Radiofrequency Exposure and Mammalian Cell Toxicity, Genotoxicity, and Transformation

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The published in vitro literature relevant to the issue of the possible induction of toxicity, genotoxicity, and transformation of mammalian cells due to radiofrequency field (RF) exposure is examined. In some instances, information about related in vivo studies is presented. The review is from the perspective of technical merit and also biological consistency, especially with regard to those publications reporting a positive effect. The weight of evidence available indicates that, for a variety of frequencies and modulations with both short and long exposure times, at exposure levels that do not (or in some instances do) heat the biological sample such that there is a measurable increase in temperature, RF exposure does not induce (a) DNA strand breaks, (b) chromosome aberrations, (c) sister chromatid exchanges (SCEs), (d) DNA repair synthesis, (e) phenotypic mutation, or (f) transformation (cancer-like changes). While there is limited experimental evidence that RF exposure induces micronuclei formation, there is abundant evidence that it does not. There is some evidence that RF exposure does not induce DNA excision repair, suggesting the absence of base damage. There is also evidence that RF exposure does not inhibit excision repair after the induction of thymine dimers by UV exposure, as well as evidence that indicates that RF is not a co-carcinogen or a tumor promoter. The article is in part a tutorial, so that the reader can consider similarities and discrepancies between reports of RF-induced effects relative to one another. Bioelectromagnetics Supplement 6:S196–S213, 2003. © 2003 Wiley-Liss, Inc.

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INTRODUCTION

The advent of cellular telephones and other wireless technologies and their wide spread and ever-increasing use, has led to increasing public, governmental, and scientific attention to the issue of whether or not adverse effects result from exposure to radiofrequency electromagnetic fields. While there are many natural and man made sources of radiofrequency fields (RFs) in the environment [Stuchly, 1977; Meltz, 1991], established scientific evidence in support of an adverse human health effect due to RF exposure is largely nonexistent. However, the very fact that there are opportunities for so many human exposures is reason enough to give the matter attention, both in the short term and to at least some extent, into the future. In addition, concern about possible adverse effects due to long duration, low level exposures continues unabated.

Research in the field of radiofrequency biological effects is not new. It has been under way for more than 40 years, both in the United States and in other countries. Until relatively recently, most of the biological research had been funded by defense agencies of various governments, since radiofrequency signals are emitted by radar and communications equipment.

Attention was usually given to understanding safety issues, since there were a number of early reports in the peer reviewed literature that suggested a possible human health issue.

This review is not all-inclusive. It focuses on publications which have appeared in the past 15–20 years that involve mammalian (human and rodent) systems. This particular review also does not deal with the entire realm of biological effects. The author for many years has advocated that a biological effect reported in the literature may not be a health hazard to humans. A biological effect can be any chemical,
structural, metabolic, physiological, or morphological alteration detected in a biological molecule, cellular structural component or organelle, or living biological system, resulting from exposure to an agent. The effect would be demonstrated by comparing a response to an exposure against a sham exposure.

A molecular alteration may or may not result in a measurable biological alteration at the subcellular or cellular level, and subcellular changes may or may not lead to measurable alterations at the cell level. If such changes were to occur and be measurable, the changes may or may not extend themselves to directly adjoining tissue, or in either case result in effects on adjoining cells or cells in tissues at a distance. Assuming that there is a tissue response, it would remain to be determined whether there will be a physiological outcome that is out of the range of normal physiological variability in any one person, and therefore result in a physiological alteration of importance to health. Any substantiated biological effect could prove to have no physiological effect, could be harmful, or could be beneficial.

The focus of this review is on a number of in vitro studies in which the possibility of toxic, genotoxic, or transforming effects of radiofrequency (microwave) exposure have been examined. In a limited number of instances, in vivo laboratory investigations related to these in vitro studies will be mentioned. There is an important reason for beginning a review of the in vitro literature with these topics. If we are concerned with adverse human health effects, we must first be concerned with whether or not the exposure of concern is toxic, i.e., the agent is able to kill cells (of which the tissues in our bodies are comprised). If this were to happen, an alteration of function of tissue(s) could lead to clinically harmful physiological alterations in the body, and ill health. If enough cells in a tissue were killed, the outright failure of an organ to function could occur, and human death could result. As will be reported below, there is an absence of reports in the literature describing cell killing by RF exposures. The available evidence is that there is no cell toxicity after acute and/or chronic in vitro and in vivo RF exposures when measurable increases in temperature do not occur (a low level exposure and therefore presumably an “athermal” situation).

