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# Teratogenic Effects of 27.12 MHz Radiofrequency Radiation in Rats

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*ABSTRACT* High-intensity 27.12 MHz radiofrequency (RF) radiation was determined to be teratogenic in rats during most of the gestation period. Eight groups of pregnant rats were exposed to a magnetic field strength of 55 amps/meter and an electric field strength of 300 volts/meter on gestation days 1, 3, 5, 7, 9, 11, 13, or 15. Exposures ceased once the dam's colonic temperature reached 43.0°C (about 20–40 minutes' duration). Eight matching control groups were sham-irradiated for 30 minutes at 0 amps/meter and 0 volts/meter. An additional group of pregnant rats received no treatment. With one exception, no significant differences occurred between sham-irradiated and untreated control groups. RF exposure, however, caused a significant incidence of fetal malformations throughout the postimplantation period (days 7 through 15). It also caused a low but significant incidence of preimplantation malformations. Fetal weight and crown-rump length were reduced in all postimplantation exposure groups but were not affected by preimplantation exposure. The incidence of dead or resorbed fetuses was significantly increased in rats irradiated on days 7 or 9. The effects observed appeared to be caused by RF-induced hyperthermia in the treated dams. Since a number of industrial, scientific, and medical devices operating at or near 27.12 MHz can cause hyperthermia in humans, women of childbearing age should avoid exposure to RF-radiation levels that exceed current US occupational standards.

The substantial increase in the number and power of radiofrequency (RF) and microwave radiation sources since World War II has led to a growing concern about the potential adverse effects of such radiation on the health of the human population. Commercial radio and television broadcast stations, commercial and governmental communication networks, citizens band and ham radios, radar, microwave ovens, medical diathermy equipment, and a variety of industrial and scientific RF and microwave devices now contribute to human exposure to RF and microwave radiation. Much of the concern about the hazards of such radiation has been directed toward effects on human reproduction and development.

A few cases of birth defects and retarded development have been reported in offspring of women exposed to high-intensity RF/microwave radiation during diathermy treatment (Marchese, '53; Ghietti, '55; Cocozza et al., '60). Other reports indicate that RF/microwave exposure may result in miscarriages (Rubin and Erdman, '59; Imrie, '71), irregular menstrual cycles (Asipov, '65), or decreased lactation in nursing mothers (Presman et al., '61; Palladin et al., '62).

Several studies have indicated that microwave radiation is teratogenic or embryotoxic in experimental mammals, usually at levels that cause some degree of heating of the exposed dam (Pobzhitkov et al., '61; Bereznitskaya, '68; Laskey et al., '70; Bereznitskaya and Rysina, '74; Rugh et al., '74, '75; Chernovetz et al., '77; Berman et al., '78; Nawrot et al., '81). Most of these studies were performed at or near 2,450 megahertz (MHz), the operating frequency of most commercial microwave ovens.

Few experimental teratology studies have been conducted in the RF region (below 300 MHz). Hofmann and Dietzel ('66) reported a high incidence of abortions in rabbits and an increased incidence of malformations in rats

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exposed to high-intensity 27.12 MHz radiation from a diathermy applicator. Dietzel and Kern ('70) reported an increased incidence of both preimplantation loss and resorptions in rats irradiated with high-intensity 27.12 MHz radiation.

In an overview of these two studies, Dietzel ('75) stated that the incidence of resorptions or malformations appeared to depend on the extent of maternal hyperthermia caused by absorption of RF energy. These reports of embryolethal and teratogenic effects from RF exposure at 27.12 MHz are a cause for concern since many industrial, scientific, and medical devices are allowed to operate at unlimited power levels at this frequency (FCC, '76).

In an early survey of RF heating and sealing devices in textile, lumber, and plastics industries (Conover et al., '75), the National Institute for Occupational Safety and Health (NIOSH) reported that nine of ten units tested exceeded the current RF/microwave radiation protection limits recommended by the American National Standards Institute (ANSI). These units operated at frequencies between 15 and 40 MHz. The ANSI limits for RF electric field strength (200 volts/meter) and RF magnetic field strength (0.5 amps/meter) correspond to the occupational exposure standard of 10 mW/cm$^2$ currently being enforced by OSHA, (OSHA, '78). In a later, more extensive survey of 82 industrial RF plastic sealers (Conover et al., '80), NIOSH reported that 60% of the units tested had radiation levels in the vicinity of the operator that exceeded the OSHA RF protection limits. The equivalent power density levels were typically in excess of 100 mW/cm$^2$. The sealers operated between 6 and 38 MHz with the majority operating around 27 MHz. All were operated by women.

At present, no Federal regulations specifically limit RF/microwave exposure of pregnant women. In direct contrast, Czechoslovakian employment practices specifically restrict the levels of RF and microwave radiation to which women of childbearing age may be exposed (Marha, '69). West German regulations also specifically restrict exposure of pregnant women and nursing mothers to RF/microwave radiation in the workplace (German Democratic Republic, '75). The maximum permissible exposure levels for pregnant workers in these two countries vary from 2 to 50 V/m, much below the ANSI standard of 200 V/m.

