28  Cytogenetic and Carcinogenic Effects of Exposure to Radiofrequency Radiation

JAMES P. MCNAMEE AND PASCALE V. BELLIER

Abstract

Radiofrequency radiation (RFR) is a portion of the electromagnetic spectrum with frequencies of 3 kHz–300 GHz. RFR is produced by many man-made sources, including mobile phones and base stations, television and radio broadcasting facilities, radar, medical equipment, microwave ovens, radio-frequency heaters as well as a diverse variety of other electronic devices within our living and working environments. Owing to ongoing public concern and the increasing prevalence of RFR-emitting devices, a great deal of research has been conducted over the past 50 years to evaluate the biological and/or health effects of thermalizing and non-thermalizing RFR exposures. In the absence of decisive epidemiological evidence to support or refute an association between RFR exposure and cancer risk, laboratory studies of possible mechanisms of carcinogenesis by RFR are important. The scientific literature on this subject is full of conflicting results and the question of whether RFR exposure can contribute to cancer risk remains unresolved. This chapter contains a literature review of the evidence for RFR-induced cytogenetic effects, but also a critique of the literature, highlighting deficiencies in the design of some studies that should be taken into account when assessing the health risk of RFR.

28.1 Introduction

Electromagnetic radiation is emitted by many natural and man-made sources and is a fundamental aspect of our lives. We are warmed by electromagnetic radiation emitted from the sun and our eyes can detect the visible light portion of the electromagnetic spectrum. The source of all electromagnetic radiation is accelerating charges and electromagnetic radiation manifests itself as oscillating electric and magnetic fields. The frequency of this oscillation (measured in hertz; 1 Hz is one oscillation per second) determines the physical properties of these fields. Radiofrequency radiation (RFR) is a portion of the electromagnetic spectrum with frequencies of 3 kHz–300 GHz, which is below that of visible light and above that of extremely low frequency fields (Fig. 28.1). RFR is
produced by many man-made sources, including mobile phones and base stations, television and radio broadcasting facilities, radar, medical equipment, microwave ovens, radiofrequency heaters as well as a diverse variety of other electronic devices within our living and working environments.

Public anxiety about exposure to RFR has existed for over 80 years. Initially, concern focused on RFR emissions from broadcast AM (530 kHz–1.6 MHz) and FM (88–108 MHz) RFR. This was followed by concerns over RFR emissions from radar installations (1–12.5 GHz), broadcast TV (54–890 MHz), microwave ovens (2.45 GHz) and video-display terminals (3–30 kHz). During the past decade, the increased use of mobile phones (850 MHz–1.9 GHz) and the emergence of new wireless technologies have reinvigorated public apprehension about the safety of human exposure to RFR. Owing to ongoing public concern and the prevalence of RFR-emitting devices, a great deal of research has been conducted over the past 50 years to evaluate the biological and/or health effects of thermalizing and non-thermalizing RFR exposures.

Unlike ionizing radiation, RFR does not have sufficient energy to directly break chemical bonds (Meltz 2003). However, sufficiently intense RFR can cause heating of materials with finite conductivity, including biological tissue. A number of well-established biological effects and adverse health effects from acute exposure to intense RFR have been documented. For the most part, these effects relate to localized heating of sensitive organs/tissues from intense RFR exposure; however, non-thermal effects such as nerve and muscle

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**Fig. 28.1** In the electromagnetic spectrum, radiofrequency radiation (RFR) encompasses frequencies ranging from 3 kHz to 300 GHz. Typical man-made sources of RFR include broadcast AM/FM/TV, mobile phones and base stations, microwave ovens and radar.
stimulation are also known to occur. The specific biological responses to RFR are generally related to the rate of RFR energy absorbed. The rate and distribution of RFR energy absorbed depends strongly on the frequency, intensity and orientation of the incident fields as well as the body size and its constitutive properties (dielectric constant and conductivity). At frequencies above 1 MHz, RFR absorption is commonly described in terms of the specific absorption rate (SAR), which is a measure of the rate of energy deposition per unit mass of body tissue and is usually expressed in units of watts per kilogram. Based on a large amount of historical knowledge, national and international exposure limits have been established to protect the general public against adverse effects associated with acute RFR exposures. However, the issue of safety of exposure to long-term, low-level RFR remains controversial and the risk of development of cancer remains the primary public health concern.

At present, there is a relative paucity of epidemiological evidence related to cancer risks associated with long-term, low-level RFR exposure. In the absence of decisive epidemiological evidence to support or refute an association between RFR exposure and cancer risk, laboratory studies of possible mechanisms of carcinogenesis by RFR are important. The scientific literature on this subject is full of conflicting results and the question of whether RFR exposure can contribute to cancer risk remains unresolved. This chapter will review the current state of the literature with respect to cytogenetic and carcinogenic effects of RFR. Since the thermal effects of RFR are well understood and cytogenetic alterations are known to be induced by heat, this chapter will focus on the evidence for cytogenetic effects from low-level, non-thermalizing RFR.

### 28.2 Challenges When Conducting RFR Research

There are many inherent challenges that must be overcome when conducting RFR bioeffects research which relate to the physical properties of this radiation. One of the most important interactions between RFR and matter is the rotational friction caused when small polar molecules (such as those in water and biological tissues) reorient themselves in the presence of the oscillating field, thereby producing heat. The rate at which heat energy is applied to the sample must not exceed the rate at which it is removed, otherwise the temperature within the sample will rise and thermal confounding may occur. If RFR energy is applied to biological samples at a low rate (e.g., below 1 W/kg) or for a short duration, then passive cooling and/or sweating may permit the tissue/body/sample temperature to remain within a normal physiological range. However, if higher SARs (above 1 W/kg) are employed and/or prolonged, then some form of active cooling mechanism will likely be required to ensure that excessive sample heating does not occur. When performing RFR experiments, it is important to include appropriate temperature controls within the experimental setup to alleviate thermal confounding in the study
Another significant challenge that must be addressed when studying the biological effects of RFR relates to the homogeneity of energy absorption within the sample (SAR distribution) and the maintenance of a relatively homogeneous, non-perturbing temperature throughout the biological sample. These are two separate, yet related, phenomena. While controlling volume-averaged temperature within the biological sample is important, it is also important to ensure that RFR energy is deposited in a homogeneous manner or “hot spots” within the biological sample can occur. This phenomenon is akin to focusing light energy on an object, whereby the localized temperature at the focal point is considerably elevated, while that of the surrounding area is not. In such a situation with RFR, the local temperature for some part of the sample may be greatly elevated, while the volume-averaged temperature remains relatively unaffected owing to heat convection and diffusion. With some in vitro exposure systems, heterogeneous RFR absorption can also cause convective flow within aqueous biological samples owing to the presence of temperature gradients within the sample. Such convection currents in a RFR-exposed sample, relative to a control, may be a source of confounding owing to a resultant stirring of the RFR-exposed samples that does not occur within the matched sham control.

Since RFR behaves in a similar fashion to light, with respect to its ability to reverberate/reflect off metallic surfaces, care must be taken to ensure that samples are exposed in a reverberant-free environment or at least that all sources of reflection are taken into account in the dosimetry analysis. This is particularly problematic for in vitro studies, where cells must be maintained under appropriate conditions (37°C, 50–60% humidity, 5% CO₂/95% air) in a tissue culture incubator. Most incubators are built with a stainless-steel-coated interior which acts as an ideal reflector for RFR. As a result, when conducting in vitro RFR research, great care must be taken to either contain RFR within a closed exposure system inside a standard tissue culture incubator or design alternative non-RFRperturbing tissue culture environments. In either case, maintenance of sample temperature, humidity, osmolarity and pH is a challenge and must be carefully controlled.

Owing to the above considerations, it is critical that RFR bioeffects studies encompass certain experimental controls to ensure that confounding variables do not result in artefactual biological observations. For in vitro studies, monitoring the temperature, pH and cell viability within the sample is paramount. For both in vivo and in vitro studies, empirical and/or numerical analysis of SAR distribution patterns within the sample is fundamentally important. The inclusion of unexposed (negative) controls, sham (non-RFR exposed) controls and positive controls is also imperative to ensure that the assay methodology is responding appropriately and experimental conditions are identical. Inclusion of these controls is particularly important when evaluating studies where RFR-induced effects were observed. On the other hand,
analysis of statistical power in studies where no evidence of RFR-induced effects were observed is important to determine whether a sufficient number of samples (independent experiments) were employed to allow the detection of significant differences between groups. For all studies, the investigators should be blinded with respect to the exposure status of the samples until all laboratory experiments, data acquisition and statistical analysis are complete.

In summary, when evaluating the literature related to RFR-induced cytogenetic and carcinogenic effects, all of the above considerations should be taken into account. This chapter contains not only a literature review but also a critique of the literature, highlighting certain deficiencies in the design of some of the studies that should be taken into account when evaluating the significance of certain RFR research findings.

28.3 Cytogenetic Effects of RFR

28.3.1 In Vitro Studies

Changes involving DNA and/or chromosome structure in somatic cells are of great relevance owing to their association with cancer and other diseases. Despite the general consensus that RFR is not sufficiently energetic to directly damage DNA, a large number of in vitro studies have been conducted to assess the genotoxic potential of RFR at a variety of frequencies. It is generally agreed that when the in vitro RFR cytogenetic studies published over the past 30 years are taken together and subjected to a weight-of-evidence evaluation, there is no compelling information to suggest that RFR exposure has any direct mutagenic, genotoxic or clastogenic effects (Brusick et al. 1998; Vijayalaxmi and Obe 2004; Moulder et al. 2005; Verschaeve 2005). In this section, the scientific literature relating to the evidence of in vitro cytogenetic effects of RFR will be reviewed and evaluated with particular focus on recent papers and/or pivotal studies (Table 28.1).