If cell killing is not an issue, then the next area of concern is genotoxicity due to RF exposure at low doses, where the cells with altered DNA would survive. Genotoxicity is often associated with cell death; however, it is also the basis for causing inherited mutations, if the DNA of the oocytes or sperm of the exposed person is altered/mutated. Evidence of genotoxicity would also indicate that the RF was a potential “initiator,” i.e., capable of being an “initiating” event leading to cancer related changes in a cell; alternatively, it could be a secondary or tertiary event, e.g., a subsequent chromosomal alteration, needed for the full development of malignant cancer.

The third area examined is mammalian cell transformation. In this type of in vitro assay, treatment with an agent results in cancer-like changes in at least some of the cells exposed. A positive result after treatment with the agent (alone) would be suggestive that the agent was a complete carcinogen. The technique could be used to examine the hypothesis that the RF was not a complete carcinogen, but either a cocarcinogen or a promoter. This would assume that RF induction of cancer was explainable by an initiation-promotion-progression model for carcino genesis; there is no evidence in the literature that this is the case. In any event, a positive in vitro result would need to be supported by appropriate in vivo studies.

This review is by design more technically critical than previous reviews [e.g., Brusick et al., 1998; Verschaeve and Maes, 1998], in that methodological and biological aspects of individual studies that have frequently only been summarized as having results which are either positive or negative, will be discussed. A positive is not a positive unless the experimental methodologies and analysis were properly done; the literature is replete with studies which do not meet this test. The same assessment can be applied to studies reporting an absence of an affect.

List of Review Criteria

A list of features which were recommended for inclusion in any published article was published in a chapter entitled “Biological Effects versus Health Effects: An Investigation of the Genotoxicity of Microwave Radiation” [Meltz, 1995]. The list, quoting from that chapter, includes:

1. Was the biological organism identified?
2. Were the experimental methods reported in enough detail to allow the study to be reproduced in another laboratory, and also to allow an investigator competent in the field to determine if it was performed properly?
3. Was the assay performed in accord with accepted (standard) protocols?
4. Was the microwave exposure system described?
5. Were the physical parameters of the exposure reported, including frequency, mode (continuous wave [CW] or pulsed wave [PW]), power, power density, location in the near or far field?
6. Was the dose reported by describing the specific absorption rate?
7. Was the temperature measured continuously during the exposure (in contrast to measurements being made before and after the exposure), and was the temperature measurement technique described?

8. Was the temperature and the time at that temperature stated?

9. Were independent treatment flasks exposed as replicates (to the same condition)?

10. Was the experiment repeated?

11. Were appropriate positive and negative controls performed?

12. Was the data statistically analyzed, and was the analysis appropriate?

13. Did the authors accept their own statistical result, or go on to make comments about specific changes that were not statistically significant?

In addition to these “criteria,” some studies are noted as being totally inconsistent with what is known from in vitro and in vivo investigations of other toxic agents; some of these inconsistencies will also be addressed herein. It could be hypothesized that the effects of RF are inconsistent with what is known about all other toxic and genotoxic agents; one would be required to demonstrate a substantiated toxic or genotoxic effect of RF before this speculative hypothesis could be addressed.

It will become evident to the reader that the author has given more critical technical and biological attention to those articles reporting evidence of toxicity, genotoxicity, and transformation due to RF exposures than to those articles reporting an absence of such evidence. This reflects the presentation of the author at the U.S. Air Force Symposium at which an overview of the article was presented. It should be evident to almost everyone that it is the positive reports of RF effects that have regularly received media attention, it is the positive reports that have concerned elected officials and regulators at the local, national, and international levels, and it is the positive reports which have been or are being replicated in international studies. This is not to say that such a technically critical review of the negative reports is not needed; in view of the possible (upcoming) decision by IARC as to whether or not RF is a known, probable, or possible human carcinogen or a noncancerogen, such a review is very important. It is planned for a future article.

