1978): Effect of surface cooling and blood flow on the microwave heating of tissue. IEEE Trans Biomed Eng 25:313–316.

Gabriel C (1995): The dielectric properties of biological materials. In Klauenberg BJ, Grandolfo M, Erwin DN (eds): "Radiofrequency Radiation Standards." New York: Plenum Press, pp 187–196.

Gandhi OP (1990): Numerical methods for specific absorption rate calculations. In Gandhi OP (ed): "Biological Effects and Medical Applications of Electromagnetic Energy." Englewood Cliffs, NJ: Prentice Hall, pp 113–140.

Gandhi OP, Hunt EL, D'Andrea JA (1977): Deposition of electromagnetic energy in animals and in models of man with and without grounding and reflector effects. Radio Sci 12(6S):39–47.

Gandhi OP, Hagmann MJ, D'Andrea JA (1979): Part-body and multi-body effects on absorption of radio-frequency electromagnetic energy by animals and by models of man. Radio Sci 14(6S):15–21.

Garn H, Gabriel C (1995): Present knowledge about specific absorption rates inside a human body exposed to radiofrequency electromagnetic fields. Health Phys 68:147–156.

Guy AW (1971): Analyses of electromagnetic fields induced in biological tissues by thermographic studies on equivalent phantom models. IEEE Trans Microwave Theor Tech 19:205–214.

Guy AW (1987): Dosimetry associated with exposure to nonionizing radiation: very low frequency to microwaves. Health Phys 53:569–584.

Guy AW, Chou CK (1986): Specific absorption rates of energy in man models exposed to cellular UHF-mobile-antenna fields. IEEE Trans Microwave Theor Tech 34:671–680.

Guy AW, Lehmann JF, McDougall JA, Sorensen C (1968): Studies on therapeutic heating by electromagnetic energy. In "Thermal Problems in Biotechnology." New York: ASME, United Engineering Centers, pp 26–45.

Guy AW, Webb MD, Sorensen CC (1976): Determination of power absorption in man exposed to high frequency electromagnetic fields by thermographic measurements on scaled models. IEEE Trans Biomed Eng 23:361–371.

Guy AW, Wallace J, McDougall JA (1979): Circularly polarized 2450-MHz waveguide system for chronic exposure of small animals to microwaves. Radio Sci 14(6S):63–74.

Guy AW, Davidow S, Yang GY, Chou CK (1982): Determination of electric current distributions in animals and human exposed to a uniform 60-Hz high-intensity electric field. Bioelectromagnetics 3:47–71.

Guy AW, Chou CK, Neuhaus B (1984): Average SAR and SAR distributions in man exposed to 450 MHz radiofrequency radiation. IEEE Trans Microwave Theor Tech 32:1182–1200.

Guy AW, Chou CK, McDougall JA, Sorensen C (1987): Measurement of shielding effectiveness of microwave protective suits. IEEE Trans Microwave Theor Tech 35:984–994.

Hartsgrove G, Kraszewski A, Surowiec A (1987): Simulated biological materials for electromagnetic radiation absorption studies. Bioelectromagnetics 8:29–36.

Hill D (1982): A waveguide technique for the calibration of miniature implantable electric field probes for use in microwave bioeffects studies. IEEE Trans Microwave Theor Tech 30:92–99.

IRPA (1993): "Electromagnetic Fields (300 Hz to 300 GHz), Environmental Health Criteria, 137." Geneva, Switzerland: International Radiation Protection Association, World Health Organization.

Johnson CC, Guy AW (1972): Nonionizing electromagnetic wave effects in biological materials and systems. Proc IEEE 60:692–718.

Justesen DR (1975): Toward a prescriptive grammar for the radiobiology of nonionizing radiations: Quantities, definitions, and units of absorbed EM energy—An assay. J Microwave Power 10:343–356.

Justesen DR, King NW (1970): Behavioral effects of low level microwave irradiation in the close-space situation: Biological effects and health implications of microwave radiation. In Cleary SF (ed): "Symposium Proceedings." BRH/DBE 70-2, pp 154–179.