The majority of studies examining the cytogenetic effects of RFR during the last 5 years have been conducted in human blood cultures. Of these studies, only a few have observed a “positive” effect resulting from RFR exposures using the cytokinesis block micronucleus (CBMN) assay. Studies by d’Ambrosio et al. (2002), Tice et al. (2002) and Zotti-Martelli et al. (2000, 2005) have all reported increased micronuclei (MN) in RFR-exposed human blood cultures using the CBMN assay. In the study by Tice et al. (2002), significant increases in MN frequency were observed following exposure of unstimulated blood cultures exposed to 837 MHz and 1.9 GHz RFR (with various paradigms) at SARs of 5 and 10 W/kg at the longest RFR exposure time of 24 h. However, the authors identified thermal confounding as a possible explanation for these results as higher localized temperatures may have occurred within the sample region containing the blood cells. It is very
Table 28.1 Summary of in vitro studies that have investigated the possible cytogenetic effects of radiofrequency radiation

| Authors                  | RFR conditions                  | SAR; duration                | Tissue /cell line       | End point | Results     | Comments                                                   |
|--------------------------|---------------------------------|------------------------------|-------------------------|-----------|-------------|------------------------------------------------------------|
| Zotti-Martelli et al. (2000, 2005) | 1.8, 2.45 or 7.7 GHz; CW        | No SAR given; 15-, 30-, 60-, 120- or 180-min exposures | Human whole blood       | MN        | Increased MN | Insufficient details on exposure design and dosimetry; no temperature monitoring in actual samples; insufficient details on control conditions |
| Maes et al. (2001)       | 900 MHz; various modulations    | SARs ranging from 0.4 to 10 W/kg; 2-h exposure | Human whole blood       | CA and SCE | No effect    | Low sample number for most exposures (N = 2); no sham or positive controls |
| Vijayalaxmi et al. (2001a, b) | 835.62 or 874.74 MHz; CW       | SARs ranging from 4.4 to 5.5 W/kg; 24-h exposure | Diluted human blood     | MN and CA | No effect    | Sample number (N = 2 for each SAR tested) |
| d'Ambrosio et al. (2002) | 1.748 GHz; CW and phase-modulated | Average SAR of 2.25 W/kg; 15-min exposure | Mitogen-stimulated diluted human blood | MN | Increased MN following exposure to phase-modulated RFR | No sham or positive controls |
| Tice et al. (2002)       | 837 MHz, 1.9 GHz; analogue, PCS, CDMA and TDMA | SARs ranging from 1 to 10 W/kg; 3- and 24-h exposures | Diluted human blood     | MN        | Increased MN at highest SARs after 24-h exposure period | Low sample number (N = 1 for each condition); possible thermal confounding due to localized hot spots |
| Study                        | Frequency | Modulation | SARs Details | Cell Type | Endpoint | Results | Notes |
|------------------------------|-----------|------------|--------------|-----------|----------|---------|-------|
| Bisht et al. (2002)          | 847 MHz   | CDMA/FDMA | ranging from 3.2 to 5.1 W/kg; 3-, 8-, 16- or 24-h exposures | C3H 10T1/2 cells | MN | No effect |       |
| McNamee et al. (2002a, b, 2003) | 1.9 GHz  | CW/2GSM-modulated | ranging from 0.1 to 10 W/kg; 2- and 24-h exposures | Diluted human blood | MN | No effect | No comment |
| Zeni et al. (2003, 2005)      | 900 or 925 MHz | CW/2GSM-modulated | ranging from 0.2, 1 or 1.6 W/kg; various exposure periods | Whole or diluted human blood and mitogen-stimulated lymphocytes | MN, CA and SCE | No effect | No sham or positive controls in the 2003 study |
| Mashevich et al. (2003)       | 830 MHz   | CW        | ranging from 1.6 to 8.8 W/kg; 72-h exposure | Mitogen-stimulated diluted human blood | Aneuploidy | Increase in chromosome 17 aneuploidy | Non-homogenous exposure; sample flasks were thermally insulated from the incubator and no active cooling was employed, resulting in a possible thermal effect |
| Koyama et al. (2003)          | 2.45 GHz  |           | ranging from 13 to 100 W/kg; 18-h exposure | CHO-K1 cells, following incubation with cytochalasin B for 24 h | MN | Increased MN at SARs of 78 and 100 W/kg; no effect below 50 W/kg | High SAR levels with little relevance towards risk assessment; did not report the number of repeat experiments |
Table 28.1 Summary of in vitro studies that have investigated the possible cytogenetic effects of radiofrequency radiation—Cont’d

| Authors          | RFR conditions                              | SAR; duration               | Tissue /cell line | End point | Results   | Comments                                           |
|------------------|---------------------------------------------|------------------------------|-------------------|-----------|-----------|----------------------------------------------------|
| Komatsubara et al. (2005) | 2.45 GHz CW and pulse-modulated | SARs ranging from 5 to 100 W/kg; 2-h exposure | Mouse m5S cells   | CA        | No effect | High SAR levels with little relevance towards risk assessment; apparently a single sample for each RFR exposure condition |
| Stronati et al. (2006) | 935 MHz; GSM-modulated | SARs of 1 and 2 W/kg; 24-h exposure | Mitogen-stimulated human whole blood | MN, CA and SCE | No effect |                                                   |

CA chromosome aberrations, CDMA code-division multiple access, CHO Chinese hamster ovary, CW continuous wave, FDMA frequency-division multiple access, GSM global system for mobile communication, MN micronuclei, PCS personal communication services, RFR radiofrequency radiation, SAR specific absorption rate, SCE sister-chromatid exchange, TDMA time-division multiple access.
important to note that blood samples from only one to two donors were used for each experimental condition; therefore, only limited conclusions can be drawn from this study. Zotti-Martelli et al. (2000, 2005) also observed increased MN in the CBMN assay following exposure of unstimulated whole-blood cultures to 1.8, 2.45 and 7.7 GHz continuous-wave (CW) RFR for up to 3 h at power densities ranging from 5 to 30 mW/cm². However, very limited information regarding the RFR exposure system and dosimetry was provided for these studies. Furthermore, while the authors measured the temperature variation within “pseudosamples” during RFR exposure, water and not whole blood was used in these experiments. This is problematic since water has very different dielectric and conductivity properties from whole blood, and much more energy would be absorbed within whole-blood cultures than in water. As such, at the RFR exposure levels described by the authors, it is possible that the observed increases in MN frequency may have been due to hyperthermia. In a well-controlled study by d’Ambrosio et al. (2002), increased MN frequency was reported in phytohemagglutinin (PHA) stimulated human lymphocytes exposed to phase-modulated 1.748 GHz RFR, but not to 1.748 GHz CW RFR. This result suggested that the cellular response to RFR may depend upon the modulation characteristics of the applied RFR field.

On the other hand, many other research groups have failed to find any evidence of RFR-induced cytogenetic effects in human blood cell cultures regardless of the modulation paradigm. Vijayalaxmi et al. (2001a, b) exposed unstimulated whole-blood cultures to 835.62 MHz frequency division multiple access (FDMA) modulated and 847.74 MHz code division multiple access (CDMA) modulated RFR for 24 h at mean SARs of either 4.4 or 5.0 W/kg and 4.9 or 5.5 W/kg, respectively. The authors found no significant changes in either cytogenetic end point between the sham and RFR-exposed samples for either of these RFR exposure conditions. Similarly, McNamee et al. (2002a, b, 2003) exposed unstimulated human whole-blood cultures to 1.9 GHz CW and pulse-modulated RFR for 2–24 h at SARs ranging from 0.1 to 10 W/kg. The formation of MN was assessed using the CBMN assay and no significant changes were found in the binucleated cell frequency, the frequency of binucleated cells with MN or the total MN incidence at any SAR level tested when compared with the sham cells. Unlike many previous studies, the studies by McNamee et al. incorporated exposure of human blood samples from five separate donors to sham and five different RFR exposure levels, allowing the possibility of a dose–response relationship to be assessed. Furthermore, real-time temperature measurements were performed every 60 s within each of the RFR-exposed and sham cultures, thereby excluding thermal effects as a possible confounding variable.

Maes et al. (2001) and Zeni et al. (2003, 2005) examined the effect of 900 MHz global system for mobile communication (GSM) modulated RFR exposure at SARs ranging from 0.2 to 10 W/kg in either PHA-stimulated or unstimulated blood cultures and found similar results. However, while the studies by Maes et al. (2001) and Zeni et al. (2003) found no evidence that
RFR exposure caused any genotoxic effects; these studies did not include the necessary controls. Since both studies lacked positive controls, the relative sensitivity of the cytogenetic assays in these laboratories to detect an effect is difficult to interpret and therefore the significance of these negative results is diminished. Furthermore, these studies failed to include sham controls and relied only upon unexposed controls. Although the importance of a sham control is obvious when a positive effect is detected, it is important to point out that the appropriate comparison for all RFR-exposed samples should be the sham control, rather than the unexposed control, since the sham and RFR-exposed samples should have been treated identically except for the RFR exposure. This comparison ensures that the exposure system and exposure environment are not the cause of possible observed effects, but rather that the RFR exposure itself is responsible. In a follow-up study, Zeni et al. (2005) included all the necessary controls when exposing unstimulated whole-blood cultures to 900 MHz GSM-modulated RFR for 2 h at 1 W/kg. No evidence of chromosomal damage, as measured by analysis of chromosome aberrations and sister-chromatid exchange (SCE), was observed. Very recently, Stronati et al. (2006) exposed PHA-stimulated whole blood to 935 MHz GSM-modulated RFR at SARs of 1 and 2 W/kg for 24 h and observed no increase in chromosome aberrations, MN frequency or SCE in RFR-exposed samples when compared with sham controls.

Several in vitro studies have also assessed the effect of RFR exposure on cytogenetic end points using a variety of animal-derived cell lines. Bisht et al. (2002) exposed a mouse embryo fibroblast derived cell line (C3H 10T1/2) to 835.62 MHz FDMA-modulated or 847.74 MHz CDMA-modulated RFR for up to 24 h at SARs of 3.2–5.1 W/kg, in both the plateau phase and the exponential growth phase of these cells. The authors found no evidence that RFR exposure had any effect on MN frequency at any SAR, modulation paradigm or exposure duration. Koyama et al. (2003) exposed Chinese hamster ovary (CHO) K1 cells to 2.45 GHz CW RFR for 18 h at SARs ranging from 13 to 100 W/kg. While the authors reported no evidence of increased MN frequency in RFR samples exposed at SARs up to 50 W/kg, an increased incidence in MN frequency was reported in cells exposed at SARs of 78 and 100 W/kg. The authors measured the average temperature of the highest RFR exposed group (100 W/kg) to be approximately 39°C. When positive control samples (exposed to a temperature of 39°C for 18 h) were assessed, a similar increased incidence of MN was reported. Thus, the increased MN frequency at the higher SARs assessed in this study appears to be due to thermal effects. Another recent study by this group (Komatsubara et al. 2005) assessed the incidence of chromosome aberrations in murine (embryonal skin derived) m5S cells following a 2-h exposure to 2.45 GHz CW RFR at SARs of 5–100 W/kg or 2.45 GHz pulse-modulated RFR at SARs of 50 and 100 W/kg. The authors reported no evidence of increased chromatid-type or chromosome-type aberrations in RFR-exposed samples at any SAR tested when compared with sham cells. It is interesting to note that the shorter exposure time (2 h) to an elevated temperature of
39–41°C had no apparent clastogenic effect in this study, while the prolonged (18-h) exposure to 39°C in the study by Koyama et al. (2003) appeared to stimulate heat-induced MN formation. In any event, the high SAR levels employed in these studies have little relevance when assessing the relative risk of RFR towards the general population since such intense exposures are not typically encountered in our working or living environment.