CELL VIABILITY (TOXICITY) AFTER IN VITRO OR IN VIVO RF EXPOSURES

If enough cells in a tissue are killed, the result could be tissue damage; and if enough tissue is damaged, the result could be severe dysfunction or organ death. It is therefore critical to know if exposure of cells to RFs can cause the death of the exposed cells.

Cells in the body exist in different proliferative states. They can be permanently nondividing and functional, such as (most) nerve and muscle cells. Alternatively, they can be the daughters of dividing cells which have differentiated and themselves are no longer able to divide; these cells now play a structural or functional role. The cells lining the villi of the intestine prior to sloughing off into the lumen are an example of this type of cell. In some cases, cells that are not typically dividing can be “called upon” after tissue damage (cell killing) to proliferate. Examples of this type of cell include endothelial cells lining the vasculature, cells of the liver, and cells in certain glands. Certain cells of the immune system also can be stimulated to proliferate, as part of their normal physiological function. Finally, the cells may be “stem cells.” These cells continually divide and are the precursor to other, more differentiated cells. Survival of these cells can be critical to both organ survival and recovery of an organ after damage. Stem cells are found in the bone marrow, at the base of the villi in the intestine, in the skin, and in the testes.

If cells never divide and are killed by an exposure, they will undergo interphase death. The same is the case for proliferating cells, which die before the first cell division after exposure. This death can be by apoptosis (programmed cell death), necrosis (death of a field of cells in the same area in a tissue), or functional death (the cells may be living, but no longer capable of performing their normal function). If a cell is in a state of proliferation when exposed to a toxic agent, it can die an interphase death, but it can also die a reproductive death: after one or more divisions, the daughter cells of the cell initially exposed will simply stop dividing. If cell division is essential to an organ, the organ can be temporarily or permanently damaged, leading to temporary dysfunction of the organ or organ death. If this were to happen, clinical symptoms would become evident.

In Vitro Studies

Viability assessed using the colony formation assay. The gold standard of measurement of cell death, prior to the realization of the importance of apoptosis, was the measurement of reproductive integrity using a colony formation assay. This approach usually does not work for normal human cells, but it can be used for studying the reproductive integrity after treatment of many continuous rodent and human cancer cell lines. In these studies, a known number of untreated (control) cells is seeded into dishes, flasks, or agar gels. After
incubating for 7–14 days, those cells that attach to the substrate can form identifiable colonies. These are counted, and the ratio of colonies to cells seeded gives the control “plating efficiency.” Usually, the number of days of incubation is sufficient to allow untreated cells to result in colonies of 50 or 100 cells. After any single treatment with a possible toxic agent, the plating efficiency of the treated cells is determined in a similar manner, and compared to the plating efficiency of the controls. The ratio gives a surviving fraction. The surviving fraction is measured for different exposure conditions. There has been some use of this viability assay in RF investigations.

Two studies appear to have been specifically designed to determine whether RFs can kill cells by a mechanism other than heating; these were by Livingston et al. [1979] and Sapareto et al. [1982]. In the Sapareto et al. [1982] study, for frequencies of 896 or 434–460 MHz, the authors found that the decreased clonal survival of two mammalian cell lines exposed in growth medium was attributable to the heating by the microwave fields, and not to an electromagnetic property of the RF. In the earlier Livingston et al. [1979] article, a comparison was made of the surviving fraction of Chinese hamster ovary cells after water bath heating vs. microwave (CW-2450 MHz) heating for increasing times at 44 °C. The RF exposure was said to be intermittent to allow maintenance of the medium temperature. No difference in the cell survival curves was observed. The exposure systems used in these studies were unique to the studies and therefore would allow concern as to the reported outcome.

