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Spontaneous and Nitrosourea-induced Primary Tumors of the Central Nervous System in Fischer 344 Rats Exposed to Frequency-modulated Microwave Fields[1]

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ABSTRACT

In a 2-year bioassay, we exposed Fischer 344 rats to a frequency-modulated (FM) signal (836.55 MHz ± 12.5 KHz deviation) simulating radiofrequency exposures in the head of users of hand-held mobile phones. We tested for effects on spontaneous tumorigenicity of central nervous system (CNS) tumors in the offspring of pregnant rats and also for modified incidence of primary CNS tumors in rats treated with a single dose of the neurocarcinogen ethylnitrosourea (ENU) *in utero*. ENU dosage (4 mg/kg) was selected to give an expected brain tumor incidence of 10–15% over the mean life span of 26 months. Pregnant dams ($n = 102$) were randomly assigned to six groups. Their offspring were treated as cohorts in each of the six groups ($n = 90$ per group; total, $n = 540$): Sham ENU/Sham Field, Sham ENU/Field Exposed, ENU/Sham Field, ENU/Field Exposed, ENU/Cage Control, and Sham ENU/Cage Control. Intermittent field exposures began on gestation day 19 and continued until weaning at 21 days, resuming thereafter at 31 days and continuing until experiment termination at 731–734 days. Energy absorption rates (SARs) in the rats' brains were similar to localized peak brain exposures of a phone user (female, 236 g, 1.0 W/kg; male, 450 g, 1.2 W/kg). Of the original 540 rats, 168 died before the termination of the experiment. In these rats, ENU significantly reduced survival from a mean of 708 days in three groups without ENU treatment to 645 days in three groups treated with ENU ($P < 0.0005$). There were no effects on survival attributable to FM field exposure in either ENU-treated or in sham-treated groups. Spontaneous CNS tumor incidence in control groups was 1.1–4.4% but sharply higher in rats receiving ENU (14.4–22.2%; $P < 0.0001$). No FM field-mediated changes were observed in number, incidence, or histological type of either spontaneous or ENU-induced brain tumors, nor were gender differences detected in tumor numbers. These negative findings with FM fields contrast with our study using standard digital phone fields pulsed on and off at 50/se, where a trend was noted toward reduced incidence of both spontaneous and ENU-induced CNS tumors (W. R. Adey *et al.*, Radiat. Res., *152:* 293–302, 1999). Although consistent but not attaining significance in the experiment overall (spontaneous CNS tumors, $P < 0.08$ one-tailed; $P < 0.16$ two-tailed; ENU-induced CNS tumors, $P < 0.08$ one-tailed, $P < 0.16$ two-tailed), the trend was significant ($P < 0.015$ one-tailed, $P < 0.03$, two-tailed) in rats that received ENU and died prior to experiment termination, with a primary brain tumor as the cause of death. We discuss differences in the signaling structure of digital and FM fields. Certain bioeffects induced by either amplitude-modulated or pulsed radiofrequency fields at athermal levels have not been seen with fields of similar average power but unvarying in intensity (continuous wave or frequency-modulated fields).

INTRODUCTION

FM[3] (or analogue) mobile phone systems have been the dominant technology for many years. Although they will be replaced eventually by digital systems, their continued use worldwide appears certain in many applications. Typically, they emit signals in the RF range from 0.1 to 1.5 GHz. Signals emanating from FM phones differ fundamentally from those generated by major digital systems in the United States and elsewhere.

No direct comparisons have been made in an animal bioassay of possible differences in their bioeffects, particularly with respect to brain tumorigenesis. FM signals remain constant in amplitude, whereas signals generated in accordance with the North American Digital Code create a train of pulses (packets) at 50/s, cycling on for one-third of an epoch and remaining silent for two-thirds (TDMA amplitude or pulse modulation). As will be discussed (1–8), a spectrum of amplitude modulation-dependent physiological responses have been reported. Most mobile phones operate in close proximity to the head. Portable FM phones, with maximum output powers of 0.6 W in the 800-MHz band, induce fields in the most exposed tissues at energy absorption rates equivalent to 1 W/kg ± 6 db, depending on the device's position and design (9).

There have been previous studies of tumor incidence in rats and mice after long-term microwave exposure (10–13). However, in none of these studies was detection of brain tumors a prime goal but was incidental in the course of whole-body analysis, both gross and histopathological. To achieve high accuracy in tumor numbers, we have examined a sequence of 20–25 sections/brain, as compared with three standard sections approved in protocols of the NTP.

In this study with Fischer 344 rats exposed to simulated FM phone fields, we have addressed four major questions: (*a*) Did lifetime exposure to the FM fields alter the spontaneous incidence of primary CNS tumors? (*b*) Did sham exposure of the rats, involving their immobilization, particularly in the near-field exposure system, cause levels of stress that might markedly alter primary CNS tumor incidence in these animals? (*c*) Did the short-lived neurocarcinogen ENU, given as a single low dose *in utero*, cause an expected increase in brain tumor incidence as a positive control? and (*d*) Did lifetime intermittent exposure to the FM fields for 24 months alter the incidence of low-level carcinogen-induced brain tumors, *i.e.*, did the FM field act as a tumor promoter or as a tumor progression agent?