In an interesting study by Mashevich et al. (2003), diluted human whole blood was stimulated with 3% PHA and then exposed to 830 MHz CW RFR for 72 h at SARs ranging from 1.6 to 8.8 W/kg. Following the culture period, the cells were harvested for cytogenetic analysis and the authors labeled the α-satellite DNA of chromosome 17 using fluorescence in situ hybridization. A linear increase in chromosome 17 aneuploidy as a function of increasing SAR was reported. The authors suggested that this effect was non-thermal in nature on the basis of their assumption that the temperature within the RFR-exposed samples did not exceed 38°C, while separate control experiments demonstrated that prolonged culturing at temperatures below 38.5°C did not affect chromosome 17 aneuploidy. However, Chou and Swicord (2003) argued the assumption that RFR sample temperatures remained below 38°C since the RFR-exposed flasks were enclosed in Plexiglas and were thereby thermally insulated from the incubator temperature. Chou and Swicord (2003) noted that such a situation would create non-uniform heating within the samples owing to inefficient heat exchange and the localized temperatures within the samples may have exceeded 40°C. As a result, the observed changes in chromosome 17 aneuploidy could have been related to thermal effects. Since no active cooling was employed in this study to ensure the sample temperature was maintained within an acceptable range and no temperature measurements were recorded during the actual exposure period, thermal confounding is a likely explanation for these observations.

28.3.2 In Vivo Studies

Despite an exhaustive number of in vitro RFR studies, there have been relatively few studies examining the cytogenetic effects in animals following in vivo RFR exposure (Table 28.2). Many early studies on RFR cytogenetic effects focused on the ability of 2.45 GHz microwave radiation to cause alterations in meiotic chromosomes in spermatogonia, as microwave hyperthermia was considered as a possible means of male contraception (Fahim et al. 1975). However, inadequate control of thermal effects has been identified as a possible confounding variable in many of the early studies that demonstrated RFR-induced genotoxic effects (Brusick et al. 1998; Royal Society of Canada 1999; Vijayalaxmi and Obe 2004; Moulder et al. 2005; Verschaeye 2005).

Among the early studies where thermal effects were unlikely, Manikowski-Czerska et al. (1985) evaluated the effect of 0.5–20 W/kg 2.45 GHz RFR exposure on mouse spermatogonia. Adult CBA/CAY mice were exposed for
| Authors                  | RFR conditions | SAR; duration | Tissue/cell line | End point | Results                                                                 | Comments                                                                 |
|--------------------------|----------------|---------------|------------------|-----------|-------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Manikowski-Czerska et al. (1985) | 2.45 GHz; CW    | Whole body average SAR ranging from 0.05 to 20 W/kg; 30 min/day, 6 days/week, 2 weeks | CBA/CAY mice | CA in sperm | Dose-response increase in translocations and univalent chromosomes | Restrained animals; no positive control                                   |
| Beechey et al. (1986)    | 2.45 GHz        | Estimated SARs of 0.05–20 W/kg; 30 min/day, 6 days/week, 2 weeks | Hybrid F1(C3H/HeH × 101/H) male mice | CA in sperm | No effect                                                              | Restrained animals; no positive control                                   |
| Saunders et al. (1988)   | 2.45 GHz        | SAR of 5 W/kg; 6 h/day; over 8 weeks, for total exposure of 120 h | Male C3H mice | CA in sperm | No effect                                                              | Animals partially restrained, no vertical movement; no positive control   |
| Vijayalaxmi et al. (1997, 1998) | 2.45 GHz; CW    | Whole body average SAR of 1 W/kg; 20 h/day, 7 days/week, 18 months | C3H/HeJ mammary cancer prone female mice | MN-PCE in blood and bone marrow | Increase observed was not deemed to be biologically significant since it was small and within the normal spontaneous range of MN-PCE in mice |
| Vijayalaxmi et al. (2001c) | 2.45 GHz; CW    | Whole body average SAR of 12 W/kg; 24-h exposure | Male Sprague-Dawley rats | MN-PCE in blood and bone marrow | No effect                                                              |                                                                           |
| Sykes et al. (2001)      | 900 MHz; pulse-modulated | Whole body average SAR | Spleen cells of pKZ1 transgenic mice | Somatic intra-chromosomal | Decrease in rate of spontaneous | Restrained animals; no positive control                                  |
| Study                      | Frequency | Modulation  | Species                  | Exposure Details                                                                 | Genotoxic Effect       | Notes                                                                 |
|----------------------------|-----------|-------------|--------------------------|----------------------------------------------------------------------------------|------------------------|----------------------------------------------------------------------|
| Trosic et al. (2002, 2004) | 2.45 GHz  | CW         | Male Wistar rats          | Whole body average SAR of 1–2 W/kg; 2 h/day, 7 days/week for up to 30 days       | Increase in MN-PCE in bone or bone marrow | No positive control; MN-PCE levels in RFR-exposed and sham groups were within the normal range for rat peripheral blood |
|                           |           |            |                          |                                                                                 |                        |                                                                     |
| Vijayalaxmi et al. (2003)  | 1.6 GHz   | CW         | Fisher 344 rats           | Brain-average SAR of 0.16 or 1.6 W/kg; 2 h/day, 2 years                         | No effect              | Restrained animals                                                   |
|                           |           |            |                          |                                                                                 |                        |                                                                     |
| Görlitz et al. (2005)      | 902 MHz   | GSM-modulated, DCS-modulated | Male and female B6C3F1 mice | Whole body average SAR of 2.8–24.9 W/kg; 2 h/day, 5 days/week for 1 or 6 weeks | No effect              | Restrained animals                                                   |
|                           |           |            |                          |                                                                                 |                        |                                                                     |

DCS digital cordless standard, MN-PCE micronuclei in polychromatic erythrocytes
30 min/day, 6 days/week for two consecutive weeks and then chromosome number and structure were evaluated in isolated spermatogonia. At the end of the exposure period, a dose-responsive increase in the number of translocations and univalent chromosomes at meiotic metaphase were reported in the RFR-exposed mice, while no translocations were observed in sham mice. In an attempt to replicate these findings, Beechey et al. (1986) employed a similar irradiation protocol using hybrid male mice but observed no evidence of increased chromosome aberrations in spermatogonia from RFR-exposed mice, where increases in rectal temperatures did not exceed 1°C. Saunders et al. (1988) examined this effect further by exposing C3H mice to 2.45 GHz RFR for 6 h/day over an 8-week period at a SAR of approximately 5 W/kg. No evidence of increased reciprocal translocations or increased numbers of cells with autosome or sex chromosome univalents was observed.

The majority of in vivo cytogenetics studies on RFR have focused on evaluating the incidence of MN in rodent peripheral blood or bone marrow. Only a limited number of chronic and/or lifetime RFR exposure studies have been conducted in experimental animals. Of these, Vijayalaxmi et al. (1997) exposed mammary cancer-prone C3H/HeJ female mice to 2.45 GHz CW RFR at a whole-body SAR of 1 W/kg for 20 h/day, 7 days/week for 18 months. Peripheral blood and bone marrow smears were assessed for the incidence of MN in polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). At this time-point, a chronic level of MN-PCE and/or MN-NCE would be anticipated in both the peripheral blood and the bone marrow of RFR-exposed mice, if RFR exposure induced a clastogenic effect. The authors reported a small, yet statistically significant increase in the number of MN-PCE in both bone marrow and peripheral blood of RFR-exposed mice; however, the authors cautioned that this difference (an increase of 0.05%) was not biologically significant since the MN-PCE frequencies for both the RFR and the control groups were within the normal range of the spontaneous incidence of MN-PCE in mice (Vijayalaxmi et al. 1998). Vijayalaxmi et al. (2003) also evaluated the incidence of MN-PCE in peripheral blood and bone marrow smears of Fischer 344 rats exposed (since birth) for 2 h/day for 2 years to a 1.6 GHz Iridium wireless communication signal at brain-averaged SARs of 0.16 and 1.6 W/kg. In this study, no significant differences were observed between RFR-exposed male and female rats relative to the sham controls. More recently, Görlitz et al. (2005) exposed B6C3F1 mice to 902 MHz GSM-modulated or 1,747 MHz digital cordless standard (DCS) modulated RFR for either 1 week at 2 h/day at whole-body SARs of 3.7–33.2 W/kg or for 6 weeks at 2 h/day for 5 days/week at SARs of 2.8–24.9 W/kg. After either a 1-week or a 6-week exposure protocol, MN in bone marrow erythrocytes, keratinocytes and spleen lymphocytes were evaluated in both male and female mice. No significant differences in the incidence of MN were observed in any of the RFR-treated groups relative to the matched sham controls.

A series of studies have also evaluated the effect of acute and/or subacute in vivo RFR exposure on cytogenetic end points in experimental animals.
Vijayalaxmi et al. (2001c) exposed male Sprague-Dawley rats continuously for 24 h to 2.45 GHz CW RFR at a whole body average SAR of 12 W/kg. At 24 h after the end of RFR exposure, the frequency of MN-PCE in peripheral blood and bone marrow smears was evaluated. RFR-exposed rats displayed elevated, yet statistically insignificant, MN-PCE in both peripheral blood and bone marrow compared with sham animals, but once again the MN-PCE frequencies in both groups were within the normal expected range for rats. In a similar study, Trosic et al. (2002, 2004) exposed adult male Wistar rats for 2 h/day, 7 days/week for up to 30 days to 2.45 MHz CW RFR at an approximate SAR of 1–2 W/kg. The authors examined MN-PCE in peripheral blood and bone marrow at various times of exposure and observed a significant increase in MN-PCE between RFR-exposed animals relative to sham controls at 8 and 15 days of exposure, but no statistically significant differences were observed after 2 or 30 days’ exposure. While the authors indicated that RFR exposure may have induced a genotoxic response at days 8–15 which was subsequently masked by an adaptive response, there are no experimental data to support this hypothesis. Since the relative MN-PCE levels in both the RFR-exposed and the sham groups were within the normal range for rat peripheral blood and there was no supporting evidence of elevated MN-PCE at other time-points, these data must be considered equivocal.