Balcer-Kubiczek and Harrison [1985, 1989, 1991] also examined the ability of RF exposures to decrease plating efficiency (survival), measured using a cloning efficiency assay. In the first of three articles on RF induced mammalian cell transformation [Balcer-Kubiczek and Harrison, 1985], they reported that a 2.45 MHz RF exposure for 24 h at an SAR of 4.4 W/kg reduced the plating efficiency by 50%, suggesting that the cell viability was decreased by 50%. However, in their next article [Balcer-Kubiczek and Harrison, 1989], using the same C3H/10T1/2 cells exposed under the same conditions for the same time, they clearly stated that they could not reproduce the initial observation. They demonstrated an absence of RF toxicity. The same was the case in a third study, in which the signal was pulse modulated at 120 Hz [Balcer-Kubiczek and Harrison, 1991].

In a series of articles by Garaj-Vrhovac et al. [1990, 1991, 1992], that are frequently quoted as reporting RF induction of chromosome aberrations or micronuclei formation, evidence was presented in one of the articles [Garaj-Vrhovac et al., 1991] that RF exposure at several different power densities with increasing exposure times caused reproductive death, as measured by colony formation assay. The studies as described, however, appear to be seriously flawed (see below). The effects reported could be due to incubating the cells over time at different temperatures. Unfortunately, the authors themselves pointed out in one of the articles [Garaj-Vrhovac et al., 1991, p. 148] that they did not know the temperature in the membrane in which the cells were located during the exposure. The situation appears to be the same for all three articles Garaj-Vrhovac et al. [1990, 1991, 1992].

Viability assessed by looking for an indication of apoptosis. Takahashi et al. [2002] explored whether or not RF causes apoptosis or programmed cell death. These authors looked for apoptosis in glial cells of “Big Blue Mice,” which had been exposed in vivo in the near field to a 1.5 GHz TDMA (mobile telephone Time Division Multiple Access pulsed signal) signal. The brains were exposed at the reported SARs of 0.67 or 2.0 W/kg for 90 min/day, 5 days/week, for 2 or 4 weeks. Using a commercial terminal end labeling assay, the authors reported that they found no evidence of apoptosis. In addition to this direct evidence, there is “circumstantial” evidence from the studies (described below) of the possible induction of DNA strand breaks after RF exposure. If apoptotic cells were present in the gels used for the comet assay, extensive DNA fragmentation would be immediately obvious when the gels were examined for distance of DNA migration. Not one of the authors mentioned observation of apoptosis.

Viability assessed using the dye exclusion assay and other methods. Several “vital” dyes, such as trypan blue, are commonly used to measure cell viability. If the cells are alive and their membranes are intact, the viable cells, treated and control, will exclude the dye whenever the assay is performed at different times after treatment. Use of such dyes immediately or a short time after acute RF exposure is likely not to reveal dead cells, for the simple reason that they have not had time to die. This assay is a “snapshot assay”; it cannot provide information as to what will happen to the viability of the treated cells at any time after the assay is performed. Cells that are not dead at the time of the assay could die shortly thereafter (or later), and the dye exclusion result would therefore provide an underestimate of cell killing.

The available data about RF killing of cells using the technique of vital dye exclusion is limited. Cleary et al. [1996] exposed CTLL-2 cytolytic T lymphocytes to a 2450 MHz signal for 2 h, at SARs ranging up to 50 W/kg. The temperature was said to be maintained at
37 °C. They reported (p. 915) that “RF exposure had no effect on CTLL–2 morphology or survival regardless of SAR.” The authors did not provide experimental data in support of this conclusion. Tice et al. [2002] exposed human blood leukocytes and lymphocytes to 837 MHz (analog), 837 MHz TDMA, 837 MHz CDMA (Code Division Multiple Access), and 1909.8 MHz PCS (Personal Communications Services) signals for 3 or 24 h, at average SARs of 1–10 W/kg. The temperature was reported to be 37 ± 1 °C. They reported that there was no evidence of toxicity for any of the RF exposure conditions examined. The authors did not provide experimental data in support of this conclusion.