Kuster N, Balzano Q (1992): Energy absorption mechanism by biological bodies in the near field of dipole antennas above 300 MHz. IEEE Trans Vehicular Technol 41:17–23.

Kuster N, Schmid T, Meier K (1993): Studies of absorption in the extreme near field of transmitters. Mannheim, Federal Republic of Germany: Proc VDE Meeting, Nov. 9–10, 1993.

Lagendijk JJW, Nilsson P (1985): Hyperthermia dough: A fat and bone equivalent phantom to test microwave/radiofrequency hyperthermia heating systems. Phys Med Biol 30:709–712.

Lehmann JF (1994): "Therapeutic Heat and Cold." Baltimore, MD: Williams and Wilkins.

Lin JC (1978): "Microwave Auditory Effects and Applications." Springfield, IL: C. Thomas.

Lin JC (1986): Computer methods for field intensity prediction. In Polk C, Postow E (eds): "Handbook of Biological Effects of Electromagnetic Fields." Boca Raton, FL: CRC Press, pp 273–313.

Lin JC, Guy AW, Johnson CC (1973): Power deposition in a spherical model of man exposed to 1-20-MHz electromagnetic fields. IEEE Trans Microwave Theor Tech 21:791–797.

Meltz ML, Eagan P, Harris CR, Erwin DN (1988): Dosimetry considerations in far field microwave exposure of mammalian cells. Physiol Chem Phys Med NMR 20:23–30.

Michaelson SM, Lin JC (1987): "Biological Effects and Health Implications of Radiofrequency Radiation." New York: Plenum Press.

Mittleman E, Osborne SL, Coulter J (1941): Shortwave diathermy power absorption and deep tissue temperature. Arch Phys Ther 22:133–139.

NCRP (1981): "Radiofrequency Electromagnetic Fields: Properties, Quantities and Units, Biophysical Interaction, and Measurements (Report 67)." Bethesda, MD: National Council on Radiation Protection and Measurements.

NCRP (1986): "Biological Effects and Exposure Criteria for Radiofrequency Electromagnetic Fields (Report 86)." Bethesda, MD: National Council on Radiation Protection and Measurements.

NCRP (1994): "A Practical Guide to the Determination of Human Exposure to Radiofrequency Fields (Report 119)." Bethesda, MD: National Council on Radiation Protection and Measurements.

NRPB (1993): "Board Statement on Restrictions on Time Varying Electromagnetic Fields and Radiation." Chilton, United Kingdom: Documents of the National Radiological Protection Board, Vol. 4, No. 5.

Olsen RG, Griner TA (1989): Outdoor measurements of SAR in a full size human model exposed to 29.2 MHz near-field irradiation. Bioelectromagnetics 10:162–171.

Phillips RD, Hunt EL, King NW (1975): Field measurements of absorbed dose and biologic dosimetry of microwaves. Ann NY Acad Sci 247:499–509.

Reilly JP (1992): "Electrical Stimulation and Electropathology." New York: Cambridge University Press.

Samulski TV, MacFall J, Zhang Y, Grant W, Charles C (1992): Noninvasive thermometry using magnetic resonance diffusion imaging: Potential for application in hyperthermic oncology. Int J Hyperthermia 8:819–829.

Schwan HP (1971): Interaction of microwave and radiofrequency radiation with biological systems. IEEE Trans Microwave Theor Tech 19:146–152.

Stuchly MA, Lecuyer DW (1985): Induction heating and operator exposure to electromagnetic fields. Health Phys 49:693–700.

Stuchly MA, Stuchly SS (1986): Experimental radio and microwave dosimetry. In Polk C, Postow E (eds): "Handbook of Biological Effects of Electromagnetic Fields." Boca Raton, FL: CRC Press, pp 229–272.

Stuchly MA, Stuchly SS (1990): Electrical properties of biological substances. In Gandhi OP (ed): "Biological Effects and Medical Applications of Electromagnetic Energy." Englewood Cliffs, NJ: Prentice Hall, pp 75–112.

Stuchly MA, Stuchly SS, Kraszewski A (1984): Implantable electric field probes—Some performance characteristics. IEEE Trans Biomed Eng 31:526–531.

