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Absence of embryotoxic effects from low-level (nonthermal) exposure of rats to 100 MHz radiofrequency radiation

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LARY JM, CONOVER DL, JOHNSON PH. Absence of embryotoxic effects from low-level (nonthermal) exposure of rats to 100 MHz radiofrequency radiation. *Scand j work environ health* 9 (1983) 120—127. Pregnant Sprague-Dawley rats were exposed to radiofrequency radiation at a frequency of 100 MHz and a power density of 25 mW/cm² for 6 h 40 min daily on gestation days 6—11. The total exposure time was 40 h. The exposure resulted in a specific absorption rate of 0.4 W/kg. This value corresponds to the maximum permissible level for specific absorption rate in the 1982 American National Standards Institute (ANSI) standard for radiofrequency/microwave exposure. The exposure produced no increase in maternal colonic temperature. Irradiated rats did not differ from sham-irradiated rats with respect to the number of implantations per litter, percentage of implantations dead or resorbed, percentage of fetuses malformed, fetal weight, fetal crown-rump length, or fetal sex ratio. The irradiated fetuses had fewer minor skeletal variations than the controls. These results suggest that radiofrequency/microwave radiation is not teratogenic or embryotoxic for rats at the maximum permissible exposure level of the 1982 ANSI standard.

Key terms: fetus, hyperthermia, microwaves, specific absorption rate, teratogenesis.

Since World War II there has been a tremendous increase in the use of radiofrequency and microwave radiation in commercial radio and television, commercial and governmental communications networks, citizens band and ham radios, radar, microwave ovens, medical diathermy, and a variety of industrial processes. Human exposure to radiofrequency/microwave radiation from these sources has increased correspondingly.

In 1974 the American National Standards Institute published a radiation protection guide for exposure to radiofrequency and microwave radiation between the fre-

quencies of 10 MHz and 100 GHz (1). It recommended that a person not be exposed to a power density greater than 10 mW/cm² or to electric and magnetic field strengths greater than 200 V/m and 0.5 A/m, respectively. This recommendation has served as the consensus American standard until the present. In 1982 a more stringent radiofrequency microwave standard was published based on the assumption that exposure to radiofrequency/microwave energy at an average whole-body specific absorption rate (SAR) of 0.4 W/kg or less will not cause adverse biological effects (2).

Specific absorption rate is defined as the amount of power (in watts) absorbed per unit of body mass (in kilograms). It is a measure of the amount of energy (in joules) absorbed by the body each second. To cause a biological effect, radiofrequency/microwave energy must be absorbed by the body. The severity of a biological effect is generally expected to

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increase as the rate of energy absorption (power) by the body increases. The amount of power absorbed per unit of body mass (SAR) is directly proportional to the power density of the incident radiation. However, in a field of constant power density, the specific absorption rate varies considerably with the frequency of the radiation and the size, shape, and orientation of the animal in the field (7). The specific absorption rate of an animal in a 10 mW/cm² field can vary by a factor of 100,000 or more depending upon the frequency of the radiation (7). For this reason, the American National Standards Institute decided to base their 1982 standard on the biological effects that occur at a given specific absorption rate rather than on those that occur at a given incident power density level or electric and magnetic field strength. Although the new limits are stated in terms of power density and electric and magnetic field strength, they vary as a function of frequency so that the average whole-body specific absorption rate of a human being will not exceed 0.4 W/kg. In the frequency range of maximum human absorption (30–300 MHz), the new limits are 63 V/m for the electric field strength, 0.16 A/m for the magnetic field strength, and 1 mW/cm² for the far-field equivalent power density level. The limits are higher at other frequencies (2).

Several investigators have reported that microwave radiation exposure at the old limit of 10 mW/cm² adversely affects embryonic development in laboratory animals (3, 4, 18). Others have reported that exposure at or near this level has no significant effect on development (11, 14, 15, 16). All of these studies have been performed at frequencies between 915 MHz and 3,100 MHz.

No low-intensity (at or below 10 mW/cm²) teratology studies have been reported in the frequency range of maximum human absorption (30–300 MHz). The present study was carried out to determine if exposure to radiofrequency radiation in this frequency range is teratogenic or embryotoxic in the laboratory rat at the new limit of 0.4 W/kg. To simulate human exposures at the 0.4 W/kg limit, we exposed rats to a 100 MHz field at a power density of 25 mW/cm².