In summary, the available in vitro studies of the effect of RF on cell viability do not indicate, where no heating (hyperthermia) is involved, evidence of cell killing by the RFs. The absence of in vitro evidence of cell toxicity after RF exposure is of relevance to in vivo effects, because any effects that were to be observed would not likely be associated with cell killing.

In Vivo Correlates of the Absence of RF Induced Cell Death

Evidence of clinical (functional) impairment. One of the most extensive studies of the effects of RF on physiological function is the chronic lifetime rodent study of Chou et al. [1992]. While the public focus about this study is on its hypothesis generating suggestion that RFs can induce tumors, its examination of 155 clinical indices over the lifetime of the exposed animals is usually overlooked. The clinical parameters monitored included, but were not limited to, serum corticosterone levels, immunological activity, hematological profile, blood chemistry, thyroxin levels, urinalysis, metabolic activity, total body analysis, organ mass, and histopathology (all tissues and organs).

In the study, Chou et al. [1992] exposed Sprague–Dawley rats, beginning at 8 weeks of age, in circular waveguides to a 2450 MHz, pulsed wave signal, square wave modulated at 8 Hz. The SAR was reported to be 0.4 W/kg, decreasing to 0.15 W/kg as the animals grew in size. The exposures were for 25 months, 21.55 h/day. The core temperature of the animals was not reported. With one exception in young animals, which disappeared over their lifetimes, there was no evidence of altered physiological function based on the 155 clinical parameters measured. The absence of such an alteration would imply that if cell killing did occur, over the period of these chronic lifetime exposures, the number of cells killed was not sufficient in each of the large number of organs associated with the 155 clinical indices examined to result in a measurable physiological alteration.

A chronic study of blood related endpoints was described by Toler et al. [1988]. The authors exposed male Sprague–Dawley rats to a 435 MHz RF signal at an estimated mean whole body SAR of 0.3–0.35 W/kg for 22 h/day, 7 days/week, for 6 months. The factors that they monitored in the blood included, but were not limited to, plasma ACTH, corticosterone, prolactin, catecholamines (misc.), hematological endpoints (misc.), and cardiovascular endpoints (misc.). They reported no differences in any of these parameters in the RF exposed animals compared to the sham exposed group.

Evidence of the absence of tissue necrosis. Another indicator of cell death after RF exposure in vivo would be the observation of tissue necrosis. An increasing number of studies have been undertaken to examine the possible induction of tumors in rodents upon RF exposures. The authors in each of the studies below listed a number of tissues that had undergone histopathological examination when the animals were terminated after extended exposures to a variety of frequencies and modulations. One of the first pieces of evidence indicating that RFs do not cause necrosis is the work of Chou et al. [1992] described above. After histopathological examination of all organs and tissues, no mention was made of any observation of RF induced tissue necrosis.

In the study by Repacholi et al. [1997], mice were exposed to a 900 MHz pulsed wave signal with a 0.6 ms pulse width, 216 Hz pulse repetition frequency. The SAR was reported to range from 0.008 to 4.2 W/kg; 0.13–1.4 W/kg (average). The animals were exposed for two 30 min periods per day, for up to 18 months. No temperature was reported. After histopathological examination of the thymus, lymph nodes (if enlarged), spleen, liver, lung, kidney, adrenal, large and small bowel, urogenital system, eyes, brain, and any tissue appearing abnormal at autopsy, no mention was made of any observation of RF induced tissue necrosis.

Toler et al. [1997] exposed female mammary cancer prone mice to a 435 MHz signal, pulse wave, with a 1.0 μs pulse width at a 1.0 kHz pulse repetition rate. The SAR reported was 0.32 W/kg. No temperature was reported. The animals were exposed 22 h/day, 7 days per week, for 21 months. After histopathological examination of the mandibular and mesenteric lymph nodes, salivary gland, femur (including bone marrow), thyroid, parathyroid, small intestine, large intestine, liver, gall bladder, ovaries, lungs and mainstream bronchi, nasal cavity, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, kidneys, adrenals, urinary bladder, pituitary, spinal cord and sciatic nerve, eyes, mammary gland, pharynx, skin, and spleen, no
mention was made of any observation of RF induced tissue necrosis.