Because a number of women are being occupationally exposed to relatively high levels of RF radiation between 6 and 40 MHz, we have carried out experiments to confirm and expand upon the reports of teratogenic and embryolethal effects in rats exposed to hyperthermic levels of 27.12 MHz RF radiation. This report presents the results of our initial study.

## MATERIALS AND METHODS

Sexually mature Sprague-Dawley rats (Harlan Industries, Cumberland, Indiana) were given Purina Laboratory Chow and water ad libitum and maintained under a 14-hour light:10-hour dark cycle at 24 ± 2°C within the animal quarters. Three sexually mature nulliparous females were caged overnight with each male and checked the following morning between 8:30 and 10:30 AM for the presence of sperm in a vaginal smear. The day that sperm was detected was considered day 0 of gestation. Mated rats were randomly assigned to designated experimental or control groups.

Pregnant rats were irradiated in a radiofrequency near-field synthesizer facility developed for NIOSH by the National Bureau of Standards (NBS). The synthesizer was operated in the dominant magnetic field mode under continuous wave conditions at 27.12 MHz. The frequency was accurately controlled by a Hewlett-Packard Model 8660C synthesized signal generator. The signal from the 8660C generator, when amplified by an Amplifier Research Model 1000L linear amplifier, provided power to the irradiation facility. Ambient temperature during irradiation was 23 ± 2°C with a relative humidity of 45 ± 10%. The air flow rate in the facility was 6 chamber volumes per hour.

Each rat was irradiated separately in a cylindrical Plexiglas holder perforated with 12-mm holes. RF energy at 27.12 MHz passes through the Plexiglas with negligible absorption. The holders were designed to prevent the rat from changing its orientation relative to the RF field and to allow circulation of air about the rat. Each rat was oriented so that its frontal plane was perpendicular to the incident magnetic field and parallel to the incident electric field. Irradiations were performed without anesthesia. Food and water were withheld during the irradiation period.

The specific absorption rate (SAR) in milliwatts/gram (mW/g) was determined for each irradiated rat by reflection coefficient measurement techniques developed at NBS (Greene, '77; Bussey and Hoer, personal communication). Colonic temperatures of irradiated rats and sham-irradiated control rats were monitored during irradiation with a Ramal liquid crystal/fiber optics microwave temperature probe system (Model LCT-1). Colonic temperatures immediately before and after irradiation were checked with a Yellow Springs Instrument model 43 meter and model 402 thermistor probe, calibrated to ± 0.1 C against an NBS calibrated mercury thermometer. Each probe was inserted into the anus to a depth of 5 cm.

Eight groups, each consisting of 16 to 28 pregnant rats, were exposed to a magnetic field strength of 55 amps/meter (A/m) and an electric field strength of 300 volts/meter (V/m) on gestation day 1, 3, 5, 7, 9, 11, 13, or 15. Exposures ceased once the dam's rectal temperature reached 43.0 ± 0.1°C (about 20–40 minutes' exposure duration). Eight control groups of 10–13 pregnant rats were sham-irradiated for 30 minutes at 0 A/m and 0 V/m on day 1, 3, 5, 7, 9, 11, 13, or 15. Sham-irradiated control groups were combined to form preimplantation (days 1, 3, 5), early organogenesis (days 7, 9, 11), and late organogenesis (days 13, 15) control groups for statistical comparisons with the experimental groups and an untreated control group. The untreated control group consisted of 29 pregnant rats left in the animal quarters throughout their gestation period to assess the possible effects of handling, transport, and restraint upon the sham-irradiated control animals.

All rats were sacrificed by cervical dislocation between 8:00 AM and noon on day 20 of gestation (22-day gestation period) to prevent cannibalization 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. The corpora lutea of pregnancy were counted in untreated dams and in dams irradiated or sham-irradiated during the preimplantation period. Each live fetus was removed, sexed, weighed, measured for crown-rump length, and examined externally for gross malformations. One-third of the live fetuses from each litter were selected at random, dissected, and examined for visceral abnormalities by a modification of Staples' ('74) technique. The remaining live fetuses were preserved in 70% ethanol and cleared and stained by the KOH-alizarin red technique of Crary ('62) for skeletal examination.

Preimplantation (days 1, 3, and 5), early organogenesis (days 7, 9, and 11), and late organogenesis (days 13 and 15) sham-irradiated control groups were statistically compared with each other and with the untreated control group. Each irradiated group was compared against the appropriate combined sham-irradiated control group. A 5% significance level was chosen for all comparisons.

The litter average was the unit of choice for data analysis. Litter averages for each parameter were compared by a one-way analysis of variance followed by Dunnett's t-test (Winer, '62). In several cases an analysis of variance could not appropriately be used because group means of zero were among those being compared. In those instances, after ascertaining that the effect (malformation rate) in a group was not due to a high incidence in only a few litters, the percentage of fetuses affected in each group was compared using Fisher's exact test with Bonferroni's correction for multiple comparisons (Neter and Wasserman, '74). The proportion of litters having at least one malformation (or one resorption) was compared between groups by a Fisher's exact test employing Bonferroni's multiple comparisons correction.