Materials and methods

Animals

Sexually mature, three- to six-month-old Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were given Purina Laboratory chow and water ad libitum and maintained under a 14-h light : 10-h dark cycle at $24 \pm 2^\circ\text{C}$ in the animal quarters. Two nulliparous females were caged overnight with each male and checked the following morning between 0830 and 1030 for the presence of sperm in the vagina. The day that sperm was detected was considered day zero of gestation. Mated rats were randomly assigned to the experimental and control groups.

Exposure facility

Pregnant rats were irradiated at 100 MHz under continuous wave, far-field conditions in a Narda model 8802 transverse electromagnetic (TEM) transmission cell. The TEM cell produces highly accurate, uniform electromagnetic fields and may be used as a standard facility for calibrating radiofrequency field strength meters (6). The signal to the TEM cell was provided by a Hewlett-Packard model 8660C synthesized signal generator through an Amplifier Research model 1000L linear amplifier. The TEM cell was housed within an environmental chamber. The temperature within the TEM cell was maintained at $24.0 \pm 0.3^\circ\text{C}$, and the relative humidity at $55 \pm 5\%$. A whisper fan drew fresh air through the TEM cell at a velocity of 0.15 m/s. The TEM cell was illuminated with a 40-W incandescent light bulb.

During irradiation, the rats were housed separately in cylindrical Plexiglas cages (20 cm long and 10 cm in diameter) perforated with 12-mm holes. Radiofrequency radiation passes through Plexiglas with negligible absorption. The cages were designed to keep the rat oriented primarily parallel to the long axis of the cage but also to allow considerable freedom of movement to minimize stress.

The direction of propagation of the electromagnetic wave in the TEM cell was vertical, with the E-field perpendicular to the septum plate. Each cage was oriented

with its long axis parallel to the E-field. As a result, each animal was oriented parallel to the E-field during most of the exposure period.

Experimental procedure

Pregnant rats were sham-irradiated or exposed to 100 MHz radiation at a power density of 25 mW/cm² for 6 h 40 min daily on each of gestation days 6–11. Total exposure time was 40 h. The exposure conditions were selected to produce an average whole-body specific absorption rate of 0.4 W/kg in each animal. The animals were not anesthetized during the treatment. Food and water were withheld during the irradiation period.

All the animals were weighed immediately before and after the treatment on gestation days 6 and 11. The colonic temperature of each animal was measured immediately before and after the exposure period on days 6 and 11 with a Yellow Springs Instrument model 43 meter and a model 402 thermistor probe, calibrated daily to $\pm 0.1^\circ\text{C}$ against a mercury thermometer calibrated by the National Bureau of Standards.

A total of 34 pregnant rats was used in the radiofrequency treatment group. A total of 32 pregnant animals was sham-irradiated in a mock TEM cell to serve as controls. They were treated identically to the radiofrequency-exposed group but were not irradiated. Approximately equal numbers of irradiated and control animals were treated during each exposure week. The treatments were carried out over a period of three months.

Four, six, or eight rats were irradiated in the TEM cell during any one treatment week. Equal numbers of rats were placed on either side of the septum plate. The power density levels at the midpoint of each cage location varied no more than 25 % from the mean value. To assure more uniform exposures, the cage location for each animal was randomly assigned on each treatment day.

All rats were sacrificed by cervical dislocation between 0800 and 1200 on gestation day 20 (22-d gestation period) to prevent cannabilization of dead or malformed offspring by the dam. The uterine horns were exposed by laparotomy and

examined for the number of implantations, live fetuses, and dead or resorbed conceptuses. Each live fetus was removed, sexed, weighed, measured for crown-rump length, and examined externally for gross malformations. Since an earlier study in our laboratory (13) indicated that radiofrequency radiation induced numerous skeletal abnormalities but few visceral abnormalities, all of the fetuses were preserved in 70 % ethanol and cleared and stained by the potassium hydroxide-alizarin red technique of Cray (5) for skeletal examination. Fetuses were scored for major skeletal abnormalities and minor skeletal variations. Minor skeletal variations were defined as all variations in skeletal development which commonly appear in untreated controls on gestation day 20.

Determinations of specific absorption rate

The specific absorption rate for radiofrequency power was determined for the exposed rats with a calorimetric technique (9) which employed 300-g 1 % saline phantom models of a medium-sized rat. The long axis of the saline model was aligned parallel to the electric field in the TEM cell. Eight phantom models were simultaneously placed at the usual cage locations in the TEM cell. An average whole-body specific absorption rate was determined for each location.