Frei et al. [1998a] exposed mammary tumor prone C3H/HeJ mice to a continuous wave 2450 MHz RF signal. The reported SAR was 0.3 W/kg. The animals were exposed 20 h/day, 7 days per week, over 18 months. No temperature was reported. After histopathological examination of the brain, trachea, esophagus, thyroid gland, salivary gland, mandibular lymph node, pancreas, pituitary gland, thymus, adrenal glands, heart, stomach, jejunum, colon, liver, gall bladder, spleen, lung, skin, mammary gland, mesenteric lymph node, duodenum, ileum, caecum, rectum, kidney, urinary bladder, ovaries, uterus, nose, skeletal muscle, bone (femur), bone marrow (femur), and all gross lesions, no mention was made of any observation of RF induced tissue necrosis.

Frei et al. [1998b] performed a second completely independent study, similar in all aspects to the above except that the SAR of the 2450 MHz signal was increased to 1.0 W/kg. The exposure time was again extensive, 20 h/day, 7 days per week, over 18 months. After histopathological examination of all of the tissues mentioned above, there again was no mention made of any observation of RF induced tissue necrosis.

Adey et al. [1999] exposed Fischer 344 rats to an 836.55 MHz, NADC RF signal. The “slot” average SARs reported at the brain were 1.0–1.6 W/kg. The exposure times were up to 24 months, beginning prior to birth, 2 h/day, 4 consecutive days/week. No temperature was reported. The brain and spinal cord were examined histopathologically. No mention was made of any observation of tissue necrosis. In a second study, performed independently, Adey et al. [2000] exposed Fischer 344 rats to an 836.66 MHz signal, modulated by recorded speech. The SARs are not certain, since the Table 1 data for this exposure is exactly the same as the Table 1 SAR data in the 1999 article, unless the SARs were exactly the same. The brain and spinal cord were again examined histopathologically, and again no mention was made of any observation of RF induced tissue necrosis.

In the Takahashi et al. [2002] study mentioned above, after exposure of the mouse brains in vivo to a 1.5 GHz, TDMA signal for 4 weeks, the authors reported that no histopathological changes, in particular gliosis or degenerative lesions, were noted in the brain tissue.

In a study designed to improve upon the methodologies of the Repacholi et al. [1997] study, Utteridge et al. [2002] exposed transgenic Pim 1 mice to an 898.4 MHz, GSM modulated signal. The animals were exposed for 1 h/day, 5 days/week, for up to 104 weeks, at SARs of 0.25, 1.0, 2.0, and 4.0 W/kg. All animals in the study, in contrast to the Repacholi study, had tissues collected for complete pathological examination. The standard set of tissues examined included thymus, spleen, enlarged regional lymph nodes, bone marrow in sternum and ribs, brain, lung, heart, liver, kidney, small and large intestine, eyes, and any identifiable tumor mass or tissue abnormality. The authors did not report any observation of RF induced tissue necrosis.

The combined negative in vitro and chronic in vivo studies indicate that RFs with different frequencies and modulations and SARs resulting in exposures at what some have called nonthermal levels of RF exposure, is evidently nontoxic. This is an extremely important statement. It should be noted that many of the above in vivo studies did not provide information about core temperature. One could assume that the authors did not expect that the whole body average SARs which they employed would result in an increase in core temperature.

**GENOTOXICITY**

**Levels of Biological Complexity in the Investigation of Genotoxic Effects**

Studies in this area have been performed at a variety of levels of biological complexity. These include, but are not limited to:

A. damage to isolated DNA (exposed in solution),
B. damage to DNA after exposure of cells (in vitro or in vivo),
C. damage to chromosomes (aberrations, micronuclei formation (due to either chromosome damage or mitotic segregation effects)),
D. sister chromatid exchange (SCE) induction,
E. induction of DNA repair synthesis (indicative of base damage), and
F. induction of phenotypic mutations.