Taflove A (1995): "Computational Electrodynamics: The Finite-Difference Time-Domain Method." Boston, MA: Artech House.

Wickersheim KA, Sun M (1987): Fiber optic thermometry and its applications. J Microwave Power 22:85–94.

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EFFECTS OF LONG-TERM RADIOFREQUENCY RADIATION ON IMMUNOLOGICAL COMPETENCE AND METABOLISM

Chung-Kwang Chou, Ph.D.
James A. Clagett, Ph.D.
Lawrence L. Kunz, D.V.M.
Arthur W. Guy, Ph.D.

Bioelectromagnetics Research Laboratory
Center for Bioengineering
School of Medicine
University of Washington
Seattle, WA 98195

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

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

JEROME H. KRUPP, D.V.M.
Project Scientist

JOHN C. MITCHELL, B.S.
Supervisor

JEFFREY G. DAVIS, Colonel, USAF, MC
Commander

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Two groups of 20 rats were exposed 21 h/day for 6 and 12 mo, respectively, to 10-μs-pulsed 2450-MHz microwaves at 800 pps and 8-Hz modulation, at an average power density of .48 mW/cm^2 (SAR 0.15-0.4 W/kg) in circularly polarized waveguides. Equal numbers of rats were sham exposed. Effects seen in the previous study (elevated corticosterone level after 6 wk exposure; increased B and T cells in rats at 13 mo exposure) were not replicated in this study. Quantitative immunological tests involving 70 parameters showed six different effects. Among them, increased hematopoietic progenitor cells in the marrow was the only consistent effect in both 6- and 12-mo exposed groups. In the second part of the study three groups of rats were exposed 21 h/day to 2450-MHz CW microwaves at an average SAR of 0, 2.5, 5, and 7.5 W/kg (10 rats each) under three environmental temperatures (17.8, 22.2, and 26.7°C) over three 6-wk periods. Metabolism--including body mass, food/water intake, oxygen consumption, and carbon dioxide production--was measured, and health profile and immunological response were monitored. Results show that the effects are highly dependent on exposure level and environmental temperature.

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SUMMARY

This report presents the results of two studies on effects of long-term radiofrequency radiation of rats. The first was an attempt to replicate the finding of enhanced immunological response after exposure to pulsed microwaves. The second study was to generate, in a parametric fashion, a dose-response demonstration of the effects on metabolism, health profile, and immunological response.

Two groups of 20 male Sprague Dawley rats were exposed to 10-μs-pulsed 2450-MHz microwaves at 800 pps and 8-Hz modulation for 21 h/day for 6 or 12 mo, at an average power density of 0.48 mW/cm^2 (SAR 0.15 to 0.4 W/kg) in circularly polarized waveguides. When compared to data from an equal number of sham-exposed rats, the following results were obtained:

1. No effect on body mass or food and water consumption
2. Effects on immune system from the 6-mo exposure:
   a. Increased number of marrow hematopoietic progenitor cells
   b. Increased proliferative responses to purified pokeweek derivative by splenic B cells
   c. Decreased total B lymphocytes in marrow
   d. Decreased proportion of small cells in marrow
3. Effects on immune system from the 12-mo exposure:
   a. Increased number of hematopoietic precursors
   b. Reduction in cell surface expression of Thy 1.1 surface antigen on thermocytes
   c. Reduction in the mean cell-surface density of s-Ig on small lymphocytes in spleen
4. No effect on hematology, serum chemistry, thyroxine, or protein electrophoresis
5. Negative effect on corticosterone level
6. No significant difference (pathological examination) between exposed and control animals except an increased peribronchiolar lymphoreticular proliferation after 12-mo exposure
7. Tumor incidence too low to allow meaningful statistical comparisons

The second part of the project involved exposing three groups of rats--at three environmental temperatures (17.8, 22.2, and 26.7^{o}C respectively)--to 2450-MHz CW microwaves for 21 h/day in circular waveguides at average power densities of 0, 5, 10, and 15 mW/cm^2 (10 rats from each group), corresponding to SARs of 0, 2.5, 5, and 7.5 W/kg, over three 6-wk periods. Metabolism--including body mass, food and water intake, oxygen consumption, and carbon dioxide production--was measured. Health profile and immunological response were also monitored. Results are summarized below:

1. Mortality occurred at high exposure levels, especially at high temperature.
2. Colonic temperature indicated that a 1°C chronic body-temperature rise was the survival threshold.
3. Temperature rise in the metabolism cage was a complex function of power level, temperature, and animal response.
4. Both power level and temperature affected growth.
5. Food consumption decreased as power density increased, at all temperatures.
6. Effect on water consumption was not consistent.
7. Effects on serum chemistry were mostly due to temperature change.
8. Radiation consistently affected BUN, ionized calcium, and alkaline phosphatase.
9. Hematology and protein electrophoresis showed no effect.
10. At 17.8^{o}C, radiation caused corticosterone level to rise; at 22.2^{o}C and 10 or 15 mW/cm^2, the corticosterone level decreased, probably due to adrenal exhaustion.
11. Both oxygen consumption and carbon dioxide production decreased during all exposures. The decrease was also a function of temperature.
12. Effects on immune system were dramatic. They included
    a. pronounced radiation effect on cellularity of the spleen,
    b. biphasic response on the cellularity of the thymus gland,
    c. effects on hematopoietic progenitor cells, and
    d. increased B cells in the thymus.

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EFFECTS OF LONG-TERM RADIOFREQUENCY RADIATION ON IMMUNOLOGICAL COMPETENCE AND METABOLISM

INTRODUCTION

During 1978-1983 the Bioelectromagnetics Research Laboratory at the University of Washington conducted the largest microwave-bioeffects evaluation study ever undertaken. The goal of that project was to investigate purported adverse health effects of long-term exposure to pulsed microwave radiation. The major emphasis was on exposing a large population of experimental animals to microwave radiation throughout their lifetimes and monitoring them for cumulative effects on general health and ultimately on longevity (Guy et al., 1983a). In this follow-up study, we have focused on the effects on immunological competence and metabolism.

A brief summary of the previous project will provide a background for the present study. A unique exposure facility was prepared that allowed 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed in individual circularly polarized waveguides. These waveguides had been developed in this laboratory as a relatively inexpensive exposure apparatus that provided highly quantifiable microwave exposure conditions (Guy and Chou, 1976; Guy et al., 1979).

The exposure facility consisted of two rooms, each containing 50 waveguides for active exposures and 50 for sham exposures. Each room contained two 2450-MHz pulsed microwave generators, each able to deliver a maximum of 10-W average power at 800 pps with a 10-us pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of .48 mW/cm^2. Whole-body calorimetry, thermographic analysis, and power meter analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 300-g rat (Chou et al., 1984).

At 3 wk of age, 200 male rats were obtained from a commercial barrier-reared colony and randomly assigned to exposed and sham-exposed treatment conditions. Exposure began at 8 wk of age and continued for 25 mo. Throughout this period all surviving animals were bled at regular intervals and blood samples were analyzed for a panel of serum chemistries, hematological values, protein electrophoretic patterns, and thyroxine and plasma corticosterone levels. Body mass and food and water consumption were measured daily, and oxygen consumption and carbon dioxide production were periodically measured in a subpopulation of the exposed and sham-exposed groups. Activity in an open-field apparatus was assessed at regular intervals throughout the study. After 13 mo, 10 rats from each treatment condition were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. The surviving 23 rats (11, sham-exposed group; 12, exposed group) were killed at the end of 25 mo for similar analyses.

The design and results of that study have been published in a series of nine technical reports covering major subtopics (Guy et al., 1983a, 1983b, 1985; Chou et al., 1983b; Johnson et al., 1983, 1984; Kunz et al., 1983, 1984, 1985). Among the 155 parameters measured during the study, most showed no statistical difference. The few endpoints that showed statistical differences were as follow:

1) Serum corticosterone values in exposed animals were elevated at the time of the first sampling session (6 wk exposure).
2) When compared with the sham-exposed group at 13 mo, the exposed animals had a significant increase in both splenic B and T cells; and mitogen-stimulation studies revealed a significantly increased response to concanavalin A (Con A) and a decreased response to pokeweed mitogen (PWM) and purified protein derivative (PPD).
3) The exposed group had statistically more total primary malignant tumors.