This experiment was designed as a 2-year bioassay to test the hypothesis that FM field exposure might alter either survival or primary CNS tumor incidence. We aimed to simulate a life-long exposure, beginning with exposures of fetal and preweanling rats. A

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[3] The abbreviations used are: FM, frequency modulated; RF, radiofrequency; TDMA, time division multiple access; NTP, National Toxicology Program; ENU, *N*-ethyl-*N*-nitrosourea; GD, gestation day; ES, ENU/Sham Field; EF, ENU/Field Exposed; EC, ENU/Cage Control; SS, Sham ENU/Sham Field; SF, Sham ENU/Field Exposed; SC, Sham ENU/Cage Control; FF, far field; NF, near field; CNS, central nervous system; T and MT, tumor and microtumor, respectively; SAR, specific energy absorption rate; ELF, extremely low frequency; CI, confidence interval; ODC, ornithine decarboxylase.

low ENU dose was selected to give maximum sensitivity for detection of a modification of tumor incidence by the FM fields as a lifetime response.

Our choice of the Fischer 344 rat was based on: (*a*) its comprehensive pathology databases in the NTP; (*b*) an overall incidence and subtypes of CNS tumors resembling those of humans (14); (*c*) reduced variations in pathological responses as an inbred strain when compared with most outbred strains (15); and (*d*) greater longevity at 24 months (80%), mainly because of a lowered spontaneous tumor incidence, a vitally important factor in long-term studies (16).

Single-strand breaks in rat brain DNA have been reported after acute low-intensity microwave exposure (17, 18) and after prolonged exposure in mice (19), but single strand breaks were not confirmed in a similar study (20). Their reported increased incidence after microwave exposure is unlikely to result from a direct genotoxic microwave action but rather to interference with enzymatic DNA repair mechanisms (21–23). In a mouse model, life-long intermittent microwave exposure has been related to an increased lymphoma incidence late in life (24).

As in our counterpart study with digital phone field exposures in this rat model (25), the major objective was to evaluate whether FM phone fields met some of the defining criteria for known neurocarcinogenic agents (26). They include: (*a*) a consistent capability to increase tumor incidence; and (*b*) a reduction in latency period of tumors (reduced rat survival time).

MATERIALS AND METHODS

Animals. Multiparous pregnant, female, virus antigen-free, Cesarian-delivered Fischer F-344 rats ($n = 104$) were obtained from Charles River Laboratories (Kingston, NY and Raleigh, NC). Pregnancies resulted from a single overnight mating session. Pregnant dams were received at the Loma Linda Veterans Affairs Medical Center on GD 14. Mean body weight on GD 18 was 246 ± 16 g (mean ± SD), and the pregnant dams were randomly assigned to six experimental groups (see below): SS, $n = 18$; SF, $n = 18$; ES, $n = 18$; EF, $n = 18$ dams; EC, $n = 16$; and SC, $n = 16$. All pregnant females and females with their respective litters were housed individually. All rats were fed Teklad 4% rat/mouse diet 7001 (Harlan Industries, Madison WI) and water *ad libitum*.

ENU Administration. A single dose (4 mg/kg body weight) of ENU (Sigma Chemical, St. Louis, MO) was administered on GD 18 to dams in groups ES, EC, and EF ($n = 52$) via the lateral tail vein as a freshly prepared solution in a phosphate/citrate/saline buffer (pH 6.0, adjusted with citric acid). The remaining 52 dams were injected with an equal volume of buffer only (Sham-ENU injection). Because no data are available for a dose-response relationship between transplacental ENU and brain tumor incidence for the Fischer 344 rat, the dosage selected was based on published data on the incidence of brain tumors in offspring of Sprague Dawley rats (20, 21) and other rat strains, such as the BD-IX (22). These strains have a brain tumor incidence ranging from 12% to as high as 40% in some experiments for ENU doses between 1 and 5 mg/kg administered on GD 20.

Experimental Groups. All pups ($n = 778$) were born on GD 22 (84%) or GD 23 (16%). Two of the 104 dams were not pregnant. Experimental groups were then set up from weanlings of litters from the assigned groups of dams. Gender distribution remained unknown until sexing at weaning. In group EF (see alphabetic group designations below), two litters (19 pups) were discarded because of unsatisfactory ENU injections and consequent dose uncertainty. There were 750 pups for distribution into the final experimental groups, 329 males and 421 females. We aimed at gender balance, based on an initial random selection of three males and three females from each litter. However, this selection method failed in group EF, where there was an aggregate of only 38 males, leading to a ratio 38:52, male:female. These progeny then became treatment cohorts in six groups, comprised of: Sham ENU/Sham Field (SS) $n = 90$, 45 males and 45 females; Sham ENU/Field Exposed (SF), $n = 90$, 45 males and 45 females; ENU/Sham Field (ES), $n = 90$, 45 males and 45 females; ENU/Field Exposed (EF), $n = 90$, 38 males and 52 females; ENU/Cage Control (EC), $n = 90$, 45 males and 45 females; and Sham ENU/Cage Control (SC), $n = 90$, 45 males and 45 females (total, $n = 540$). The cage control groups EC and SC were established to test for effects of reported stresses associated with tube restraint of the type used in the near-field FM field exposures (groups SS and ES).