## RESULTS

### *Dosimetry*

This study was designed to determine the extent and severity of RF teratogenic and embryotoxic effects under "worse case" conditions. The exposure conditions were selected to deliver a radiation dose that was nearly lethal because of the high temperature induced in the dam. Previous pilot studies conducted under various combinations of electric and magnetic field strengths and exposure durations revealed that the occurrence of teratogenic effects was better correlated with the rate of power absorption (i.e., the SAR) than with the total energy absorbed. High intensity RF radiation of short duration (10–30 minutes) caused a significant incidence of fetal malformations and embryonic resorptions, whereas moderate intensity radiation of much longer duration (2–4 hours) caused very few effects even though the total energy absorbed was greater. Teratogenic effects appeared to depend more upon the degree of maternal hyperthermia than upon exposure duration. Most of the malformed litters were found in rats irradiated to a temperature of 43.0°C or higher. No malformations occurred if the rat's temperature was less than 41.9°C. Colonic temperatures greater than 43.0°C were increasingly lethal with no rat surviving above 43.5°C. Subsequently, the main study was designed so that rats were irradiated to a constant temperature (43.0°C) rather than for a constant time period.

*TABLE 1. Power absorption and exposure duration of rats treated with 27.12 MHz radiofrequency radiation*[1]

| Gestation day | Average SAR[2] (mW/g ± SE) | Average exposure[3] duration (minutes ± SE) |
|---|---|---|
| 1 | 11.2 ± 0.5 | 28 ± 1.2 |
| 3 | 12.3 ± 0.9 | 27 ± 1.3 |
| 5 | 11.1 ± 0.5 | 30 ± 1.1 |
| 7 | 11.9 ± 0.6 | 32 ± 1.3 |
| 9 | 11.5 ± 0.6 | 31 ± 1.5 |
| 11 | 12.5 ± 0.4 | 26 ± 0.9 |
| 13 | 11.6 ± 0.6 | 28 ± 0.9 |
| 15 | 11.4 ± 0.4 | 27 ± 0.8 |

[1]Rats exposed continuously to a 55 A/m, 300 V/m field until their colonic temperature reached 43.0 ± 0.1°C.
[2]Specific absorption rate (SAR) defined as RF power absorbed divided by total body weight.
[3]Significant difference among exposure groups ($P \leq 0.01$).

In the principal study, the colonic temperature of the rats at the cessation of irradiation averaged 43.0 ± 0.1°C. The temperature of individual rats ranged from 42.6°C to 43.4°C because of some instability in the Ramal temperature probe. The initial colonic temperature averaged 38.6°C in sham-irradiated control rats. The temperature of the control rats rose an average of 0.5°C during the 30-minute exposure period owing to restraint and the irritation caused by the continuous presence of the Ramal temperature probe in the colon.

Eleven percent of the rats exposed to RF radiation died during the irradiation period or shortly thereafter from excessive hyperthermia. All but four of the 26 rats that died had a final colonic temperature of 43.0°C or higher. None of the sham-irradiated or untreated control rats died during the course of the experiment.

The SAR for the RF irradiated animals was relatively constant among groups, averaging from 11.1 to 12.5 mW/g (Table 1). The average irradiation period was also similar among treatment groups, ranging from 26 to 32 minutes.

### *Embryotoxicity*

No significant differences in indicators of embryotoxicity were found among the sham-irradiated and untreated control groups (Table 2). For irradiated animals, however, a number of indicators of embryotoxicity were significantly different from those of the respective sham-irradiated controls. Although no difference was observed in the incidence of preimplantation loss in rats irradiated on days 1, 3, or 5, postimplantation loss was significantly higher in rats irradiated on day 7 (29% incidence) and day 9 (49% incidence). Mean fetal weight was significantly lower than in controls in every group irradiated during postimplantation development (days 7 through 15) with the greatest reduction occurring after irradiation on day 9. Mean crown-rump length of irradiated animals was correspondingly reduced on all postimplantation days and was slightly (but significantly) reduced on preimplantation days 1 and 5. No selective embryotoxicity was observed with respect to sex since the sex ratio was normal in every group.

### *Teratogenicity*

The incidence of fetal abnormalities is listed in Table 3. No significant differences in teratogenicity were found among the sham-irradiated and untreated control groups except for a higher incidence of major visceral abnormalities in the early organogenesis sham controls compared to the untreated controls (4% vs. 0%). No external abnormalities were found in any of the control groups, and the incidence of major skeletal and visceral abnormalities was very low. The type and incidence of abnormalities found in control fetuses are summarized in Table 4.

The incidence of external abnormalities was significantly higher than the respective control incidence in all groups irradiated during postimplantation development, reaching a maximum of 67% on gestation day 9. The incidence was also significantly higher on preimplantation day 3 (3% vs. 0%). The incidence of litters having at least one abnormal fetus was correspondingly higher on days 3, 9, 11, 13, and 15 and approached significance on day 7 ($P \leqq 0.08$). Approximately 87% of the litters were affected on day 9 and 94% on day 13.