In this follow-up study, the first two effects were repeated, but not the third which requires an experiment of a considerably different design.

The technical reports referenced in the last paragraph provide details of the exposure facility, dosimetry, and animal procedures.

OBJECTIVES

The major objective of the current study was to replicate the finding of enhanced immunological response after exposure to pulsed microwave radiation and to determine the temporal variation of the observed effects after 6- and 12-mo exposures. All research was conducted under experimental conditions identical to those in the original experiment, with an enlarged sample size and refined assay techniques.

A second objective was to generate, in a parametric fashion, a dose-response demonstration of effect for those endpoints where no effects were observed at the low power density used in the original experiment. Particular emphasis was placed on measures of metabolism.

SCOPE OF WORK

We replicated the exposure conditions under which enhanced immunological responses had been observed. Two groups of 20 animals each were exposed for 6 and 12 mo respectively. An equal number of sham-exposed animals served as controls. For best use of the existing exposure facility, we started the 6- and 12-mo exposure groups at the same time in one exposure room. To replicate the experimental procedures used in the original study, we conducted regular bleedings at 6-wk intervals during the exposure periods; however, these blood samples were analyzed only at the final bleeding immediately prior to sacrifice of animals for removal of spleen, thymus, and bone marrow for immunological testing. As in the original study, we determined B- and T-cell enumeration and mitogen-stimulation responses of the splenic cells. We also measured the response of thymic lymphocytes and marrow stem cells. Gross pathological and histopathological evaluations of each animal were made following sacrifice.

To determine a dose-response relationship for demonstrable microwave effects, we exposed nine groups of 10 rats each to one of three power densities at one of three ambient temperatures for up to 6 wk.

Ten sham-exposed animals were used as control subjects for each ambient temperature. We collected measurements of metabolism including body weight, food and water consumption, and respiratory gas exchange. Serum chemistry, hematology, protein electrophoresis, thyroxine, and corticosterone were measured at death. Following sacrifice of the animals we made gross pathological and histopathological examinations and removed spleen, thymus, and bone marrow tissue for immunological competence testing.

IMMUNOLOGY

The biological endpoints evaluated in this phase of the project are concerned directly with the immune competence evaluation, including serum chemistry, hematology, protein electrophoresis, thyroxine, corticosterone, and gross pathological and histopathological examinations. These endpoints are the same as those in the original project. As part of the replication, we measured body mass and food and water consumption daily.

Body Mass and Consumption of Food and Water

The animal population, housing facilities, and maintenance procedures are described in detail in Volume 1 of the original project (Guy et al., 1983a). At 21 days of age, 100 male SPF rats (Sprague-Dawley) were obtained from a commercial supplier (Camm (SD) BR, Camm Research Labs, Wayne, NJ) and housed individually throughout the study in a barrier facility. For 21 h/day these animals were in Plexiglas cages placed inside wiremesh waveguides; 50 under microwave-exposure and 50 under sham-exposure conditions. Ten rats in each group served as spare animals. Each morning all animals were removed from these cages at staggered times over a 2-h period, weighed, and placed in standard filter-bonneted Plexiglas cages while their waveguide cages were being cleaned. The facility was maintained at 21 $\pm$ 1°C and 55 $\pm$ 10% relative humidity. Fluorescent room lighting was maintained on a 12/12 light/dark cycle (lights on 0700-1900).

During the 21 h/day that the animals were housed in the waveguide, they had ad libitum access to autoclaved pelleted chow (Certified Autoclavable Rodent Chow #5014, Ralston Purina Co., St. Louis, MO) and distilled water.

Figures 1 and 2 show the mean body mass of the 6- and 12-mo exposures. Analysis of variance with repeated measures did not reveal any significant difference ($p > 0.05$). The same results are observed on food and water consumption, as shown in Figures 3-6. No effect was found.