FM Far Field (FF) and Near Field (NF) Exposures. Daily (7 days/week) FF exposures of the pregnant females in groups SF and EF began on GD 19 and continued until parturition 3 or 4 days later. Thereafter, FF exposure of dams and offspring continued until the pups were aged 21 days (25 FF exposures). All FF and sham exposures were performed on rats in their home cages. To accommodate all dams with their litters, three exposure time periods were used: 1600–1800, 1900–2100, and 2200–2400 h. NF exposures began at age 31 days. In the 10-day interim, the offspring were weaned and individually identified by numbered tail tattooing but received no field exposures. NF exposures were resumed on 4 consecutive days weekly until experiment termination at 731–734 days (total, 384 NF exposures). NF and sham exposures occurred at the same time of day for each group. Weekly weighings and colony inspections were carried out on the fifth day.

Animal Handling and Veterinary Surveillance. Rats were weighed weekly and housed by sex, exposure group, and injection group, with two males or three females per cage. No substitutions were made after tail tattooing. Rats were observed daily. With the appearance of one or more clinical signs, such as progressive weight loss, head tilt, ocular discharge, and others, they were evaluated by the project veterinarian (C. J. K.), who determined whether immediate euthanasia was required. All procedures (a.m.) were in compliance with a protocol approved by the Animal Care Committee of the Loma Linda Veterans Affairs Medical Center. Animal facilities at this Center are fully accredited by the American Association for Advancement of Laboratory Animal Care. The experiment was performed in as blind a manner as possible in that at no time prior to the conclusion of the experiment did the veterinarian or the pathologist know to which groups the animals were assigned.

Necropsy Procedures and Pathological Examination. Termination of the experiment took place over 4 days when the rats were 731–734 days of age. To the extent possible, the same numbers of animals from each experimental group were euthanized on each of the 4 days. All animals that were terminated ($n = 372$) were lightly anesthetized in a CO_2 chamber and then received 1 ml heparin i.p. (1000 IU/ml), followed by deep pentobarbital anesthesia (55 mg/kg i.p.). They were then perfused with a peristaltic pump via the left ventricle with 240 ml of 10% phosphate-buffered formol saline at a sustained intracardiac pressure of 120 mm Hg. A complete gross necropsy of extraneural organs was performed, and each carcass was then stored in fresh perfusion fixative until the brain and spinal cord were removed for histopathological examination. Using a rat brain matrix (Harvard Apparatus, Holliston, MA), transverse sections of the brain 1.0-mm thick were cut and processed sequentially (approximately 25 and 20 sections for males and females, respectively). Transverse spinal cord sections were cut every 1 cm. Additional sections were taken at extraneural sites with gross lesions. All sections were cut at 5-μm thickness and stained with H&E and evaluated microscopically (R. J. H.). Thus, a greater number of brain sections/brain was examined in this study as compared with standard procedures in the carcinogenesis studies in Fischer 344 rats in the NTP, which limited examinations to three coronal sections of the brain at the levels of: (*a*) the frontal cortex and the basal ganglia; (*b*) the parietal cortex and the diencephalon; and (*c*) the cerebellum and pons.

Primary tumors of the CNS were classified into histological subtypes using guidelines reported previously (14, 27, 28). Tumors grossly visible on external examination or after transverse sectioning of brain or spinal cord were classified by size as Ts or as MTs if only detected microscopically (29). The number of primary brain tumors in each rat was evaluated by sequential arrangement of histological brain sections. Causes of death in rats not surviving to the end of the experiment were based on gross autopsy findings, supplemented where appropriate by histological evaluation of gross lesions. Additional independent peer review of tumor numbers and classification of CNS tumor subtypes was done by a panel organized by Experimental Pathology Laboratories, Inc. (Research Triangle Park, NC).

Data Analysis Methods. Experimental design parameters and all statistical analyses were contributed by our statistician (G. Z.). In planning this study, a calculation of experimental power was made, based on tumor incidence observed in our preceding Fischer rat study, where we tested spontaneous and ENU-induced brain tumor incidence in exposures to digital phone fields (25).

Here, with an assumed spontaneous brain tumor incidence of 8.3% from the previous study and sample sizes of 90 rats in each group, we would be able to detect an increase of 13% with a power of 80%. Similarly, with an ENU-induced tumor incidence of 15% (25), we would be able to detect an increase of 15% with a power of 80%. Using two-tailed tests, the corresponding increases are 15 and 18% for a sample size of 90 rats and a power of 80%.

Kaplan-Meier survival analysis (30) was used to compare all six groups and selected data subsets. As a product limit, nonparametric estimate of the probability of survival, it avoids assumptions of normality in follow-up studies where survival time is known only for individuals dying during the study period. This estimate is based on constructing time intervals containing only one death in each interval. Here, the test's usefulness is limited by severe censoring of data. Incidence rates were compared using Z-statistics and χ^2 analysis. Both one-tailed and two-tailed tests of the null hypothesis were calculated, with the initial premise that the one-tailed test would be appropriate for our hypothesized increase in tumor incidence.

Microwave Exposure Systems. These have been described in detail elsewhere (18) and will be summarized here. An ideal simulation of exposure conditions for the human cellular phone user would be restricted to a NF model. However, because fetal tissue is believed to be maximally sensitive to other known neurocarcinogens and because some groups received a transplacental dose of the carcinogen ENU on GD 18, exposure of pregnant animals was required. We therefore developed two complete exposure systems. System I approximated FF conditions. It provided initial exposures for pregnant dams, and later, for offspring up to weaning at 21 days of age. After weaning, System II produced a NF exposure from weaning to the end of the experiment, with each rat in individual restraints.