**Importance of DNA Damage**

The molecule in the cell that has received the most attention with respect to potential RF damage, because of its importance for cell function, cell proliferation, cell viability, mutation and cancer, is deoxyribonucleic acid (DNA). This is the molecule in which the genetic information of the cell and the entire biological organism, comprised of different cells, is maintained and conserved over time. Alterations in the genetic information in the reproductive cells of an organism, e.g., the sperm or ovum, can lead to inherited mutations in the next or subsequent generations of offspring. In a
developing embryo or fetus, DNA alterations, at least in some of the cells, can lead to death before birth, gross structural abnormalities, mental retardation or decreased IQ, or temporary or permanent growth retardation. DNA alterations in the cells of a child or adult could lead to cancer in that individual. Death of a large number of cells in one organ of an exposed individual, due to DNA damage, could lead to changes in the physiological function of that organ, affecting health and the physiological state of the person exposed.

Possible Types of DNA Damage

Different organisms have different amounts of DNA, found in different numbers of chromosomes. There are several alterations that can occur in the DNA molecule if it were to be "attacked" directly by an agent. These include local denaturation (separation of small regions of the two DNA strands), base damage (of the thymine, adenine, guanine, or cytosine bases), sugar damage (of the deoxyribose sugar), cross linking of the two strands, DNA–protein cross links, or DNA single (SSB) or double (DSB) strand breaks. The damage could be alkali labile, i.e., occurring as a result of initial DNA base damage by agent treatment of the cell, and appearing as DNA single strand breakage upon alkali treatment to denature the strands during the SSB assay procedure.

Direct Damage Due to Absorption of the RF by DNA in Solution

If the DNA is to be directly damaged, then there must be some evidence that DNA in solution can absorb RF energy directly. If this absorption was occurring in a different manner than the way that RF is absorbed in water, one would expect that the absorption might be frequency dependent. This was proposed theoretically by Prohofsky and his coworkers [Kohli et al., 1981] and Mei et al. [1981], among others. The theoretical idea was that there could be acoustic absorption modes and that these could depend on the size of the DNA molecule.

After some initial reports that frequency specific absorption in plasmids of DNA could occur [Swicord and Davis, 1982; Edwards et al., 1984, 1985], follow-up investigations revealed that this was not the case [Foster et al., 1987; Gabriel et al., 1987]. This is an important observation, since without evidence of a direct and unique absorption mechanism that could result in redistribution in some manner of the absorbed energy in the molecule, the alternative is that some type of direct bond breakage was occurring. This direct bond breakage would require an amount of energy per photon or wave equivalent to that found in ionizing radiation, such as X-rays or gamma rays. Since the frequency of ionizing radiation is some thousands to millions of times higher than the typical frequencies in the microwave range, such bond breakage is energetically impossible.

DNA Exposed in Solution: Experimental Results

Studies of DNA breakage due to RF exposure were performed in solution by Sagripanti and Swicord [1986]. The initial results reported were positive. Unfortunately, the use of copper electrodes immersed in the solution containing the DNA led to the possibility/probability of the generation of free radicals due to the presence of the copper in solution. The breakage was not due to the direct action of the RF [Sagripanti et al., 1987].

Indirect DNA Damage Hypothesis

In a living organism, the DNA molecules in the cell are “bathed” in the complex nucleoplasm of the nucleus. The DNA itself is highly compacted and in a complex local environment of water molecules (bound and/or unbound), histones (basic proteins), RNA molecules, and numerous other biomolecules involved in controlling the RNA transcription and DNA replication processes (including periodic attachments to the nuclear matrix). If direct action resulting in DNA damage does not occur, one could still postulate an indirect action. This would be based on the hypothesis that the exposure resulted in the generation of free radicals due to the ionization of water or other molecules. The latter would require bond breakage, and as mentioned above, this is not energetically possible.

Finally, one could hypothesize that the reactive species, if they were to be produced, would be the result of a biochemical reaction initiated in the cell as a result of the RF exposure. If this were occurring, genetic damage other than DNA SSBs would be readily evident. As will be described below, there is no established biological evidence that such genetic damage occurs. This still remains an intriguing hypothesis and needs to be further explored in connection with reported, although not established biological effects.