The incidence of major skeletal abnormalities was significantly higher in irradiated animals than in their respective controls on days 1, 7, 9, 11, and 15. The incidence approached significance on day 13 ($P \leqq 0.08$). The highest incidence of skeletal abnormalities again occurred on day 9 (60%). The incidence of litters having at least one major skeletal abnormality was significantly higher than controls in rats irradiated on days 7, 9, 11, and 13.

Numerous variations in skeletal development occurred in both control and irradiated fetuses. There were differences in the numbers of metacarpals, metatarsals, sternebrae, and caudal vertebrae. Paired or dumbbell-shaped vertebral centra, rudimentary 14th ribs, and delayed or irregular ossification of various cranial bones were also common variations. The

*TABLE 2. Embryotoxicity of 27.12 MHz radiofrequency radiation in rats*

| Gestation day | Total litters | Total implants | Total viable fetuses | Preimplant. loss (%) | % Dead or resorbed | Mean fetal weight (g ± SE) | Mean crown-rump length (mm ± SE) | Sex ratio[1] (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 17 | 163 | 143 | 19 | 22 | 3.79 ± 0.14 | 37.2 ± 0.5* | 50 |
| 3 | 21 | 215 | 191 | 25 | 12 | 3.90 ± 0.10 | 38.1 ± 0.4 | 46 |
| 5 | 28 | 332 | 293 | 13 | 17 | 3.68 ± 0.08 | 37.4 ± 0.3* | 50 |
| Preimplantation sham controls | 35 | 359 | 311 | 22 | 16 | 3.81 ± 0.05 | 38.8 ± 0.3 | 49 |
| 7 | 23 | 278 | 197 | — | 29* | 3.33 ± 0.15* | 36.0 ± 0.5* | 44 |
| 9 | 16 | 186 | 95 | — | 49* | 3.08 ± 0.15* | 34.1 ± 1.1* | 57 |
| 11 | 19 | 209 | 171 | — | 18 | 3.59 ± 0.09* | 36.4 ± 0.4* | 54 |
| Early organogenesis sham controls | 31 | 328 | 293 | — | 11 | 3.96 ± 0.07 | 38.6 ± 0.3 | 53 |
| 13 | 18 | 230 | 189 | — | 18 | 3.36 ± 0.11* | 36.5 ± 0.4* | 50 |
| 15 | 18 | 201 | 157 | — | 22 | 3.35 ± 0.10* | 36.6 ± 0.3* | 45 |
| Late organogenesis sham controls | 21 | 234 | 218 | — | 12 | 4.01 ± 0.04 | 38.8 ± 0.3 | 51 |
| Untreated controls | 29 | 336 | 315 | 12 | 6 | 3.91 ± 0.07 | 38.0 ± 0.3 | 44 |

*Differs significantly from the respective sham control value (≤ 0.05).
[1]Sex ratio defined as male fetuses/total fetuses × 100%.

*TABLE 3. Incidence of fetal abnormalities in rats exposed to 27.12 MHz radiofrequency radiation*

| Gestation day | % External abnormalities | % Litters with external abnormalities | % Major skeletal abnormalities | % Litters with major skeletal abnormalities | % Skeletal variations | % Major visceral abnormalities | % Litters with major visceral abnormalities |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 11* | 29 | 49* | 0 | 0 |
| 3 | 3* | 19* | 3 | 10 | 44* | 8 | 10 |
| 5 | 0 | 0 | 5 | 15 | 52* | 3 | 8 |
| Preimplantation sham controls | 0 | 0 | 2 | 7 | 21 | 0 | 0 |
| 7 | 2* | 18 | 11* | 32* | 69* | 8 | 19 |
| 9 | 67* | 87* | 60* | 85* | 83* | 65* | 86* |
| 11 | 22* | 56* | 32* | 76* | 73* | 11 | 22 |
| Early organogenesis sham controls | 0 | 0 | 0 | 0 | 25 | 4** | 13 |
| 13 | 42* | 94* | 6 | 28* | 57* | 1 | 6 |
| 15 | 17* | 67* | 7* | 22 | 58* | 7 | 11 |
| Late organogenesis sham controls | 0 | 0 | 0 | 0 | 18 | 1 | 0 |
| Untreated controls | 0 | 0 | 1 | 7 | 24 | 0 | 0 |

*Differs significantly from the respective sham control value (P ≤ 0.05).
**Differs significantly from the untreated control value (P ≤ 0.05).

incidence of such skeletal variations in control groups ranged from 18% to 25%. However, the incidence was higher in every irradiated group than in their corresponding controls, ranging from 44% on day 3 to 83% on day 9.

The incidence of major visceral abnormalities in irradiated fetuses was significantly higher than in controls only on gestation day 9 (64% vs. 4%). On the basis of litters affected, the difference on day 9 was also significant (86% vs. 13%).