System I: Large Horn Radiator for Simulated FF Exposures. FF conditions were simulated with an approximately plane wave in a large tapered horn. Rat cages were positioned in a vertically oriented 3×3 matrix at the square aperture of the horn (2.0 m on a side). Sham exposures were made in a square chamber of identical dimensions and materials. A power input of 37.2 W produced a mean field power density at the horn aperture of 2.6 ± 0.50 mW/cm^2 (mean $\pm$ SD). The horn was excited in circular polarization to reduce possible orientation-dependent coupling to the animals, because dams and pups were free to move about their cages. Wavefront circularity (axial ratio) was within 2.3 dB, and power density was within 1.6 dB across the cage exposure area.

System II: Carousel NF Exposure System. Long-term, intermittent exposures (2 h/day, 4 days/week) to the NF began at age 33 days, using exposure platforms with 10 rats oriented radially around a central antenna (the carousel system). The antenna is a standard production half-wave sleeved dipole (Motorola). To accommodate 120 rats simultaneously (60 field exposed, 60 sham), 12 exposure platforms were used. A plastic tubular restraint confined each rat for the duration of the exposure. They faced the antenna at a fixed distance from the tip of the nose (30 mm from weaning to 120 days, 45 mm thereafter). After a short training period (1 week) they would freely enter the tubes and often slept during exposure. To accommodate the 360 exposed/sham exposed rats in this study, exposures were conducted in three shifts.

SARs in the NF. We have tested a FM 836.55 MHz signal, with $\pm$12.5 KHz maximum deviation. Modulation was by a recorded pattern of "balanced speech" that generated all major speech components in a 2-min epoch that recycled continuously. The average antenna power of 2.5 ± 0.1 W was selected to produce the same average SARs in the exposed brains as the slot average SAR in our previous study with digital cell phone fields (25). Energy absorption levels were tested by two different techniques, each of which was verified by an independent method: numerical modeling verified by electric probe measurements, and thermography verified by thermometric probes.

Numerical dosimetry (31, 32) was based on magnetic resonance imaging data sets of a rat cadaver with a resolution of 0.125 mm^3 in the brain and 1.0 mm^3 in the rest of the body. The results have been validated at 30 specific points within a cadaver brain, using an electric field probe. Thermography exposed hemisected rat cadavers to a 235-W field at 836 MHz for $\leq$90 s and acquisition of a series of infrared images of the cut surfaces for 2 min. Thermography suffered from "smearing" of the thermal image from effects of thermal diffusion. Thermometry was based on a Vitek thermistor probe (BSD Medical Devices, Salt Lake City, UT) placed on the cut surface of the brain and readings compared with the thermographic images. These measurements were complicated by imperfect contact between the sensor and the tissue and by radiative cooling from thermistor leads. However, within the described limitations, the dosimetric assessments agree well (Table 1).

RESULTS

Overall Experimental Findings: Survival Curve Analysis. The FM field exposures in this experiment covered an epoch of 731 days, ~80% of the natural life span of the Fischer 344 rat. Of the original 540 rats, 168 (31%) either died spontaneously ($n = 45$; 8%) or were euthanized ($n = 123$; 23%) prior to the end of the experiment because of severe clinical impairment. Deaths in the course of the experiment are summarized in Fig. 1. With the use of Kaplan-Meier statistics, the survival curves for all six groups were compared. Because most rats in each group survived to be sacrificed at the end of the study, each Kaplan-Meier analysis has considerable censored data.

Survival curves were clustered in two families that separate three groups of rats receiving ENU (ES, EF, and EC) from three undosed groups (SS, SF, and SC). Dosage with ENU significantly decreased mean survivals of 714, 706, and 706 days (groups SS, SF, and SC, respectively) to 632, 643, and 661 days (groups ES, EF, and EC, respectively; $P < 0.0005$). There were no effects on survival attributable to FM field exposure in either ENU or sham groups. No effects of these FM fields were noted on survival of rats with either spontaneous or ENU-induced brain tumors. No effects on survival were noted in comparison of two cage control groups (SC and EC) with rats subjected to possible stresses reportedly associated with tube restraint as used in the course of NF FM field exposure (SS and ES).

There were the expected rates of a large granulocyte lymphocyte leukemia and pituitary adenomas; both are endogenous diseases in the Fischer 344 rat strain. Large granulocyte lymphocytes were detected in 137 of 540 rats (25%), based on hematocrits, differential WBC counts, and necropsy findings. There was no evidence of a differential

Table 1 *Calculated and measured brain and whole-body SAR for rat NF FM exposures*

	Calculated[a] SAR (W/kg) +25% nose to antenna spacing				Measured SAR (W/kg) rat cadaver[c]
	30 mm		45 mm		45 mm
Rat size class (g)	WB[b]	Brain	WB	Brain	Brain
150	0.72	1.1	0.57	0.74	
250	0.51	1.4	0.42	1.0	1.8[d]
300	0.44	1.5	0.37	1.1	
450	0.31	1.6	0.27	1.2	2.3[e]

[a] Calculated and measured brain and whole-body SAR for rat NF FM exposures. Values are scaled for power input used in this study, *i.e.*, 2.5 ± 0.1 W. The uncertainty of the numerical dosimetry was estimated to be less than $\pm$1.5 dB for the brain average and less than $\pm$1 dB for the whole-body average SAR. Uncertainty in thermography is less than $\pm$3 dB. Brain spatial resolution of magnetic resonance imaging-based model, 0.125 mm^3; remainder of body, 1.5 mm^3. The 30-mm nose-to-antenna distance was used from weaning to 120 days, thereafter increased to 45 mm.
[b] WB, whole body.
[c] Comparison of SARs (W/kg) of thermographic imaging of a rat cadaver with a modeled rat-shaped mass of simulated muscle tissue.
[d] Female, 236 g.
[e] Male, 462 g.