In an interesting study, Sykes et al. (2001) used pKZ1 transgenic mice, which possessed a β-galactosidase reporter gene in reverse orientation, to assess somatic intrachromosomal recombination inversion events. The authors exposed the mice to 900 MHz pulse-modulated RFR for 30 min/day for 1, 5 or 25 days at a whole body average SAR of 4 W/kg. While no effect of RFR exposure was observed on intrachromosomal recombination in spleen cells of pKZ1 mice after 1 or 5 days’ exposure, a significant decrease in the rate of spontaneous recombination was observed after a 25-day RFR exposure period. However, this result appears to have arisen from the statistical approach employed in this study. The authors found no significant difference in the number of recombination events between the non-exposed control groups at days 1, 5 and 25 and therefore chose to combine control data from all time-points into a single pooled control group for comparison with the RFR-exposed groups at each time-point. As a result, the RFR-exposed samples for each time-point were not directly compared with their matched non-exposed time-point controls. Comparison of the RFR-exposed samples with the time-point matched controls would not likely have yielded significant differences. In any event, it is unclear what the biological significance of a reduced rate of spontaneous intrachromosomal recombination may be.

### 28.3.3 Human Studies

Only a limited number of studies have evaluated cytogenetic aberrations in human blood lymphocytes following occupational exposure to RFR (Table 28.3).
Table 28.3 Summary of human studies that have investigated the possible cytogenetic effects of radiofrequency radiation

| Authors                        | RFR conditions | SAR; duration | Tissue/cell line               | End point | Results                  | Comments                                                                 |
|--------------------------------|----------------|---------------|--------------------------------|-----------|--------------------------|--------------------------------------------------------------------------|
| Garson et al. (1991)           | 0.4 MHz–20 GHz | SAR unknown, occupational exposure | Radio linemen and age-matched controls | CA        | No effect                |                                                                          |
| Fucic et al. (1992)            | 1.25–1.35 GHz | SAR unknown, occupational exposure | Non-smoking men | MN        | Increased MN frequency   |                                                                          |
| Garaj-Vrhovac and Fucic (1993) | 1.25–1.35 GHz; pulsed | SAR unknown, occupational exposure | Male air traffic control workers | CA        | Increased CA in all subjects |                                                                          |
| Maes et al. (1995)             | 450–900 MHz   | SAR unknown, occupational exposure | Male transmission antennae maintenance workers | CA        | No effect                | Pilot study, only 6 exposed and 6 control individuals                      |
| Lalic et al. (2001)            | Frequencies reaching 8 GHz | SAR unknown, occupational exposure | Subjects working in radio-relay stations | CA        | Increased CA relative to background, based on literature references | Authors did not assess the background aberration frequencies in their laboratory to ensure it was similar to literature values; insufficient information on exposure conditions |
| Study          | Frequency Range | SAR Information | Exposure Type and Controls | Endpoints | Findings                  | Details |
|---------------|-----------------|-----------------|-----------------------------|-----------|---------------------------|---------|
| Gadlia et al. (2003) | 890–960 MHz     | SAR unknown, minimum 2-year exposure | Mobile-phone users (non-smoker, non-alcoholic or smoker-alcoholic) and matched controls | CA and SCE | Increase in CA and SCE for mobile phone users, compared with controls | Insufficient details on stratification of subjects |
| Maes et al. (2006) | 150 MHz–40 GHz; various modulations | SAR unknown, occupational exposure | Radio-field engineers and administrative staff of mobile phone companies | SCE and CA | No effect |         |
Two early studies by Fucic et al. (1992) and Garaj-Vrhovac and Fucic (1993) reported an increased incidence of chromosome aberrations and MN in the blood of male subjects who were occupationally exposed to RFR (1.25–1.35 GHz). Gadchia et al. (2003) recently reported an increased incidence of chromosome aberrations and SCE frequencies in normal and smoker–alcoholic mobile-phone users (890–960 MHz). In contrast, Maes et al. (1995) found no evidence of excess aberrations in peripheral blood from antenna maintenance workers exposed to 450–950 MHz RFR, while Garson et al. (1991) found no evidence of increased aberrations in peripheral blood from radio linemen exposed to 0.4-MHz–20 GHz RFR. However, these studies are generally considered inconclusive owing to a variety of methodological shortcomings such as inadequate RFR exposure dosimetry, lack of detailed information regarding enrolled subjects and inadequate sample size based on statistical power analysis (Verschaeve 2005).

More recently, Lalic et al. (2001) reported increased chromosome aberrations (chromatid breaks, acentric fragments and dicentric chromosomes) in peripheral blood from subjects working in radio-relay stations. The authors reported increased levels of acentric fragments and dicentric chromosomes in the RFR-exposed subjects relative to literature reference values for the background incidence of these forms of aberrations in control populations. However, since the authors did not assess aberration frequency in any control samples within their laboratory, it cannot be determined whether the authors detected an RFR-induced cytogenetic effect or whether the background frequency for detecting these forms of aberrations was considerably higher in their laboratory relative to reference values from other laboratories. For these reasons, these data must be considered methodologically flawed.

Maes et al. (2006) evaluated a variety of cytogenetic end points in peripheral blood samples obtained from 38 radio field engineers and 11 administrative staff of a mobile-phone company and 25 unrelated controls. The radio field engineers were exposed to a variety of antennas where they were frequently exposed to a variety of frequencies ranging from 450 MHz to several gigahertz; administrative staff were exposed to RFR in the 150–170 MHz and 6–40 GHz range; while the controls were not occupationally exposed to RFR. No statistically significant increase in chromosome aberration (which included analysis of gaps, breaks, interchromatid exchanges, translocations, dicentric chromosomes and acentric fragments) or SCE frequencies was observed in RFR-exposed subjects, relative to control subjects. However, it is important to point out that no dosimetric analysis was carried out, although measurements indicated that RFR exposures were typically below the limits of the ICNIRP guidelines (International Commission on Non-Ionizing Radiation Protection 1998).

In conclusion, the vast majority of in vitro studies using human blood cultures and mammalian cell lines have found no consistent evidence of cytogenetic effects from non-thermal RFR exposures. Of the limited number of in vitro studies reporting RFR-induced cytogenetic effects, thermal
confounding appears to be a likely explanation. Of the few studies which have assessed the effect of in vivo RFR exposure on cytogenetic end points in animals and humans, many suffer from obvious methodological and/or statistical shortcomings. Future well-characterized prospective human and animal RFR exposure studies would be beneficial to permit a more complete RFR health risk assessment.

28.4 DNA Strand Breaks

28.4.1 In Vitro Studies

Since the publication of in vivo studies by Lai and Singh (1995, 1996, 1997), a large number of studies have assessed the ability of in vitro RFR exposure at a variety of frequencies to induce DNA strand breaks in a variety of mammalian cell lines and in human peripheral blood (Table 28.4). Unlike the in vivo data, the results of in vitro investigations of RFR-induced DNA damage and/or repair have yielded nearly uniform negative results (Brusick 1998; Vijayalaxmi and Obe 2004; Moulder et al. 2005).

Phillips et al. (1998) reported alterations in primary DNA damage at clearly non-thermal RFR exposure levels in Molt-4 cells, following in vitro exposure to a variety of conditions (813–836 MHz; time division multiple access, TDMA, modulated or Integrated Digital Enhanced Network (iDEN) modulated; SAR 0.002–0.02 W/kg). However, this study has been criticized owing to a lack of internal consistency as some RFR frequency/modulation paradigms were reported to statistically increase the level of DNA damage, while others were found to significantly reduce the level of DNA damage. Furthermore, there was no dose dependency for these observations and the fold-differences in control values between experiments often exceeded the fold-differences between sham and RFR-exposed samples. Thus, the results from this study must be considered equivocal. Hook et al. (2004) re-evaluated the ability of RFR (813–847 MHz; CDMA-, FDMA-, iDEN- or TDMA-modulated; SAR 0.0024–3.2 W/kg) to elicit DNA damage in Molt-4 T-lymphoblastoid cells, but failed to find any evidence of a RFR-induced genotoxic effect at any frequency/modulation paradigm employed. Most other studies assessing the effect of in vitro RFR exposure on mammalian cell lines have also found no evidence of genotoxic effects. Malyapa et al. (1997a, b), Li et al. (2001) and LaGroye et al. (2004b) found no evidence of increased DNA damage, as determined by the alkaline comet assay, in either mouse C3H 10T1/2 fibroblasts or human glioblastoma U87MG cells following in vitro exposure for 2–24 h to 835 MHz–2.45 GHz RFR at a variety of modulations in the SAR range of 0.6–5.1 W/kg. More recently, Sakuma et al. (2006) exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts to 2.1425 GHz RFR for 2–24 h at SARs of 0.08–0.8 W/kg, but also found no evidence of any RFR-mediated effect on DNA damage.
Table 28.4 Summary of in vitro studies that have investigated the possible DNA-strand breaks effects of radiofrequency radiation

| Authors                     | RFR conditions | SAR/duration | Tissue/cell line | End point      | Results                  | Comments |
|-----------------------------|----------------|--------------|------------------|----------------|--------------------------|----------|
| Malyapa et al. (1997a, b)   | 2.45 GHz, 835.6 MHz and 837 MHz, various modulations | SAR ranging from 0.6 to 1.9 W/kg; 2-4 and 24-h exposures | Human glioblastoma and mouse U87MG cells | DNA strand breaks | No effect | Exponentially growing and plateau-phase cells |
| Maes et al. (1997)          | 953.2 MHz, CW | SAR of 0.3-0.4 W/kg; 2-h exposure | Human whole blood | DNA strand breaks | No effect | Exponentially growing and plateau-phase cells |
| Phillips et al. (1998)      | 813.6 and 836.5 MHz, TDMA- and iDEN-modulated | SAR ranging from 0.002 to 0.026 W/kg; intermittent exposures of 2, 3 and 21 h | Molt-4 T-lymphoblastoid cells | DNA damage, for some conditions | Increased/ decreased | Low sample number, inconsistency in control values |
| Vijayalaxmi et al. (2000)   | 2.45 GHz; pulse-modulated | SAR of 2.1 W/kg; 2-h exposure | Whole human blood | DNA strand breaks | No effect | Low sample number (N = 1 or 2 for all conditions) |
| Li et al. (2001)            | 847.7 and 835.6 MHz, CDMA- and FDMA-modulated | SAR ranging from 3.8 to 4.9 W/kg; for CDMA and 5.1 W/kg for FDMA; 2-4 and 24-h exposures | Mouse CH10712 fibroblasts | DNA strand breaks | No effect | |
| Tice et al. (2002)          | 837 MHz, 1.9 GHz, and 1.9 GHz; pulse-modulated | SAR ranging from 1 to 10 W/kg; 3-4 and 24-h exposures | Diluted human blood | DNA strand breaks | No effect | |
| McNamee et al. (2002b, b)   | 1.9 GHz; CW and pulse-modulated | SAR ranging from 0.1 to 10 W/kg; 2-4 and 24-h exposures | Diluted human blood | DNA strand breaks | No effect | |
| Study                  | Frequency | Modulation | SARs | Cell Type                | Effect                  |
|------------------------|-----------|------------|------|-------------------------|-------------------------|
| LaGroye et al. (2004a) | 2.45 GHz; CW | Average SAR of 1.9 W/kg; 2-h exposure | Mouse C3H 10T1/2 fibroblasts | DNA strand breaks | No effect |
| Hook et al. (2004)     | 813.6–847.7 MHz; various modulations | SARs ranging from 0.002 to 3.2 W/kg; various exposure times up to 24 h | Molt-4 T-lymphoblastoid cells | DNA strand breaks | No effect |
| Diem et al. (2005)     | 1.8 GHz; intermittent or continuous CW and GSM | SARs of 1.2 and 2 W/kg; 4-, 16- or 24-h exposure | Human diploid fibroblasts and rat granulosa cells | DNA strand breaks | Increased single-strand and double-strand breaks in both cell types |
| Zeni et al. (2005)     | 900 MHz; GSM-modulated | SARs of 0.3 and 1 W/kg; 2-h exposure | Human peripheral blood lymphocytes | DNA strand breaks | No effect |
| Sakuma et al. (2006)   | 2.14 GHz; CW and W-CDMA-modulated | SARs of 0.08, 0.25 and 0.8 W/kg; 2- and 24-h exposures | Human glioblastoma A172 cells and human IMR-90 fibroblasts | DNA strand breaks | No effect |
| Stronati et al. (2006) | 935 MHz; GSM-modulated | SARs of 1 and 2 W/kg; 24-h exposure | Human whole blood | DNA strand breaks | No effect |