Statistical methods

To test for differences in maternal body weight between groups during the study, a multivariate analysis of variance was performed on the six weight measurements taken over time for each dam. The multivariate analysis of variance was also used to test for group differences in pre- and postexposure temperatures over the 6-d treatment period. The Wilcoxon distribution-free test on ranked litter proportions was used to test for group differences in the proportion of dead or resorbed fetuses per litter, the proportion of external malformations per litter, the proportion of major skeletal abnormalities per litter, the proportion of minor skeletal variations per litter, and the sex ratio of

each litter. The Student's t-test was used to test for differences in mean implantations per animal, mean fetal weight per litter, and mean fetal length per litter. A 5% significance level was chosen for all the comparisons.

Results

Specific absorption rate

The measurements of specific absorption rate for the eight saline phantom rat models ranged from 0.35 to 0.47 W/kg with a mean value of 0.41 W/kg. According to the *Radiofrequency Radiation Dosimetry Handbook* (7) the predicted whole-body specific absorption rate for a medium-sized rat (320 g) exposed to a 25 mW/cm² field at 100 MHz is 0.5 W/kg. The measured values for the rat models were very close to the predicted values. It is therefore expected that the average whole-body rate for the live rats during the irradiation period was approximately 0.4 W/kg.

Biological effects

There were no differences in maternal body weights between the irradiated and sham-irradiated rats at any time during the gestation period (table 1). However, a large decrease in body weight occurred in both groups during the treatment period. The animals lost approximately 10 g over the 6-h 40-min treatment period on both day 6 and day 11. This decrease was consistent among individual animals as well. Body weights were sampled for

a few animals on treatment days other than days 6 and 11 and the same 10-g decrease in weight was observed after each treatment.

No differences in maternal colonic temperature were observed between irradiated and sham-irradiated rats either before or after the treatment (table 1). In both groups the mean colonic temperature dropped 0.6 to 0.8°C during the treatment period. However, since the distribution of radiofrequency energy absorption within the rat was not known, localized temperature increases in other areas of the body cannot be ruled out.

There was no evidence that the radiofrequency radiation exposure was embryotoxic or teratogenic. No differences were detected between the irradiated and sham-irradiated groups with respect to mean implantations per litter, percentage of implantations dead or resorbed, percentage of live fetuses externally malformed, percentage of live fetuses with major skeletal abnormalities, mean fetal weight, mean crown-rump length, or fetal sex ratio (table 2). The percentage of minor skeletal variations in live fetuses was significantly higher in the control group than in the irradiated group (76 vs 64%).

Three externally malformed fetuses, each in separate litters, were found in the irradiated group, but none were seen in the control group (table 3). Four to five percent of the fetuses in each group had major skeletal abnormalities (table 2), most of which can be attributed to significant delays in the rate of skeletal ossification.

Table 1. Weights and colonic temperatures of pregnant rats exposed for 40 h to 100 MHz radiofrequency radiation on gestation days 6–11.^a

	Irradiated group		Control group	
	Mean	SE	Mean	SE
Mating weight (g)	288	4	285	4
Preexposure weight on gestation day 6 (g)	306	5	307	4
Postexposure weight on gestation day 6 (g)	295	4	295	4
Preexposure weight on gestation day 11 (g)	320	4	321	4
Postexposure weight on gestation day 11 (g)	311	4	311	4
Gestation day 20 sacrifice weight (g)	389	7	386	5
Preexposure colonic temperature on gestation day 6 (C°)	38.2	0.1	38.1	0.1
Postexposure colonic temperature on gestation day 6 (C°)	37.4	0.1	37.5	0.1
Preexposure colonic temperature on gestation day 11 (C°)	38.2	0.1	38.1	0.1
Postexposure colonic temperature on gestation day 11 (C°)	37.4	0.1	37.3	0.1

^a 6 h 40 min of daily exposure to 25 mW/cm² (specific absorption rate = 0.4 W/kg).