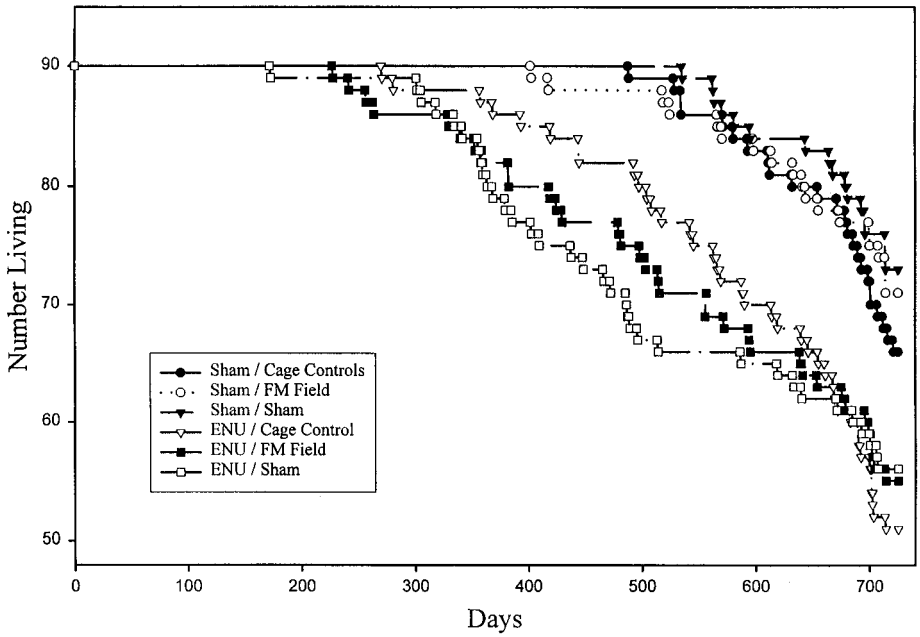

Fig. 1. Survival curves in a 2-year bioassay for six groups of rats (n = 540) tested in an 800-MHz FM microwave field: group SS (n = 90); group SF (n = 90); group ES (n = 90); group EF (n = 90); EC (n = 90); and group SC (n = 90). All surviving rats (n = 372) were sacrificed at 731–734 days.

incidence attributable to separate or combined exposures to ENU or FM fields. Incidence of pituitary tumors was assessed on necropsy inspection of the sella region and the adjoining hypothalamus and confirmed histologically, but histopathology was not done in normal-appearing pituitary glands. In 243 (45%) of 540 rats, no pituitary tumors were detected. In 194 rats (36%), macroscopic tumors were observed, and in 103 rats (19%), the lesions were large enough to produce hypothalamic compression. No significant difference in gender incidence was detected.

Incidence of Primary CNS Tumors. There was a total of 60 primary tumors of the CNS in all groups. We detected a total of 54 glial tumors (48 in the brain and 6 in the spinal cord; Table 2). These 54 tumors occurred in 52 rats. With the exception of two rats each having two tumors (one rat with two astrocytomas in group EC, and one rat with two mixed gliomas in group EF), all other rats had only a single tumor. No FM field-mediated increase in number or incidence of either spontaneous brain tumors (SS *versus* SF) nor of ENU-induced brain tumors (ES *versus* EF) was observed (Table 2).

No significant differences were found between incidence of spontaneous CNS tumors in the control groups (1.1% in the SS group and 4.4% in the SC group) nor in comparison of the groups SS *versus* SF (1.1 *versus* 4.4%; P = 0.17). Thus, this FM field produced no measurable change in incidence of spontaneous brain or spinal cord tumors.

By comparison, CNS tumor incidence increased sharply in rats receiving ENU (ES, 22.2%; EF, 17.8%; EC, 14.4%). This increase was highly significant in comparison with the spontaneous rates ($P < 0.0001$), but as in the spontaneous tumor groups, there were no statistically significant effects attributable to FM field exposures. Sham exposure, where rats were placed in an unenergized NF expo-

Table 2 *Numbers, incidence, and types of primary CNS tumors in all F344 rats*[a]