*iden Integrated Digital Enhanced Network, W-CDMA wideband CDMA*
Studies by Maes et al. (1997), Vijayalaxmi et al. (2000), Tice et al. (2002), McNamee et al. (2002a, b, 2003), Zeni et al. (2005) and Stronati et al. (2006) have also assessed the ability of non-thermal RFR, at a variety of frequencies and modulations, to induce DNA damage in human peripheral lymphocytes using the alkaline comet assay. The study by Tice et al. (2002) possessed an inadequate number of independent experiments ($N = 1–2$ for each exposure paradigm) upon which to accurately assess a possible RFR-induced genotoxic effect. However, the remaining studies have provided strong evidence that non-thermal RFR exposure has no effect on inducing DNA damage in cultured human lymphocytes. In contrast, Diem et al. (2005) reported increased DNA damage (DNA single-strand and double-strand breaks) in human fibroblasts and rat granulosa cells following in vitro exposure of these cells to 1.8 GHz RFR at SARs of 1.2–2 W/kg for 4–24 h, under a variety of exposure conditions. In this study, the authors classified DNA “comets” into one of five categories based upon visible inspection, then applied a transformation factor to achieve a “normalized tail factor” for each sample. Vijayalaxmi et al. (2006) raised several concerns for this approach since such transformation of each classification group by arbitrary transformation factors did not control for differences in S/G2-phase or apoptotic cells. These cells would be classified into the “higher” damage categories owing to either the presence of DNA replication forks or orderly DNA fragmentation, respectively. As such, small differences in the rate of cell growth/death between control and RFR-exposed groups may have been responsible for the observed differences between the sham and RFR-exposed samples. In any event, additional studies are required to validate these observations.

In summary, there is no consistent evidence in the literature for non-thermal, RFR-induced DNA damage in either mammalian-derived cell lines or primary human lymphocytes. This information is supported by a large database of studies using traditional bacterial/mammalian cell lines and recent transgenic in vivo mutagenesis assays, where there is virtually no evidence of any mutagenic effects from RFR exposure.

28.4.2 In Vivo Studies

Analysis of RFR-induced DNA strand breaks (using the alkaline comet assay) in animal tissue has been much more controversial (Table 28.5). An early study by Sarker et al. (1994) reported RFR-induced alterations in $Hinf$1 digested genomic DNA, relative to control DNA, in both brain and testis from mice exposed to 2.45 GHz RFR at a SAR of 1.18 W/kg for 2 h/day for 120–200 days. This was followed by a series of highly publicized studies by Lai and Singh (1995, 1996, 1997), who reported increased levels of primary DNA damage (which may have included DNA single-strand and double-strand breaks, alkali-labile sites and DNA cross-links) in rat brain cells at 0–4 h after a 2-h in vivo exposure to 2.45 GHz CW or pulse-modulated RFR at SARs...
Table 28.5 Summary of in vivo studies that have investigated the possible DNA-strand breaks effects of radiofrequency radiation

| Authors            | RFR conditions | SAR; duration | Tissue/cell line | End point | Results | Comments |
|--------------------|----------------|---------------|------------------|-----------|---------|----------|
| Sarker et al.      | 2.45 GHz; CW   | Average SAR of 1.18 W/kg; 2 h/day, up to 200 days | Inbred male Swiss albino mice; isolated cells from brain and testis | DNA transformations | Distinct DNA alterations in exposed samples | Restrained animals; sample number N = 2 per time-point |
| Lai and Singh      | 2.45 GHz; PW or CW | Whole body average SAR 0.6 and 1.2 W/kg; 2-h exposure | Male Sprague-Dawley rats; isolated brain cells | DNA strand breaks immediately and 4 h after exposure | Increased DNA damage 4 h after CW and PW exposure | Non-restrained animals; no positive control; injection of free-radical scavengers blocked RFR DNA damaging effect; used proteinase K in lysis buffer |
| Malyapa et al.     | 2.45 GHz; CW   | Whole body average SAR of 1.2 W/kg; 2-h exposure | Male Sprague-Dawley rats; isolated brain cells | DNA strand breaks immediately and 4 h after exposure | No effect | Non-restrained animals; observed an increased background level of DNA damage in rats euthanized with CO₂ as done in the Lai and Singh studies; observed that the time from euthanasia to dissection needs to be minimized, where increased time results in increased damage; did not use proteinase K in lysis buffer |

(Continued)
| Authors           | RFR conditions | SAR; duration | Tissue/cell line | End point                   | Results                  | Comments                                                        |
|-------------------|----------------|---------------|------------------|-----------------------------|--------------------------|                                                                |
| LaGroye et al.    | 2.45 GHz; PW   | Whole body    | Male Sprague-    | DNA strand breaks 4 h       | No effect                | Non-restrained animals; lysis                                    |
| (2004b)           | average SAR of | average SAR of | Dawley rats;     | after exposure              |                          | buffer with and without proteinase K                            |
|                   | 1.2 W/kg; 2-h exposure | 1.2 W/kg; 2-h exposure | isolated brain cells |                             |                          |                                                                  |
|                   |                | 900 MHz       | Male CD1 Swiss   | DNA strand breaks and DNA   | No effect                | Non-restrained animals; no positive control                    |
| Aitken et al.     | Whole body     | isolated      | mice; isolated   | integrity                   |                          |                                                                  |
| (2005)            | average SAR    | spermatozoa   | DNA lesions in a |                                            |                          |                                                                  |
|                   | of 0.09 W/kg;  |              | segment of the   |                                            |                          |                                                                  |
|                   | 12 h/day, 7 days|              | β-globin gene    |                                            |                          |                                                                  |
|                   |                |              | and in the      |                                            |                          |                                                                  |
|                   |                |              | mitochondrial    |                                            |                          |                                                                  |
|                   |                |              | genome           |                                            |                          |                                                                  |
| Paulraj and       | 2.45 and 16.5 | Whole body    | Male Wistar rats;| DNA strand breaks            | Increased DNA damage     | Restrained animals; no positive control                        |
| Behari (2006)     | GHz; amplitude | average SAR   | isolated brain   |                             |                          |                                                                  |
|                   | modulated      | of 1 or 2 W/kg;| cells            |                             |                          |                                                                  |
|                   |                | 2 h/day, 5 days/week,|              |                             |                          |                                                                  |
|                   |                | 7 weeks       |                  |                             |                          |                                                                  |
| Belyaev et al.    | 915 MHz; GSM   | Whole body    | Male Fisher 344  | DNA double-strand breaks     | No effect                | Non-restrained animals; no positive control                    |
| (2006)            |                | average SAR   | rats; isolated   | and chromatin conformation   |                          |                                                                  |
|                   |                | of 0.4 W/kg;  | cells from spleen,|                                            |                          |                                                                  |
|                   |                | 2-h exposure  | thymus and brain |                                            |                          |                                                                  |

PW pulsed wave
of 0.6–1.2 W/kg. Of particular interest was the observation that injection of melatonin or the spin-trap compound N-tert-butyl-α-phenylnitrone (PBN), either immediately before or after RFR exposure, could prevent the occurrence of RFR-induced DNA damage, indicating a role for free radicals in this effect. These studies generated a flurry of controversy, alarming media reports and research to confirm these findings and to identify a mechanism by which RFR exposure may have influenced DNA damage formation and/or repair.

In an attempt to confirm these observations, Malyapa et al. (1998) exposed rats to 2.45 GHz CW RFR for 2 h at a SAR of 1.2 W/kg, but found no evidence of immediate or delayed RFR-induced DNA damage in the rodent brain. However, the authors did note that the background level of DNA damage increased in brain cells from CO₂-asphyxiated animals relative to that in guillotined animals and that the time from CO₂ asphyxiation until dissection was an important parameter in the extent of background DNA damage. This is considered an important observation since the studies by Lai and Singh (1995, 1996, 1997) employed CO₂ asphyxiation and included numerous wash steps to remove red blood cells, resulting in an apparent large time-lag between CO₂ asphyxiation and processing of samples via the alkaline and neutral comet assays. As a result, there is considerable potential that the observations by Lai and Singh were not related to RFR exposure, but rather were the result of post-euthanasia confounding by the time-dependent generation of free radicals in the tissue extracts, despite the inclusion of PBN in the mincing buffer. On the other hand, unlike Lai and Singh, Malyapa et al. (1998) did not use proteinase K in their comet assay protocol. Since proteinase K digestion removes nuclear proteins, DNA from nuclear-protein associations and DNA–protein cross-links would be free to migrate and the studies by Lai and Singh may have detected DNA damage that Malyapa’s group could not observe. To address this possibility, LaGroye et al. (2004a) used both versions of the alkaline comet assay to evaluate the ability of in vivo 2.45 GHz pulsed-wave RFR exposure at a SAR of 1.2 W/kg to induce DNA damage in the rat brain at 4 h after an acute 2-h exposure. In this study, animals were killed one at a time by guillotine and no DNA damage was detected following RFR exposure, indicating that the comet assay methodology did not appear to be a significant factor in explaining the conflicting results between these laboratories.