Table 2. Embryotoxicity in rats exposed for 40 h to 100 MHz radiofrequency radiation on gestation days 6–11.^a

	Irradiated group	Control group
Number of litters	34	32
Mean implantations per litter ($\pm$ SE)	13.8 $\pm$ 0.7	13.0 $\pm$ 0.7
Percentage of implantations dead or resorbed per litter	12	13
Percentage of live fetuses externally malformed per litter	0.7	0
Percentage of live fetuses with major skeletal abnormalities per litter	4	5
Percentage of live fetuses with minor skeletal variations per litter	64 ^b	76
Mean fetal weight per litter (g $\pm$ SE)	3.31 $\pm$ 0.06	3.26 $\pm$ 0.06
Mean fetal crown-rump length per litter (mm $\pm$ SE)	35.9 $\pm$ 0.3	35.5 $\pm$ 0.2
Fetal sex ratio per litter (males/total $\times$ 100 %)	47	42

^a 6 h 40 min daily exposure to 25 mW/cm² (specific absorption rate = 0.4 W/kg).

^b Differs significantly from that of the control group ($p \leq 0.05$).

Table 3. External malformations and major skeletal abnormalities in fetal rats exposed for 40 h to 100 MHz radiofrequency radiation on gestation days 6–11.^a

External malformations and major skeletal abnormalities	Irradiated group ^b	Control group ^b
Number of fetuses examined	415 (34)	375 (32)
Total fetuses affected	20 (14)	20 (11)
Thoracogastropagus (conjoined twins), acephaly, severely bent tails	1 (1)	— (—)
Umbilical hernia, stunting	1 (1)	— (—)
Arthrogryposis of hind limb, mandibular micrognathia, swollen and partially resorbed tail, tapir-like head, cranial edema, bulge above eyes	1 (1)	— (—)
Basihyoid missing	9 (6)	17 (8)
Full 14th ribs	3 (2)	— (—)
Rudimentary 13th ribs	3 (3)	1 (1)
Pubis missing	1 (1)	3 (2)
Both pubis and ischium missing	1 (1)	1 (1)
Only two metacarpals in each forepaw	2 (2)	2 (1)
Only two metatarsals in each hindpaw	1 (1)	1 (1)
All metatarsals missing	1 (1)	— (—)
Only five lumbar vertebrae	2 (2)	— (—)
Two or more sacral vertebrae missing	2 (2)	— (—)
All caudal vertebrae missing	2 (2)	1 (1)
Four or more sternebrae missing	2 (2)	1 (1)
Small left orbit	— (—)	1 (1)

^a 6 h 40 min daily exposure to 25 mW/cm² (specific absorption rate = 0.4 W/kg).

^b Number of litters affected in parentheses.

Discussion

The results of the present study suggest that exposure to radiofrequency/microwave energy at levels which result in a whole-body specific absorption rate of 0.4 W/kg or less is not harmful to the developing rat embryo. There was no indication that the radiofrequency exposure at 0.4 W/kg was embryotoxic or

teratogenic. Although three externally malformed fetuses appeared in the irradiated group, while none appeared in the control group, the incidence was not statistically significant, and the malformations were unlikely to be caused by the radiation. Malformed fetuses have occurred sporadically in untreated Sprague-Dawley rats in our laboratory in the past, although the incidence has been very low

(approximately one fetus per thousand examined).

One interesting finding of this study was the relatively constant 10-g weight loss experienced by both irradiated and sham-irradiated dams during the 6-h 40-min treatment period (table 1). This decrease occurred even though the rats slept throughout most of the treatment period and rarely defecated or urinated during this period. It is assumed that the decrease was due to the unavailability of food and water during the treatment period combined with the rather high basal metabolic rate of the rats. Subsequent observations of nonpregnant females deprived of food and water for 6 h 40 min, but left in the animal quarters, revealed a similar weight loss (approximately 8 g). Further study of this phenomenon is suggested since many toxicologic studies are designed so that food and water are unavailable to the animal for eight or more hours during treatment.

The daily weight loss during treatment may have contributed significantly to the rather low fetal weight at sacrifice in both groups (table 2). In past studies in our laboratory, untreated or sham-irradiated Sprague-Dawley rat fetuses weighed approximately 3.8 g at sacrifice rather than the 3.3 g reported in the present study. The reduced fetal size may have been caused by a delay in embryonic growth during the treatment period on gestation days 6–11 when food and water were withheld and maternal weight dropped. However, since no untreated (as opposed to sham-irradiated) controls were included in this study, no definite conclusion can be reached about the lower than expected fetal weights.

The irradiated animals differed from the controls only by having a lower percentage of minor skeletal variations (table 2). The incidence of skeletal variations generally increases with decreased body weight due to delayed ossification. On a litter-by-litter basis, the same trend was observed in this study. The lower incidence of skeletal variations in the irradiated group may therefore have been due to the slightly higher mean body weight for the group (table 2).