	All rats[b] (n = 540)					
Site and histological classification	SS (n = 90)	SF (n = 90)	ES (n = 90)	EF (n = 90)	EC (n = 90)	SC (n = 90)
CNS						
Total tumors (rats)	1 (1)	4 (4)	20 (20)	17 (16)	14 (13)	4 (4)
CNS tumor incidence (%)	1.1	4.4	22.2	17.8	14.4	4.4
No. of CNS glial tumors	1	2	19	15	13	4
Brain tumor incidence (%)	1.1	3.3	18.9	15.6	14.4	3.3
No. of brain tumors	1	3	17	15	14	3
Glial						
Oligodendroglioma	0	0	1	1	3	0
Astrocytoma	1	0	5	3	5[c]	3
Mixed glioma	0	0	8	10[d]	5	0
Ependymoma	0	1	2	0	0	0
Nonglial						
Meningioma	0	0	0	0	1	0
Granular cell tumor	0	2	1	1	0	0
Spinal cord incidence (%)	0	1.1	3.3	2.2	0	1.1
No. of spinal cord tumors	0	1	3	2	0	1
Glial						
Astrocytoma	0	1	1	0	0	1
Mixed glioma	0	0	2	1	0	0
Nonglial						
Undifferentiated	0	0	0	1	0	0

[a] Only tumors of brain and spinal cord are shown. The number of rats/group is 90. Incidence is in percentage; all other entries are numbers of tumors.
[b] All rats had one tumor except where indicated.
[c] One rat with two astrocytomas.
[d] One rat with two mixed gliomas.

Table 3 *Causes of death in preterm rats (unscheduled sacrifice) in all groups*

	Treatment groups[a] $n = 168$ rats					
	SS (17)	SF (19)	ES (34)	EF (35)	EC (39)	SC (24)
1. Primary neural tumor	0	1	20	14	10	0
2. Primary tumor with hydrocephalus	4	4	1	1	7	6
3. LGL leukemia metastatic to CNS	1	1	1	0	0	0
4. Extraneural tumor	10	9	7	13	16	14
5. Extraneural nontumor lesions	0	0	2	2	0	1
6. Nontumor CNS lesions	0	0	0	0	1	0
7. No lesion detected	2	4	3	5	5	3

[a] Numbers in parentheses, numbers of rats.

sure apparatus, did not alter tumor incidence when cage controls (EC and SC) were compared with their counterpart groups (ES and SS). Analysis of overall brain tumor incidence alone, with exclusion of spinal cord tumors, did not differ from the combined tumor analysis for either spontaneous or ENU-induced tumors. Here also, the presence of the FM field produced no measurable effect. No significant gender differences were detected in incidence of any of the six types of primary nervous system tumors listed in Table 2.

In comparing the relative size of CNS tumors in rats that survived to full term, there were 2 Ts and 2 MTs in the SC group, 10 Ts and 6 MTs in the EC group, 20 Ts and 1 MT in the ES group, and 16 Ts and 1 MT in the EF group.

In comparing the incidence of tumor subtypes, comparison of sham and control rats with those receiving ENU indicated increased numbers of mixed gliomas in the ENU rats, but there were no effects on any of these characteristics attributable to FM field exposure.

Findings in Rats Dying before Experiment Termination (Unscheduled Sacrifice, Preterm Rats). In our previous research with animal tumor bioassays of 60-Hz magnetic field effects, we noted that some magnetic field effects were most apparent early during the copromotion phase, but their statistical significance declined later in the carcinogenic process (33). In the present study, rats dying prior to experiment termination or found dead (unscheduled sacrifice, referred to hereafter as "preterm" rats) have therefore been grouped separately in analysis of tumor incidence and histopathology.

Six major categories of probable causes of death were identified in rats dying before experiment termination at 731–734 days (Table 3). Of the 168 rats, 17 died in the SS group, 19 in the SF group, 34 in the ES group, 35 in the EF group, 39 in the EC group, and 24 in the SC group. There were no obvious differences in death rates within the three groups not receiving ENU nor within the three groups that had received ENU, nor did the additional factor of FM field exposure modify death rates in either of these two clusters. Where primary neural tumors were determined to be the cause of death (Table 3, category 1), no significant differences were detected in tumor incidence or tumor histopathology attributable to FM field exposure (Table 4). Rats in category 7 (Table 3) had no gross lesions at necropsy, and confirmation of cause of death awaits complete histopathological examination of all extraneural tissues.

In comparing the relative size of CNS tumors in this preterm group, there were three Ts and no MTs in the EC group, five Ts and one MT in the ES group, and one T and five MTs in the EF group. No tumors were found in the SC group.

DISCUSSION

By reason of inconsistencies attributable to study design, lack of exposure details, and uncontrolled confounding factors, epidemiological evidence on a possible causal role of RF/microwave exposures in human cancer remains inconclusive (34). Occupational exposure to RF/microwave fields has been associated with increased brain tumor incidence and was significantly elevated to 10-fold among those used for 20 years or more. All of the excess risk for RF/microwave-exposed subjects related to jobs involving design, manufacture, repair, or installation of electrical or electronic equipment, where there may have been concurrent exposure to soldering fumes, solvents, and a variety of other chemicals (35). The excess risk was restricted to astrocytic tumors. Risks were not elevated in those similarly exposed while working in non-electrical/electronics jobs. Among electrical tradesmen exposed to ELF fields, risks for astrocytic tumors were slightly elevated but not statistically significant. A nested case-control study among United States Air Force personnel has compared brain tumor risks from exposures to either ionizing or nonionizing radiation (36). With exposures to ELF fields, the odds ratio was 1.28 (95% CI, 0.95–1.74), after adjustment for age, race, and senior military rank. For RF/microwave exposures, the similarly adjusted odds ratio was 1.39 (95% CI, 1.01–1.90). By contrast, the odds ratio for ionizing radiation (based on medical exposures) was 0.58 (95% CI, 0.22–1.52), with the conclusion that there is a small association between ELF and RF/microwave exposures and brain tumors and none with ionizing radiation. Increased brain tumor incidence in workers exposed to power frequency fields have also been reported (37–39), with the conclusion from a comparative analysis of five studies from France, Canada, and the United States that the relative risk/10 μT-years was 1.12 (95% CI, 0.98–1.28; Ref. 40).