More recently, Paulraj and Behari (2006) reported increased DNA damage in brain cells of Wistar rats following exposure to 2.45 or 16.5 GHz RFR for 35 days at 2 h/day at SARs of approximately 1.0 and 2.0 W/kg, respectively. In contrast, Belyaev et al. (2006) found no evidence of DNA double-strand breaks or changes in chromatin conformation in rat brain following a 2-h acute exposure to 915 MHz GSM-modulated RFR at 0.4 W/kg. Similarly, Aitken et al. (2005) observed no evidence of single-strand or double-strand DNA breaks in spermatofzoa of mice exposed to 900 MHz RFR for 12 h/day for 7 days; however, Aitken et al. (2005) noted altered PCR amplification of a
9-kb fragment of the β-globin gene and a 10-kb fragment of the mitochondrial genome in RFR-exposed mouse spermatozoa, relative to control animals. The authors argued that these changes were indirect evidence of RFR-induced DNA lesions, which may have been undetectable using the conventional genotoxicity assays. Obviously, additional in vivo studies are required to delineate the inconsistencies in the literature.

### 28.5 Epigenetic Effects of RFR

Despite the apparent inability of non-thermal RFR to induce direct (genotoxic) DNA and/or cytogenetic damage, it is possible that RFR may interact with biological systems in an indirect (non-genotoxic or epigenetic) fashion to modulate the response of cells or tissues to known genotoxins. Proposed mechanisms for RFR-induced epigenetic effects include changes in the fidelity and/or efficiency of DNA repair processes, changes in cell-cycle progression and/or cell proliferation, changes in the expression of certain genes (such as heat-shock proteins and proto-oncogenes) and changes in cell survival. The evidence for these RFR-induced effects is contradictory and outside the focus of this review. This section will focus solely on the cytogenetic evidence of RFR-induced epigenetic effects.

Relatively few studies have examined the consequence of combined effects of exposure to RFR and known physical and/or chemical mutagens on cytogenetic alterations. Early studies by Balcer-Kubiczek and Harrison (1985, 1989, 1991) reported that 2.45 GHz RFR exposure at SARs of 0.1–4.4 W/kg for 24 h, could increase the rate of neoplastic transformation of C3H 10T1/2 cells when coexposed to either benzo[a]pyrene or X-rays in the presence of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Subsequent studies by Cain et al. (1997) and Roti Roti et al. (2001) failed to observe similar effects, although these studies employed lower SARs and different RFR frequencies. More recently, Wang et al. (2005a) exposed C3H 10T1/2 cells to 2.45 GHz RFR at SARs of 5–200 W/kg for 2 h, either with RFR alone or in the presence of RFR and 3-methylcholanthrene. No evidence of RFR-induced neoplastic transformation was observed at any SAR level when cells were exposed to RFR in the presence or absence of TPA; however, increased transformation was observed in cells co-exposed to 3-methylcholanthrene and RFR at SAR levels greater than 100 W/kg. While the temperature in the 100–200 W/kg groups reached 40 and 44°C respectively, the authors argued that the RFR-induced increase in neoplastic transformation was not due to thermal effects since control samples were exposed to similar temperatures but no increase in neoplastic transformation was observed in these samples. It is important to point out that while the volume-averaged temperature was reported not to have exceeded 40–44°C, the presence of hot spots within the sample cannot be ruled out since insufficient information on SAR uniformity was reported.
Initial studies by Maes et al. (1996, 1997) reported a strong synergistic increase in the frequency of SCE in human whole-blood cultures following exposure to 900- or 954 MHz RFR at SARs of 0.3–1.5 W/kg and mitomycin C. Similar epigenetic effects were reported by Zhang et al. (2002) and Wang et al. (2005b), where increased levels of MN and primary DNA damage were observed in human whole-blood cultures following co-exposure to RFR and a variety of mutagenic compounds. Unfortunately, these studies suffer from a number of serious deficiencies, including inadequate sample size, inappropriate statistics and insufficient detail relating to the exposure system design, dosimetry and SAR distribution to exclude the possibility of thermal effects. On the other hand, an increased frequency of MN was also reported in CHO-K1 cells following coexposure to bleomycin and 2.45 GHz RFR at high SARs (78–100 W/kg), in a well-controlled study by Koyama et al. (2003). It is interesting to note from this study that high RFR SAR exposures seem to induce non-thermal epigenetic cytogenetic effects; however, the relevance of these findings to health risk assessment is questionable owing to the magnitude of these exposures.

In contrast, several recent studies where dosimetry was well defined and possible thermal influences were eliminated failed to detect evidence of RFR-induced epigenetic effects on cytogenetic end points. In a follow-up study to their original work, Maes et al. (2001) exposed human blood cultures to mitomycin C or X-rays and 900 MHz RFR with a variety of modulation paradigms for 2–24 h at SARs of 0.4–10 W/kg, but failed to find any evidence of excess chromosome aberrations or SCE in RFR-exposed samples. Similarly, LaGroye et al. (2004b) reported no evidence of increased primary DNA damage (as measured by the alkaline comet assay) in C3H 10T1/2 cells co-exposed to bleomycin and 2.45 GHz RFR at a SAR of 1.9 W/kg and either 137Cs γ-rays or cisplatin. More recently, Stronati et al. (2006) assessed the ability of 935 MHz GSM-modulated RFR exposure at a SAR of 1–2 W/kg for 24 h to alter the magnitude of the cytogenetic and primary DNA damage induced by 1 Gy X-irradiation in human blood cultures. No evidence of increased unstable chromosome aberrations, SCE, MN or primary DNA damage was observed in the RFR plus X-ray co-exposed groups, relative to the X-ray only controls.

Recently, Verschaeye et al. (2006) were the first group to evaluate the ability of in vivo RFR exposure to modulate the cytogenetic effects induced by a known mutagen, namely, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). In this study, female Wistar rats were supplied with drinking water containing 19 µg/ml MX and were either sham or exposed to 900 MHz RFR for 2 years at 2 h/day, 5 days/week at SARs of 0.3 and 0.9 W/kg. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were acquired at the end of the study. No evidence of either increased RFR-induced primary DNA damage or MN formation was observed when compared with MX-only controls. However, it is important to point out that MX treatment also had no significant effect on primary DNA damage levels in the blood or liver of exposed animals relative to controls.
and was only found to increase DNA damage in the rat brain samples at 24 months. Similarly, there was no evidence of an increased frequency of MN in PCEs in the MX-treated rats at any time-point relative to control animals, suggesting that the dose of MX applied to the drinking water may not have been optimal for evaluation of the epigenetic effects of RFR in vivo. Thus, while this study is novel in design and scope, the importance of this study is limited for the evaluation of the human health risks of RFR exposure.

28.6 Cancer Studies in Animals

Although the analysis of human populations has been a powerful tool for detecting previously unidentified human carcinogens, current epidemiological studies of RFR have been hindered by the relatively recent introduction (within the last 10 years) of a wide variety of RFR-emitting devices and the long latency phase (more than 20 years) for the development of most human cancers. Furthermore, since new wireless technologies and applications are emerging at a tremendous rate, it is difficult for any epidemiology study to capture the effect of long-term human exposure to specific RFR conditions. As such, cancer studies in experimental animals are particularly important in evaluating whether an association between RFR exposure and cancer exists as laboratory animal studies provide an integrated system in which RFR experimental variables can be controlled, specific hypotheses can be investigated and exposures can be precisely measured. In this section, the evidence for a causal association between long-term, low-level RFR exposure and cancer in rodents will be reviewed (Tables 28.6–28.8).

Prior to 1997, only a limited number of studies assessed the effect of long-term, low-level RFR exposure in animals and most of these studies suffered from inadequate dosimetry, confinement or thermal stress and high rates of inherent disease in the animals (Moulder et al. 2005); however, two studies merit consideration. Chou et al. (1992) reported that following 25 months’ exposure of Sprague-Dawley rats for 21.5 h/day to 2.45 GHz pulse-modulated RFR at a whole body averaged SAR of 0.15–0.4 W/kg, a significant increase in overall malignant tumor incidence was observed in the RFR-exposed rats when compared with the sham group. However, the authors cautioned that the biological significance of these results was limited as there were no accompanying differences in lifespan, total number of tumors or type of tumor. Wu et al. (1994) exposed Balb/c mice to 2.45 GHz RFR for 3 h/day, 6 days/week for 5 months at SARs of 10–12 W/kg in the presence of the known colon cancer inducing compound dimethylhydrazine. The authors did not find any evidence that RFR exposure had an effect on the incidence or size of chemically induced tumors, or on the incidence of protuberant or infiltrative tumors in tumor-bearing mice when compared with control groups.
Table 28.6 Summary of cancer studies in animals that have investigated the effects of radiofrequency radiation – 1992–2000

| Authors          | RFR conditions         | SAR; duration           | Animal model        | End point                        | Results                                           | Comments                                                                 |
|------------------|------------------------|-------------------------|---------------------|----------------------------------|--------------------------------------------------|-------------------------------------------------------------------------|
| Chou et al.      | 2.45 GHz; pulse-modulated | Whole body average SAR between 0.15 and 0.4 W/kg; 21.5 h/day for 25 months | Male Sprague-Dawley rats; 100 rats per group | Tumor incidence in all major organs | Increased incidence of primary malignant tumors in RFR-exposed rats | No differences in lifespan, total number of tumors or tumor type; unrestrained animals; all animals were histopathologically assessed; no positive control |
| Wu et al.        | 2.45 GHz; CW           | SARs of 10–12 W/kg; 3 h/day, 6 days/week for 5 months | Dimethylhydrazine-induced colon cancer in Balb/c mice; 26–32 mice per group | Incidence of colon cancer | No effect | Animal movement partially restricted during exposure; all animals were histopathologically assessed; low number of animals per group; no positive control |
| Repacholi et al. | 900 MHz; pulse-modulated | Average SARs ranged from 0.13 to 1.4 W/kg; 2×30 min/day for 18 months | Ejμ-Pim1 (lymphoma-prone) mice; 100 mice per group | Incidence of lymphoma; tumor incidence in all major organs | Increased incidence of lymphoma in RFR-exposed mice | Unrestrained animals; not all animals were histopathologically assessed; non-homogeneous SAR between and within animals; no positive control |