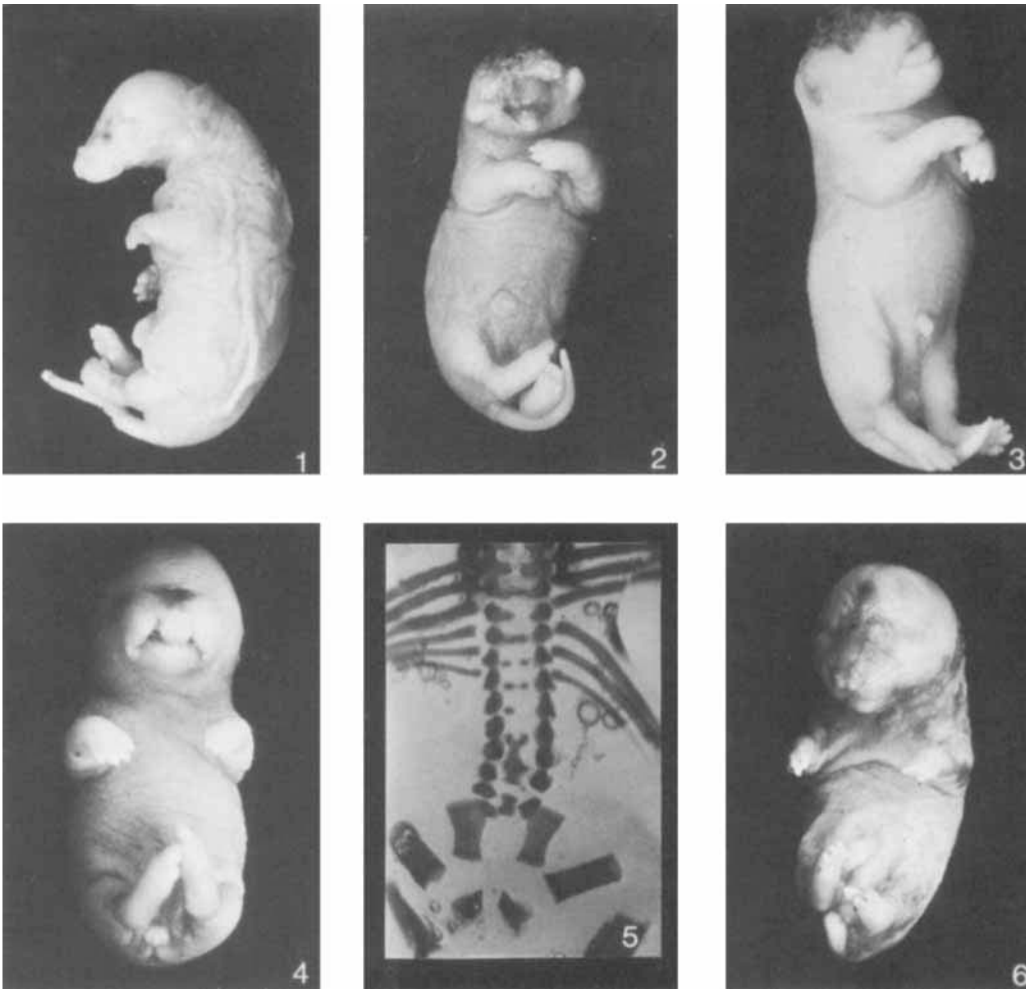
More than 200 different types of abnormalities were observed in irradiated fetuses, most of which occurred only once. The type and incidence of the predominant abnormalities are listed in Table 5. Only defects that occurred in two or more fetuses were included. All of the defects in rats irradiated on day 1 involved

*TABLE 4. Abnormalities in control rat fetuses*

| Control groups | Abnormality | Number fetuses affected |
|---|---|---|
| Preimplantation sham irradiation | Basihyoid missing | 3 |
| Early organogenesis sham irradiation | Renal and ureteral agenesis | 1 |
| | Agenesis of brain ventricles | 1 |
| | Agenesis of 13th ribs | 1 |
| | Small vesicles in lens | 1 |
| | Abnormal growth between skull and skin, malformed cerebral hemispheres | 1 |
| Late organogenesis sham irradiation | Very short 13th rib | 1 |
| Untreated | Parietals, interparietal, and supraoccipitals irregularly and poorly ossified | 2 |
| | One-half of one thoracic centrum missing | 1 |