No previous long-term studies of microwave exposures at athermal levels in rats and mice have evaluated brain tumor incidence as a primary goal, reporting only findings incidental to whole-body gross and histopathological examinations. We have taken special care to minimize failures in recognizing small CNS tumors by examining a sequence of 20–25 sections/brain. This contrasts with the three standard sections used in the NTP. Other studies have used carrier frequencies from 435 MHz to 2.45 GHz with both continuous wave and pulsed fields. They tested effects on general health and longevity, immune status, and mammary tumor incidence, with negative findings. No primary brain tumors were reported in 200 exposed and sham-exposed Sprague Dawley rats exposed to pulsed and ELF-

Table 4 *Numbers, incidence, and types of primary CNS tumors in rats dying before experiment termination (unscheduled sacrifice, preterm rats)*[a]

Site and histological classification	Preterm rats[b] ($n = 168$)					
	SS ($n = 17$)	SF ($n = 19$)	ES ($n = 34$)	EF ($n = 35$)	EC ($n = 39$)	SC ($n = 24$)
CNS						
Total tumors	0	1	19	14[c]	10	1
CNS tumor incidence (%)	0	5.3	55.9	37.1	25.6	4.2
No. of CNS glial tumors	0	1	16	13	10	4
Brain tumor incidence (%)	0	0	47.1	31.4	25.6	4.2
No. of brain tumors	0	0	16	12	10	1
Glial						
Oligodendroglioma	0	0	1	1	2	0
Astrocytoma	0	0	4	2[c]	3	1
Mixed glioma	0	0	8	9	5	0
Ependymoma	0	0	2	0	0	0
Nonglial						
Granular cell tumor	0	0	1	0	0	0
Spinal cord incidence (%)	0	5.3	8.8	5.7	0	0
No. of spinal cord tumors	0	1	3	2	0	0
Glial						
Astrocytoma	0	1	1	0	0	0
Mixed glioma	0	0	2	1	0	0
Nonglial						
Undifferentiated	0	0	0	1	0	0

[a] All lesions in animals that died prior to the termination of the experiment are shown. The numbers of rats/group are shown in parentheses. Incidence in percentage; all other entries are numbers of tumors.

[b] See Table 3 for causes of death.

[c] Two tumors in one rat. All other rats had one tumor.

modulated (8 Hz) 2.45 GHz fields for 25 months (10). In a series of studies in mice prone to mammary cancer, exposure to circularly polarized 2.45-GHz fields (SARs, 0.3–1.0 W/kg) for 18–21 months had no effects on tumor incidence, tumor growth rate, latency to tumor onset, or longevity (11–13). In Eμ-*Pim1* transgenic mice, exposures simulating Global System for Mobile Communications (GSM) digital cell phone fields were associated with a significant increase in follicular lymphomas appearing after the age of 10 months (24). Autopsy on an adult squirrel monkey exposed to 2.45 GHz fields for 90 min each week for 3 years revealed a frontoparietal primitive neuroectodermal tumor (41).

A review of published data on the genetic toxicology of RF radiation with a focus on mobile phone carrier frequencies concluded that they are not genotoxic; they do not induce genetic effects *in vitro* or *in vivo*, at least under athermal conditions, and do not seem to be teratogenic nor to induce cancer (42). RF/microwave fields lack necessary photon energies to disrupt chemical bonds. The threshold for disruption of chemical bonds by electromagnetic fields occurs at photon energies around 7–10 electron V, first seen in UV spectral regions and at progressively shorter wavelengths, extending into X-ray spectral zones.

Single strand breaks in rat brain DNA reported after acute low-intensity microwave exposure suggest a role for defective DNA repair mechanisms in carcinogenesis. Both animal models and clinical disorders suggest a role for defective DNA repair mechanisms in carcinogenesis. There was a 90% reduction in ENU-induced CNS gliomas if exposure to ionizing radiation (2 Gy) occurred 24 h before ENU dosage (21). The reduction correlated with activation by the radiation of the DNA repair enzyme alkylguanine-DNA-6-alkyltransferase. Defects in cloned repair genes have been associated with a predisposition to certain cancers (22). Moreover, free radicals of the oxygen and nitrogen species may act as complete carcinogens, the outcome depending on interactions between DNA damage, antioxidant levels, and DNA repair systems (6, 23).