The exposure conditions in this study (specific absorption rate = 0.4 W/kg) did

not result in an increase in colonic temperature in the dam (table 1). This result is significant since radiofrequency/microwave radiation exposure is teratogenic and embryo-lethal in laboratory animals when it results in appreciable heating of the exposed dam (10, 13, 17). In past studies in this laboratory (13) pregnant rats received a single 20–40 min exposure to radiofrequency radiation of 27.12 MHz at a specific absorption rate of 11.1 to 12.5 W/kg. The exposure elevated the dam's temperature from 38.5 to 43.0°C and caused a high incidence of fetal malformations (up to 67 % on gestation day 9). The malformations were presumed to be caused by heat since similar teratogenic effects have been reported in several species after hyperthermia was induced by conventional heat sources (8).

Most radiofrequency/microwave teratology studies in the literature have reported exposure conditions in terms of the power density level incident upon the animal. However, at a given power density level (eg, 10 mW/cm²), the specific absorption rate of an animal can vary considerably depending upon the size of the animal and the frequency of the radiation. In the present study the power density level was 25 mW/cm², but the specific absorption rate was only 0.4 W/kg. Other teratology studies reporting power density levels at or close to 10 mW/cm², however, actually had much higher predicted specific absorption rates than the present study. The results of these studies are summarized in table 4. Estimates of the specific absorption rate were calculated for each study from the *Radiofrequency Radiation Dosimetry Handbook* (7).

Although the *Radiofrequency Radiation Dosimetry Handbook* only provides an approximate estimate of the specific absorption rates associated with each of the studies in table 4 and the actual rates are unknown, an interesting pattern can be seen when the studies are compared in terms of the estimated rates. The most adverse developmental effects occurred at an estimated specific absorption rate of 10 W/kg. Lower fetal body weights and lower relative brain weights were reported at an estimated rate of 2 W/kg. No significant effects have been reported at a rate lower than 2 W/kg. Based on past research in

Table 4. Literature reports of developmental effects from exposure of animals to low-intensity (about 10 mW/cm²) microwave radiation. (SAR = specific absorption rate)

Developmental effects	SAR (W/kg) ^a	Species	Frequency (MHz)	Power density and duration of exposure	Reference
Increased frequency of fetal death and developmental anomalies, decreased litter size, increased neonatal mortality, retarded behavioral development	10	Mouse	3,000	10 mW/cm ² for 2 h/d on gestation days 1—15	Bereznitskaya & Rysina (4)
Increased incidence of stillbirths and postnatal deaths	10	Mouse	3,000	10 mW/cm ² for 1 h twice each day for five months before and during pregnancy	Bereznitskaya (3)
No significant effects	5—11	Mouse	3,100	5—11 mW/cm ² for 18—306 h before pregnancy	Miro et al (15)
No significant effects	5	Rat	915	10 mW/cm ² for 8 h/d during gestation (110 h total)	Jensh et al (11)
No significant effects	3—5	Rat	1,700	10—15 mW/cm ² for 1 h/d on gestation days 5—8 and 12—16	Rioch (16)
Early eye opening, behavioral deficit	2.5	Rat	918	5 mW/cm ² for 20 h/d on gestation days 1—19	Johnson et al (12)
Decreased body weight and relative brain weight	2	Rat	2,450	10 mW/cm ² for 5 h/d on gestation days 3—19	Shore et al (18)
No significant effects	2	Rat	2,450	10 mW/cm ² for 1 h on gestation days 9 and 16	Michaelson et al (14)
No significant effects	1—2	Rat	3,100	5—11 mW/cm ² for 18—306 h before pregnancy	Miro et al (15)

^a Calculated from Durney et al (7).

our laboratory (13) and this analysis of the literature, it appears that a threshold for teratogenic effects exists at a specific absorption rate of approximately 10 W/kg, although some embryotoxic effects may occur at levels as low as 2 W/kg.

In the present study exposure of rats to radiofrequency radiation at the new limit of 0.4 W/kg set by the American National Standards Institute did not cause an increase in colonic temperature and was not teratogenic or embryotoxic. There is no convincing evidence in the literature that teratogenic or embryotoxic effects from radiofrequency/microwave exposure can occur in the absence of thermal stress on the maternal animal or developing embryo. Additional studies are needed to confirm this observation however. It is recommended that, in all future radiofrequency/microwave teratology research, determinations of the specific absorption rate and colonic temperature measurements of the dam during exposure be included to provide a more definitive basis for comparing results among studies.

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