*TABLE 5. Predominant abnormalities in rat fetuses exposed to 27.12 MHz RF radiation*

| | Fetuses affected | |
|---|---|---|
| | % | No. |
| Day 1 | | |
| Greatly retarded cranial ossification | 2 | 2 |
| Supernumerary cranial bones | 2 | 2 |
| Day 3 | | |
| Twisted or kinked tail | 1 | 4 |
| Mandibular agnathia or micrognathia | 1 | 2 |
| Day 5 | | |
| Basihyoid missing | 1 | 3 |
| Hydronephrosis | 1 | 2 |
| Supraoccipital missing | 1 | 2 |
| All caudal vertebrae missing | 1 | 2 |
| Day 7 | | |
| Sacral or all caudal vertebrae missing | 2 | 3 |
| Kinked tail | 1 | 2 |
| Day 9 | | |
| Microphthalmia | 39 | 35 |
| Small, narrow orbits | 26 | 15 |
| Anophthalmia | 25 | 23 |
| Exencephaly | 22 | 20 |
| Protruding tongue | 22 | 20 |
| Majority of upper cranial bones missing | 17 | 10 |
| Mandibular micrognathia | 14 | 13 |
| Maxillary micrognathia | 13 | 12 |
| Microcephaly | 13 | 12 |
| Facial aplasia | 9 | 8 |
| Atlas fused to exoccipital | 7 | 4 |
| Mandibular agnathia | 6 | 6 |
| Cleft palate | 6 | 6 |
| Encephalocoele | 5 | 5 |
| Maxillary agnathia | 4 | 4 |
| Anotia | 4 | 4 |
| Hydrocephaly | 4 | 4 |
| Day 11 | | |
| Sacral vertebrae fused | 12 | 15 |
| Sacral vertebrae missing | 8 | 10 |
| Lumbar vertebrae missing | 7 | 9 |
| All caudal vertebrae missing | 7 | 9 |
| Kinked, twisted, or hooked tail | 7 | 12 |
| Brachyury | 6 | 11 |
| Anury | 5 | 9 |
| Irregularly ossified lumbar vertebrae | 4 | 5 |
| Imperforate anus | 2 | 4 |
| Day 13 | | |
| Brachyury | 24 | 45 |
| Kinked, twisted, or hooked tail | 14 | 27 |
| Oligodactyly of hindpaws | 5 | 10 |
| Oligodactyly of forepaws | 5 | 9 |
| Syndactyly of forepaws | 4 | 8 |
| Brachydactyly of forepaws | 4 | 7 |
| Cleft palate | 2 | 4 |
| Day 15 | | |
| Cleft palate | 11 | 17 |
| Mandibular micrognathia | 8 | 12 |
| Bent or hooked tail | 3 | 5 |
| Maxillary micrognathia | 2 | 3 |
| Edema | 2 | 3 |



Figs. 1–6. Day-20 rat fetuses irradiated at 27.12 MHz.

Fig. 1. Fetus irradiated on day 3. Note narrow cranium, mandibular agnathia, and kinked tail.

Fig. 2. Fetus irradiated on day 9. The fetus had severe facial aplasia, mandibular micrognathia, anophthalmia, anotia, and exencephaly.

Fig. 3. Fetus irradiated on day 9. Note exencephaly, anophthalmia, maxillary micrognathia, and protruding tongue.

Fig. 4. Fetus irradiated on day 11. Note hydrocephaly, bilateral cleft lip, and missing tail.

Fig. 5. Fetus irradiated on day 11. Note missing or fused lumbar vertebrae and missing sacral and caudal vertebrae.

Fig. 6. Fetus irradiated on day 13. Note oligodactyly of left forepaw and short tail.

alterations in skeletal development. Tail defects and mandibular agnathia or micrognathia were characteristic of the few malformed fetuses observed in rats irradiated on day 3. No single characteristic defect was noted among the few abnormal fetuses found in the day-5 group. In the day-7 group, the predominant defects involved the tail and posterior vertebrae. The greatest number and variety of effects occurred in fetuses irradiated on gestation day 9. Microphthalmia or anophthalmia with associated small, narrow cranial orbits were present in 25–39% of all viable fetuses. Exencephaly and the associated defects of protruding tongue and aplasia of the upper cranial bones accounted for 17–22% of the fetal abnormalities. Another 6–14% of the fetuses exhibited other severe malformations including maxillary and mandibular micrognathia, mandibular agnathia, microcephaly, facial aplasia (cleft face), cleft palate, and fusion of the atlas to the exoccipitals. In the day-11 group most of the defects involved fusion of the sacral vertebrae, agenesis of lumbar, sacral, or caudal vertebrae, and various tail defects (anury, brachyury, and kinked, twisted, or hooked tails). Fetuses irradiated on day 13 had a 24% incidence of brachyury and 14% incidence of kinked, twisted, or hooked tails. Numerous paw abnormalities including oligodactyly, syndactyly, and brachydactyly were also prominent. In the day-15 group, cleft palate and mandibular micrognathia predominated, but a few tail defects occurred, which affected only the extreme distal portion of the tail. Typical malformations observed in rat fetuses exposed to RF radiation at different stages of embryonic development are illustrated in Figures 1 through 6.

## DISCUSSION

The results of this study generally agree with those of Hofmann and Dietzel ('66) and Dietzel and Kern ('70) for rats irradiated at 27.12 MHz. Hofmann and Dietzel reported an increased incidence of fetal malformations in rats irradiated during postimplantation development, and Dietzel and Kern reported an increased incidence of postimplantation resorptions. The specific malformations reported were related to the treatment day and were similar to malformations we observed for corresponding treatment days. As in our study, the incidence of malformations appeared to be related to the degree of maternal hyperthermia attained during treatment.