(Continued)
| Authors          | RFR conditions       | SAR; duration | Animal model                        | End point                          | Results       | Comments                                                                 |
|------------------|----------------------|---------------|-------------------------------------|------------------------------------|---------------|---------------------------------------------------------------------------|
| Toler et al.     | 435 MHz; pulse-modulated | Whole body average SAR of 0.32 W/kg; 22 h/day, 7 days/week for 21 months | Female C3H/HeJ (mammary tumor prone) mice; 200 mice per group | Tumor incidence in mammary glands and all major organs | No effect | Unrestrained animals; all animals were histopathologically assessed; no positive control |
| Frei et al.      | 2.45 GHz; CW         | Whole body average SAR of 0.3 and 1.0 W/kg; 20 h/day, 7 days/week for 18 months | Female C3H/HeJ (mammary tumor prone) mice; 100 mice per group | Tumor incidence in mammary glands and all major organs | No effect | Unrestrained animals; all animals were histopathologically assessed; no positive control |
| Adey et al.      | 836 MHz; CW or pulse-modulated | Brain-average SAR of 1.0–1.6 W/kg; in utero and postweaning exposure for 2 years | ENU-treated and untreated Fischer 344 rats; 60–90 rats per group | Incidence of primary CNS tumors | No effect | Restrained animals; histopathological assessment performed on CNS tissue from all animals |

CNS central nervous system, ENU ethylnitrosourea
### Table 28.7  Summary of cancer studies in animals that have investigated the effects of radiofrequency radiation – 2001–2002

| Authors             | RFR conditions | SAR; duration | Animal model | End point | Results | Comments |
|---------------------|----------------|---------------|--------------|-----------|---------|----------|
| Zook and Simmens    | 860 MHz;       | Brain average | ENU-treated  | Incidence | No effect| Restrained animals; all animals were histopathologically assessed |
| (2001, 2006)        | CW and pulse-  | SAR of 1.0 W/kg; 6 h/day, 5 days/week for 2 years | and untreated Sprague-Dawley rats; 60 rats per group | tumors in CNS and in all major organs |
|                     | modulated      |               |              |           |         |          |
| Heikkinen et al.    | 902 MHz;       | Whole body    | Female CBA/S mice initiated with ionizing radiation; 50 mice per group | Incidence of neoplastic lesions in all major organs |
| (2001)              | CW or GSM-     | average SARs of 0.35 and 1.5 W/kg; 1.5 h/day, 5 days/week for 78 weeks |           |           |         | Restrained animals; all animals were histopathologically assessed |
|                     | modulated      |               |              |           |         |          |
| Imaida et al.       | 1.5 GHz;       | Whole body    | DMBA-induced skin tumors in female ICR mice; 30–48 mice per group | Incidence of tumors on skin and in several major organs |
| (2001)              | pulse-modulated| average SAR of 0.08 W/kg; 90 min/day, 5 days/week for 19 weeks |           |           |         | Restrained animals; all animals were histopathologically assessed |
| Utteridge et al.    | 898.4 MHz;     | SARs of 0.25, 1.0, 2.0 and 4.0 W/kg; 1 h/day, 5 days/week for 104 weeks | Eμ- Pim1 (lymphoma-prone) mice; 120 mice per group | Incidence of lymphoma; tumor incidence in all major organs |
| (2002)              | GSM-modulated  |               |              |           |         | Restrained animals; all animals were histopathologically assessed; ENU-positive control included |
|                     |                |               |              |           |         |          |
| Bartsch et al.      | 900 MHz;       | Whole body    | DMBA-induced mammary tumors in female Sprague-Dawley rats; 60 rats per group | Incidence of mammary tumors |
| (2002)              | GSM-modulated  | average SAR ranging from 0.017 to 0.07 W/kg; continuous exposure for 1 year |           |           |         | Unrestrained animals; histopathological assessment only performed when mammary tumors became 1–2 cm in diameter |

DMBA 7,12-dimethylbenz[a]anthracene
| Authors          | RFR conditions   | SAR; duration               | Animal model       | End point                  | Results   | Comments                                                                 |
|------------------|------------------|-----------------------------|--------------------|---------------------------|-----------|---------------------------------------------------------------------------|
| Anane et al.     | 900 MHz; GSM-modulated | Whole body average SARs of 0.1–3.5 W/kg; 2 h/day, 5 days/week for 9 weeks | DMBA-induced mammary tumors in female Sprague-Dawley rats; 16 rats per group | Incidence of mammary tumors | Equivocal results | Animal movement partially restricted during exposure; histopathological assessment only performed on mammary glands with visible (macroscopic) tumors; insufficient number of animals per group; inconsistency across replicate experiments |
| La Regina et al. | 835 MHz FDMA- and 847 MHz CDMA-modulated | Brain-average SAR of 1.3 W/kg; 4 h/day, 5 days/week for 2 years | Fischer 344 rats; 80 rats per group | Incidence of tumors in all major organs | No effect | Restrained animals; no positive control; all animals were histopathologically assessed |
| Heikkinen et al. | 849 or 902 MHz; pulse-modulated | Whole body average SAR of 0.5 W/kg; 1.5 h/day, 5 days/week for 52 weeks | Female transgenic and normal K2 mice coexposed to UV radiation; 8–27 mice per group | Incidence of skin tumors | No effect | Restrained animals; only macroscopic skin lesions were histopathologically assessed; low animal numbers (8–27 per group) |
| Sommer et al.    | 900 MHz; GSM-modulated | Whole body average SAR of 0.4 W/kg; continuous exposure for 46 weeks | Female AKR/J (lymphoma-prone) mice; 160 mice per group | Incidence of lymphoma | No effect | Unrestrained animals; all animals were histopathologically assessed; no positive control |
| Author(s)          | Frequency | SAR Type   | Methodology                                                                                     | Incidence                                                                 |
|-------------------|-----------|------------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Anderson et al.   | 1.6 GHz;  | Iridium signal | Brain-average SAR ranging from 0.16 to 1.6 W/kg; in utero and postweaning exposure (2 h/day) for 2 years | Fischer 344 rats; 90 rats per group; Incidence of tumors in CNS and all major organs; No effect; Restrained animals; all animals were histopathologically assessed; no positive control |
| Shirai et al.     | 1.6 GHz;  | Pulse-modulated | Brain-average SAR of 0.67 and 2.0 W/kg; 90 min/day, 5 days/week for 104 weeks                    | ENU-treated and untreated Fischer 344 rats; 100 rats per group; Incidence of tumors in CNS and all major organs; No effect; Restrained animals; all animals were histopathologically assessed |
| Huang et al.      | 849 MHz or 1.763 GHz; | CDMA-modulated | Whole body average SAR of 0.4 W/kg; 2×45 min/day, 5 days/week for 19 weeks                        | DMBA-induced skin tumors in male ICR mice; 20 mice per group; Incidence of skin tumors; No effect; Insufficient information on animal exposure conditions; low animal numbers (20 per group) |
| Yu et al.         | 900 MHz;  | GSM-modulated | Whole body average SARs of 0.44, 1.33 and 4.0 W/kg; 4 h/day, 5 days/week for 26 weeks            | DMBA-induced mammary tumors in female Sprague-Dawley rats; 100 rats per group; Incidence of mammary tumors; No effect; Restrained animals; histopathological assessment performed on mammary glands from all animals |

*UV* ultraviolet
Since 1997, a relatively large number of studies have assessed the effect of long-term, low-level RFR exposure on the induction or promotion of tumors in both normal and tumor-prone rodents. Repacholi et al. (1997) exposed unrestrained mice genetically predisposed to develop lymphomas (Eµ-Pim1) to 900 MHz pulse-modulated RFR for 2 × 30 min/day for up to 18 months at SARs ranging from 0.008–4.2 W/kg. In this highly publicized study, the authors reported that while lymphoblastic lymphoma was not significantly affected, an increased incidence of non-lymphoblastic lymphoma was observed in RFR-exposed mice when compared with sham controls. This study was criticized for its poor RFR exposure homogeneity (SAR range 0.008–4.2 W/kg) among the experimental animals and inadequate histopathological assessment (e.g., only ill or dead animals were examined pathologically), which may have inadvertently introduced bias into the analysis. In an effort to address these shortcomings, Utteridge et al. (2002) exposed restrained Eµ-Pim1 transgenic mice and wild-type mice to 898.4 MHz GSM-modulated RFR for 1 h/day, 5 days/week for 104 weeks at SARs of 0.25, 1.0, 2.0 and 4.0 W/kg. Under tightly controlled exposure conditions and following pathological examination of all animals, the authors reported that long-term RFR exposure had no significant effect on lymphoma induction in normal or lymphoma-prone mice when compared with sham controls. While this study was also criticized since it was not a direct replication of the study by Repacholi et al. (1997) and therefore could not discount the previous findings by this group, the findings from the current study deserve merit in their own regard. Recently, Sommer et al. (2004) continuously exposed unrestrained lymphoma-prone AKR/J mice to 900 MHz GSM-modulated RFR for 46 weeks at a whole body average SAR of 0.4 W/kg. The AKR/J mouse strain was chosen since it is genetically predisposed to develop thymic lymphoblastic lymphoma as its genome carries the AK virus. The authors found no evidence of decreased survival rates or increased incidence of lymphoma in RFR-exposed mice, relative to sham mice, at any SAR level tested. While these results are consistent with those of Utteridge et al. (2002), it must be noted that Repacholi et al. (1997) reported an increased incidence of non-lymphoblastic lymphoma which was not evaluated in this study.