In a life-term rat model, we have assessed possible brain tumor promotion by simulated FM mobile phone microwave fields and compared the findings with a comparable exposure to digitally encoded (TDMA) phone fields (18). Taken jointly, a summary of the experimental evidence points to a perturbation in brain tumor incidence by the digitally encoded signal not detected in exposure to FM fields. Although consistent but not attaining significance in the experiment overall (spontaneous CNS tumors, $P < 0.08$ one-tailed, $P < 0.16$ two-tailed; ENU-induced tumors, $P < 0.08$ one-tailed, $P < 0.16$ two-tailed), the trend was significant ($P < 0.015$ one-tailed, $P < 0.03$ two-tailed) in rats that received ENU and died prior to experiment termination, with a primary brain tumor as the cause of death. The effect was seen among glial-derived but not in nonglial histological subtypes. There were no effects on the incidence of primary nervous system tumors attributable to FM field exposure in spontaneous CNS tumors nor in the higher incidence in cohorts that received the carcinogen ENU. In a similar Fischer 344 rat study testing the TDMA digital field, we observed trends toward reduced incidence of both spontaneous and drug-induced CNS tumors (25). However, nonglial CNS tumors in that study numbered only four.

As already noted, there are fundamental physical differences between a digital microwave signal transmitted as a series of packets or pulses and the unvarying intensity of analogue (FM) fields. There may also be fundamental differences in their modes of eliciting bioeffects. Resonant interactions with molecules or portions of molecules may be expected in the millimeter wave/far infrared spectral regions. But for typical microwave fields with carrier frequencies far below these spectral regions, theoretical considerations and experimental data support the concept of collision-broadened, rather than resonant, spectra in their biomolecular interactions (43). Heating is thus their prime mode of interaction. As reviewed elsewhere (6), in cell culture preparations with SARs less than 5 W/kg, cellular responses have been reported primarily from exposures to microwaves that were amplitude or pulse modulated at ELF frequencies (*i.e.*, typically <300 Hz), where heating does not appear to be the mediator of these bioeffects. In contrast, effects on cell cultures have been reported with microwave fields lacking amplitude or pulse modulation (continuous wave or FM), all using SARs equal to or greater than 10 W/kg.

From initial observations of modulation frequency-dependent responses of calcium binding in cerebral tissue (1–3), there are now many reports that RF fields, when either pulse or amplitude modulated at ELF, may regulate a wide range of *in vitro* responses, including cell membrane ion transfer, enzyme activation, neurotransmitter release, and cell growth regulation, even where these fields are below levels where tissue heating may mediate the responses (so-called "athermal exposures"; Ref. 4–6). Exposures here and in the comparable digital field study (25) were in this range (brain SAR, 1 W/kg). There is the implication that some form of envelope demodulation occurs in tissue recognition of ELF components. A suggested basis for envelope demodulation at cell surfaces may reside in the spatial anisotropy of the intensely anionic charge distribution on strands of glycoprotein protruding from the cell interior (4–6, 44).

Thus, the aggregate evidence suggests a biological role for ELF amplitude- or pulse-modulated microwave fields with absorbed energies below 5 W/kg, where heating is not the mediator of these bioeffects. If the concept of amplitude or pulse modulation frequency dependence gains further experimental support, elucidation of the tissue detection mechanisms for these responses becomes important (6). Low-level microwave fields with 16-Hz amplitude modulation (SAR, 0.08 W/kg) increased ODC activity in CHO hamster ovary cells by 50%, but responses decreased to control levels at 6 and at 100 Hz in a modulation frequency-dependent manner (45). ODC and its polyamine products are responsive to both 60-Hz ELF fields and to radiofrequency fields with 60-Hz amplitude modulation (7, 8, 46, 47).

A direct comparison of ODC activity in murine fibroblasts (L929) exposed to either a simulated TDMA digital phone field producing 50 pulses (packets)/s or to an FM phone field at athermal levels (SAR, 2.5 W/kg, 60-KHz deviation) showed a 90% increase with digital exposures but no response to the FM fields (47). This study also reported the ODC response to be a function of modulation frequency, significantly increased at 16, 55, 60, and 65 Hz but not at 6 or 600 Hz. Iridium cell phone fields (11 packets/s) modified activity of ODC and levels of its polyamine product putrescine in fetal rat brains (48). These electromagnetic sensitivities of ODC and its key functions in regulation of cell growth suggest possible links between overexpression of ODC in initiated cells and tumorigenesis in the absence of any other promoter (49).

The observed range of spontaneous CNS tumor incidence of 1.1–4.4% and brain tumor incidence of 1.1–3.3% approximated the range of 0.77–3.3% (CI upper bound, 6.5%) for brain tumors for the Fischer 344 rat in 2-year carcinogenicity studies of the NTP (50, 51). These studies report widely differing incidences, with the highest incidence in a single study of 4% (52, 53). In our study, tumor incidence was sharply increased to 15–22% over the lifetime of rats receiving a single dose of ENU *in utero*. They thus became a positive control in seeking possible promotional effects of FM field in tumorigenesis. Accuracy of tumor counts in the present study may have benefited from use of 20–25 sections/brain, permitting detection of MTs possibly missed in the NTP standard procedure with a maximum of three sections/brain. Other studies have reported trends toward a higher tumor incidence when at least five to eight sections/brain are used (54).

In summary, there were no effects on incidence of either spontaneous or ENU-induced primary tumors of the CNS associated with lifetime exposure of rats to FM microwave fields simulating electrical characteristics of hand-held mobile telephone transmissions at the head of the user.

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Spontaneous and Nitrosourea-induced Primary Tumors of the Central Nervous System in Fischer 344 Rats Exposed to Frequency-modulated Microwave Fields

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