Several differences between our respective studies should be noted, however. We observed a much higher incidence and greater variety of fetal malformations and a higher incidence of postimplantation resorptions. In our study, gestation day 9 was most sensitive to RF teratogenesis with craniofacial malformations predominating. Hofmann and Dietzel, however, reported maximum sensitivity on days 13 and 14 (corresponding to our days 12 and 13), with limb and tail malformations predominating. We observed a low but significant incidence of malformations after preimplantation RF exposure but they reported no preimplantation malformations. Dietzel and Kern reported high preimplantation embryonic loss, whereas we found no preimplantation loss above control levels. We observed a relatively high incidence of resorptions (up to 49%) after irradiation on day 7 or 9 but not on other days. Dietzel and Kern, on the other hand, reported a greater incidence of resorptions after late gestation RF exposure with a maximum at day 15. The observed differences in our respective studies may be related to the higher temperature attained by our animals (43°C vs. 42°C or less) or to differences in experimental procedures and exposure conditions. It should be noted that our animals received whole-body radiation exposure whereas animals in the other two studies were exposed topically in the abdominal region. The electric and magnetic field distributions in our respective studies were presumably different.

Since these investigators made no field strength measurements or power absorption measurements, we cannot estimate similarities or differences in exposure conditions between our respective studies. Because the relationship between the exposure conditions (and the resulting internal temperature distributions) in our respective studies is not known, a direct comparison of results based on colonic temperature may not be valid. Nevertheless, it appears from our respective studies that 27.12 MHz RF radiation sufficiently intense to cause appreciable body heating is definitely teratogenic in rats. It also appears that the extent and severity of malformations produced depend on the intensity and duration of the radiation exposure.

Since most of the malformations caused by postimplantation RF exposure tend to be relatively day-specific, the majority probably result from direct damage to the embryo during the brief period of exposure. The embryo is likely to be significantly heated, although at this time the exact relationship between embryonic temperature and the measured colonic temperature is unknown. Heat itself is thought to damage the developing embryo and fetus directly, although maternal reactions to heat exposure may modify this effect (Edwards, '78).

A similar spectrum of malformations and similar day specificity is present in our RF experiments and in hyperthermia teratology studies that have used conventional means of heating. In rats, maternal temperature elevations of 3–5°C cause numerous fetal malformations including anophthalmia or microphthalmia on gestation days 8–10; anencephaly on day 10; ectrodactly on day 12; cleft palate, micromelia, syndactyly, ectrodacyly, hydrocephaly, and short or absent tails on day 13; and microphthalmia, short digits, and short tails on day 14 (Edwards, '78). Cockcroft and New ('75) subjected rat embryos explanted and grown in culture during days 10–12 of development to various conditions of hyperthermia. Embryos incubated at 2–3°C above the normal temperature of 38°C developed numerous abnormalities. Half of the embryos grown at 40°C were abnormal, and nearly all of those grown at 41°C were abnormal. These results suggest that hyperthermia may have a direct effect on the developing embryo. In general, the results of hyperthermia studies in rats are similar to the results of RF studies, and suggest that the teratogenic effects induced by RF radiation are caused by heat.

Core temperature elevations of 3.5°C or more above normal in guinea pigs inhibit or delay mitosis and cause clumping of chromosomes and ultimate destruction of rapidily dividing cells, particularly in the central nervous system (Edwards et al., '74). Since the core temperature of our RF-irradiated rats averaged about 4.5°C above normal, inhibited mitosis or cell death might be expected in embryonic tissues. Interference with or delay in cellular proliferation during embryonic development could easily alter crucial developmental pathways and result in growth retardation, malformations, or embryonic death. Although the postimplantation teratogenic and embryolethal effects we observed could possibly result from alterations in maternal homeostasis or placental injury caused by RF-induced temperature elevation, they are most likely caused by direct injury to embryonic tissues at the time of treatment.

A mechanism underlying the observed preimplantation malformations is more difficult to conceptualize. Malformations caused by preimplantation exposure to teratogens rarely occur. Most have resulted from maternal exposure to x-ray radiation (see Brent, '77, for a review). Rugh and McManaway ('77) also reported a few malformations in rat fetuses irradiated with high-intensity 2,450 MHz microwave radiation during preimplantation development. RF and microwave radiation could alter preimplantation development by heating the embryo, but death would appear to be a more likely outcome than the induction of immediate or subsequent malformations.

Preimplantation malformations could occur if RF heating denatures heat-sensitive proteins without killing the embryo. If key proteins are inactivated (e.g., enzymes, inducing molecules, or receptor sites), there could be an impairment in cellular differentiation at a later stage of development. Such an impairment could result in fetal malformations.

An alternative explanation is that the observed preimplantation malformations result from damage to maternal tissues or alterations in maternal homeostasis due to RF absorption. Damage to the uterus, accumulations of noxious metabolites, or hormonal imbalances could interfere with the implantation process or with subsequent postimplantation development.