Six recent studies have assessed the ability of long-term RFR exposure to influence the development of mammary tumors in rodent models. Frei et al. (1998a, b) exposed C3H/HeJ mammary tumor-prone mouse to 2.45 GHz CW RFR for 18 months at 20 h/day, 7 days/week at SARs of 0.3 and 1 W/kg. In both studies, there were no significant differences between RFR-exposed and sham mice with respect to tumor incidence, latency to tumor onset, growth rate of mammary tumors or animal longevity. In a related study by the same group, Toler et al. (1997) found no evidence that latency to tumor onset, growth rate of mammary tumors or animal longevity was affected by a 21-month exposure (22 h/day, 7 days/week) of C3H/HeJ mammary tumor-prone mice to 435 MHz pulse-modulated RFR at a SAR of 0.32 W/kg. In an attempt to investigate the possible tumor-promoting capability of RFR, several groups have used the well-characterized 7,12-dimethylbenz[a]anthracene (DMBA) induced
mammary tumor model to study this issue in female Sprague-Dawley rats. Bartsch et al. (2002) reported the results of three independent experiments, initiated in three consecutive years, where unrestrained rats were continuously exposed to 900 MHz GSM-modulated RFR at whole body averaged SARs ranging from 0.017 to 0.07 W/kg. Histopathological evaluation of all tumors was performed as the tumors reached 1–2 cm in diameter during the course of the study and the milk line was verified for the presence of any additional tumors in all animals. The authors found a significantly delayed malignant tumor median latency in RFR-exposed rats in their first experiment that was absent in their other two studies. However, as a whole, the authors concluded that RFR exposure had no effect on either DMBA-induced mammary tumor latency or the cumulative tumor incidence since this prolonged latency to tumor onset was not replicated in their subsequent studies. In a similar study, Anane et al. (2003) performed two independent studies where female Sprague-Dawley rats with DMBA-induced mammary tumors were exposed to 900 MHz GSM-modulated RFR for 2 h/day, 5 days/week for 9 weeks at SARs of 0.1–3.5 W/kg. A total of 16 animals were included in each exposure group. All macroscopic mammary tumors were histopathologically evaluated; however, mammary glands without visible tumors were not evaluated and microscopic tumors may have been missed. The authors reported no differences in the latency to tumor onset between the sham and RFR-exposed groups in each study, nor between either of the two studies. A significant increase in tumor incidence was observed in rats exposed to RFR at SARs of 1.4 and 2.2 W/kg in the first experiment, but the opposite effect was observed in their second experiment, where a decreased tumor incidence was reported in rats exposed to RFR at a SAR of 1.4 W/kg. Furthermore, tumor multiplicity on rats bearing tumors was inconsistent in the 1.4-W/kg RFR-exposed groups between the two studies. In the second experiment, the tumor-bearing rats in the 1.4-W/kg RFR-exposed group had fewer malignant tumors than tumor-bearing rats in the sham group, whereas no such difference was observed in the first study. The inconsistency in the results, which may be partially attributable to low animal numbers per group, led the authors to conclude that this study added no new evidence as to the co-promoting effect of RFR. More recently, Yu et al. (2006) also investigated the effects of 900 MHz GSM-modulated RFR exposure on mammary tumor promotion in female rats with DMBA-induced mammary tumors. The authors exposed rats (100 per group) to cage conditions or to RFR for 4 h/day, 5 days/week for 26 weeks at SARs of 0, 0.44, 1.35 and 4.0 W/kg. All mammary glands were palpated weekly and analyzed histopathologically. The authors reported that no statistically significant differences were observed between RFR-exposed and sham rats for latency to tumor onset, mammary tumor incidence, tumor multiplicity or tumor size on tumor-bearing rats. However, the investigators did observe a statistically significant increase in body weight and tumor incidence in the cage control group and the latency to tumor onset was also significantly shorter in these animals compared with that in the sham and RFR-exposed groups. The authors mentioned that these
differences may have been due to daily food deprivation of the sham and RFR-
exposed groups, which may have led to reduced tumorigenesis in these groups
relative to the cage controls. In summary, when these studies using mammary
tumor prone mice and chemically induced mammary tumors are assessed
collectively, there is no compelling evidence of altered mammary tumor
incidence or development by RFR exposure.

The possible association between RFR exposure and brain tumor risk has
been of great public and scientific concern for the past decade, owing to the
large and ever-increasing number of mobile-phone users worldwide who are
exposed to RFR in close proximity to the head. Since 2000, several animal
cancer studies have investigated whether RFR exposure may influence brain
tumor initiation and/or promotion. Adey et al. (2000) exposed ethyni-
trosourea (ENU) treated and normal Fischer 344 rats continuously to 836 MHz
CW RF fields in utero on gestational day 18 for 3–4 days until parturition,
then at 10 days after weaning the pups were exposed intermittently to RFR for
the remainder of their lifespan (approximately 2 years). The brain-averaged
SARs were estimated to range from 1 to 1.2 W/kg. The authors found no
evidence that RFR exposure had any effect on either spontaneous or ENU-
induced brain tumor incidence. In an earlier study by the same group (Ady
et al. 1999), the authors employed a similar experimental design but the rats
were exposed to 836 MHz pulse-modulated RFR at brain-averaged SARs of
1.0–1.6 W/kg. In their earlier study, the authors also reported no evidence
of any tumorigenic effects from RFR exposure, but did note that RFR exposure
caused a trend towards a reduced incidence in ENU-induced brain tumors.
More recently, Shirai et al. (2005) exposed normal and ENU-treated Fischer 344
rats to 1.439 GHz pulse-modulated RFR at brain-averaged SARs of 0.67 and
2 W/kg. RFR exposures commenced at 5 weeks of age for 90 min/day, 5 days/week
for 104 weeks. The authors reported that there were no differences in survival
rates, incidences or numbers of brain and/or spinal cord tumors or tumor types
in ENU-treated rats among the RFR-exposed groups relative to sham animals.
Anderson et al. (2004) conducted a similar study, where pregnant Fischer 344
rats were exposed to a 1.6 GHz Iridium signal. Similar to the previous studies,
rats were exposed in utero on gestational day 19 until weaning (about 23
days) for 2 h/day, 7 days/week, then the offspring were exposed for 2 h/day
for 5 days/week under near-field conditions to 1.6 GHz RFR at brain-averaged
SARs ranging from 0.16–1.6 W/kg for 2 years. No statistically significant
differences in the incidence of cancer in RFR-treated normal rats relative to
sham rats was observed in this 2-year study.

La Regina et al. (2003) exposed normal adult Fischer 344 rats to either 835
or 847 MHz RFR for 4 h/day, 5 days/week for 2 years at a time-averaged brain
SAR of 1.3 W/kg. At the end of the study, all major organs were examined
grossly and histopathologically for the incidence of spontaneous tumor for-
mation. The authors found no evidence of altered tumor formation or tissue
hyperplasia in the brain or other organs of RFR-exposed rats, relative to sham
controls. Zook and Simmens (2001) also examined the effect of long-term
exposure to 860 MHz pulse-modulated or CW RFR on tumor initiation and promotion in the brains of rats during a 2-year bioassay. ENU-treated and untreated rats were exposed to RFR for 6 h/day, 5 days/week from 2 up to 24 months of age. The brain-averaged SAR was calculated to be approximately 1 W/kg. At the end of the exposure period, all but two rats (898 in total) were necropsied and all major tissues were studied histopathologically. The authors reported no statistically significant differences in tumor incidence, volume, multiplicity, histological type, malignancy or fatality due to brain tumors from RFR exposure. Furthermore, there was no evidence of RFR-induced neoplasia in any other tissue examined. However, animals exposed to 860 MHz pulse-modulated RFR in the highest-dosed ENU group tended to develop fatal brain tumors at a shortened latency compared with the sham group. Although the trend was not statistically significant, the authors further examined this possible RFR-promoting effect in a more recent study (Zook and Simmens 2006). In this study, a similar experimental design was employed, however an equal number of RFR-exposed and control animals were sacrificed at 30-day intervals between 171 and 325 days of exposure. As with their previous study, histopathological analysis was performed on all rats (1,080 in total). The authors again found no statistically significant differences in tumor incidence, volume, multiplicity, histological type, malignancy or fatality associated with any type of neurogenic tumor. Furthermore, Zook and Simmens (2006) found no evidence of RFR-induced shortening of brain tumor latency in this study. The authors concluded that their studies found no evidence that RFR could induce a tumor-promoting effect.

Heikkinen et al. (2001) examined the tumor-promoting ability of RFR on tumors initiated with ionizing radiation. The authors exposed 200 female CBA/S mice to 4-Gy ionizing radiation (given in weekly 1.33-Gy fractions) followed by 902 MHz GSM-modulated or CW RFR at SARs ranging from 0.35–1.5 W/kg for 1.5 h/day, 5 days per week for 78 weeks. No significant differences were reported in the incidence of any neoplastic lesions in mice exposed to RFR, relative to the sham control group. In a later study, Heikkinen et al. (2003) examined the ability of RFR to influence the promotion of ultraviolet (UV) light induced skin tumorigenesis in transgenic ornithine decarboxylase overexpressing mice and in normal mice. In this study, mice were exposed to UV radiation in the presence/absence of 849 or 902 MHz pulse-modulated RFR for 1.5 h/day, 5 days/week for 52 weeks. The authors observed no statistically significant changes due to RFR exposure in any of the parameters examined, although a trend of accelerated tumor development was seen in both RFR groups. However, the authors had low animal numbers in all of their groups, ranging from eight in their non-transgenic cage control group to only 27 in their non-transgenic GSM-modulated RFR group, thereby reducing the statistical power of the study to detect differences between groups. Imaida et al. (2001) investigated the co-promoting ability of 1.5 GHz pulse-modulated RFR (whole body average SAR 0.08 W/kg; peak skin SAR 2 W/kg) in ICR mice initiated with DMBA. The animals were
exposed to RFR for 90 min/day, 5 days/week for 19 weeks. At 20 weeks, all animals were killed and skin tumors were analyzed histopathologically. No evidence of RFR-induced skin tumor co-promotion was observed. Similarly, Huang et al. (2005) assessed skin tumor co-promotion in ICR mice exposed to either 849 or 1,763 MHz CDMA RFR (whole body average SAR 0.4 W/kg) for 2 × 45 min/day, 5 days/week for 19 weeks following DMBA initiation and found no evidence of RFR-induced skin tumor promotion.

Overall, the results of animal cancer studies relating to RFR exposure are overwhelmingly negative, but it is important to note that these types of animal studies have inherent limitations. Rodent strains react differently to restraint stress, food deprivation, acclimatization as well as to environmental conditions in various testing laboratories. This phenomenon is readily observable by comparing the low reproducibility in the rate of tumor incidence by known carcinogens in these animal studies, even when conducted within the same laboratory. Therefore, the limited number of studies with positive effects should not be dismissed entirely without additional replication studies.

28.7 Summary

Over the past 30 years, hundreds of studies have been conducted in laboratories around the world to evaluate the ability of non-thermalizing RFR to induce DNA strand breaks, chromosome aberrations, MN, SCE and cancer in a variety of animal and human systems. The vast majority of in vitro, in vivo and human RFR investigations conducted to date have found no consistent evidence of RFR-induced cytogenetic, genotoxic or carcinogenic effects. Of the limited number of in vitro studies reporting RFR-induced cytogenetic effects, thermal effects appear to be a possible confounding variable. Relatively few studies have assessed cytogenetic end points in animal and human tissue following in vivo RFR exposure and obvious methodological and/or statistical shortcomings are evident in many of these studies. The results of animal cancer studies relating to RFR exposure have reported a nearly uniform lack of association between RFR exposure and cancer initiation and/or promotion. However, current epidemiological studies of RFR have been hindered by the relatively recent introduction (within the last 10 years) of a wide variety of RFR-emitting devices and the long latency phase (more than 20 years) for the occurrence of most humans cancers. Therefore, despite the extensive body of experimental studies conducted to date, the issue surrounding the safety of RFR will likely remain a significant public concern for years to come. In the absence of conclusive epidemiology, future well-characterized prospective human and animal RFR cytogenetics studies would be beneficial to permit a more complete RFR health risk assessment.
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