Any proposed mechanisms for RF teratogenesis are speculative at this time. Much work remains to be done before the causes can be determined with any degree of certainty. Both preimplantation and postimplantation RF effects may very well result from a combination of maternal pathogenesis and direct damage to the developing embryo.

Although most of the teratogenic and embryotoxic effects of RF radiation are probably due to temperature elevation caused by RF absorption in tissues, effects caused by "nonthermal" interactions with the electromagnetic field cannot be ruled out. Several studies have reported fetal abnormalities, increased embryonic or fetal mortality, or decreased fetal weight in rodents exposed to microwave radiation at levels that cause no significant increase in maternal core temperature (Bereznitskaya and Rysina, '74; Shore et al., '77; Berman et al., '78). Other studies performed at equal or higher exposure levels, however, have reported no adverse effects on embryonic development (Miro et al., '65; Rioch, '74; Michaelson et al., '76; Jensh et al., '77, '78; Berman et al., '81). Even though adverse developmental effects caused by exposure to low-intensity microwave radiation may occur, they are not necessarily "nonthermal." The effects could be due to localized heating that is insufficient to measurably raise the maternal core temperature.

Given that high-intensity RF radiation at 27.12 MHz is teratogenic in the rat, should RF radiation in general be considered a serious threat to the human embryo? Since there is evidence in the literature to implicate heat as a potential human teratogen, RF radiation should be considered a potential hazard to the human embryo if it is sufficiently intense to cause heating of body tissues. Many RF sources can cause significant heating in the body. For example, medical diathermy devices operating in the RF range (primarily at 27.12 MHz) can cause appreciable heating of exposed tissues.

Diathermy treatment during pregnancy is positively contraindicated (Hofmann and Dietzel, '66). In addition, workers exposed to high-intensity RF radiation while operating RF heat sealers or while climbing active television broadcast antennas have reported definite body heating (unpublished results from NIOSH surveys).

Radiofrequency heating is potentially much more dangerous than conventional heating (conduction, convection, infrared radiation, etc.). Most conventional heat sources warm the surface of the body, allowing the thermoregulatory system to effectively regulate core temperature via heat receptors in the skin. RF radiation, however, penetrates much deeper into the body and can cause significant heating of internal organs without a corresponding increase in skin temperature. Consequently, heat receptors in the skin may fail to activate normal cooling mechanisms. Horvath et al. ('48) reported that human volunteers whose thighs were exposed to high-intensity 2,450 MHz microwave radiation experienced only mildly pleasant sensations of warmth, even though internal temperatures increased by as much as 10.7°C.

The thermoregulatory response to RF heating is likely to be delayed until the temperature of the blood entering the hypothalamus increases. The thermoregulatory system may therefore be ill equipped to rapidly regulate deep-body heating caused by RF radiation. In addition, significant heating of the amniotic fluid relative to surrounding tissues is expected to occur under some RF exposure conditions (Hofmann and Dietzel, '66), and the blood supply to the amnion may not be adequate to remove the heat from the amniotic sac rapidly. This could cause a significant elevation of embryonic temperature relative to maternal core temperature. It is therefore conceivable that significant heating of the human embryo could occur without the mother's awareness.

Unfortunately, even if all RF-induced teratogenic effects were due to heat, it would be difficult to predict the teratogenic potential of different RF radiation sources. The degree of absorption of RF energy by the body depends on a number of factors including the frequency, modulation, and operating cycle of the source. It depends on the electric and magnetic field strength of the incident radiation. It likewise depends on the size, shape, temperature, and physiological state of the exposed animal, upon its orientation with respect to the RF field, upon its coupling to the RF source, and upon the presence or absence of a ground plane (Durney et al., '78, and unpublished observations). In addition, environmental factors such as temperature, humidity, and air velocity critically affect the thermal burden a body can tolerate under given RF exposure conditions (Deichmann, '66).

Although many people are occupationally exposed to high-intensity RF radiation, most of the general population is exposed to very low-intensity RF in the environment. Body heating from such low-level environmental exposure is negligible, and there is little likelihood that thermal damage to the human embryo will occur. However, no low-intensity teratology studies, to our knowledge, have been performed in the RF range (below 300 MHz), so the possibility of adverse effects from low-level RF radiation cannot be dismissed.

Because of the absence of studies of low-intensity RF radiation effects below 300 MHz, the difficulty of predicting RF power absorption and heating from a given RF source, and the usual difficulties in extrapolating teratology data from animals to man, an accurate assessment of potential RF teratogenic effects in human beings cannot be made at this time. More research is needed to determine the threshold levels for RF teratogenesis in animals. In addition, epidemiology studies need to be conducted to determine if workers exposed to RF radiation are exhibiting adverse reproductive effects. (NIOSH is currently conducting such a study of workers exposed to high-level radiation from RF heat sealers.) Until the potential for human RF/microwave teratogenesis is determined and safe exposure levels are established, care should be taken to ensure that pregnant women are not exposed to levels that exceed the current U.S. occupational standard.

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