In addition to or as a result of the different types of DNA damages listed above, molecular mutations also could occur. These could be deletions of short sequences of DNA, base changes, or base deletions. These types of DNA alterations could lead to phenotypic mutations in those cells with the altered DNA that remain viable. As will be indicated below, there is also no evidence that RF causes such phenotypic mutations.
Recent Evidence That RF Does Not Induce DNA SSBs or DSB

The issue of DNA strand breaks due to RF was raised dramatically by the series of reports from the laboratory of Lai and Singh [1995, 1996, 1997]. The studies were performed using whole animal exposures in circular waveguides at a frequency of 2450 MHz. The first article reported only on the induction of SSBs in the cells of the brain of the exposed animals, with a reported whole body average SAR of 0.6 W/kg. The brain SARs, based on measurements in another article, were said to range from 0.5 to 2.0 W/kg. The total exposure time to either a 2450 MHz CW signal or to a 2450 MHz PW signal (2 μs pulse width, 500 pulses per s) was 2 h. In the first Lai and Singh [1995] article (and only in the first article), the brain tissue was removed from the animals in an effort to detect the DNA SSBs immediately after the 2 h RF exposure. In addition, the brains of both the CW and PW RF exposed animals were removed for the SSB assay after waiting an additional 4 h after the RF exposure ended.

The results reported were of great interest for three reasons. First, it was the first published report of the induction of SSBs by RFs. Second, immediately after the RF exposure, there was clear evidence of SSBs after the CW exposure, but not after the PW exposure. This was the first clear report of a difference between CW and PW exposures. Third, for the PW exposure, even though there was no evidence of SSBs immediately after the exposure, SSBs were present in a large proportion of the cells at 4 h after the exposure. It would have been of considerable interest if the difference in the effect between the CW and PW exposures immediately after the end of the 2 h RF exposure and 4 h post exposure had been further examined, especially with regard to the kinetics of the appearance (or lack thereof) of the breaks during the first 2 h of exposure and 4 h later. An examination of a dose response of the observations in the initial article would also be of great interest.

In Vitro Studies Showing the Absence of RF Induced DNA Strand Breaks

The studies listed below, which have been undertaken in different laboratories using different rodent and human cell types at different frequencies and modulations and with SARs ranging from 0.3 to 10 W/kg and exposure times from 2 to 24 h, did not reveal induction of DNA strand breaks by RF exposure.

The work of Phillips et al. [1998] in the Molt-4 human cell line, has often been mentioned as supporting the work of Lai and Singh [1995, 1996, 1997]. There is no consistency in the data, with more evidence of a decrease in migration of DNA in RF exposed cells compared to control cells than increases in the migration of the DNA in the RF exposed cells compared to control cells; in all instances, only small percentage increases in migration were reported. If the argument presented in the article by the authors was accepted, i.e., that the RF exposure might be stimulating DNA repair in the cells, the article to a large extent is actually a direct contradiction of the work of Lai and Singh [1995, 1996, 1997]. It is very possible that the contradictory results within the article itself are simply the result of experimental noise; there is no consistent pattern even within the data tables presented by Phillips et al. [1998].

Maes et al. [1997] exposed whole blood samples to a 935.2 MHz, GSM signal for 2 h, with the SARs reported to be 0.3–0.4 W/kg. The temperature in the medium was not reported. The technique used to detect DNA SSBs was the alkaline comet assay. The cells were assessed for DNA SSBs after the 2 h RF exposure. No SSBs were detected.

Vijayalaxmi et al. [2000] exposed human blood samples to a 2450 MHz, PW signal for 2 h, with a mean of 2.14 W/kg (range: 8.0 µW/kg). The temperature in the medium was 36.9 ± 0.3 °C. The technique used to detect the DNA SSBs was the alkaline comet assay. The cells were examined for DNA SSBs immediately after RF exposure or 4 h post exposure. No DNA strand breaks were detected. Tice et al. [2002] exposed diluted human blood to 837 MHz (analog), 837 MHz TDMA, 837 MHz CDMA, and 1008 MHz PCS signals for 3 or 24 h, at average SARs of 1–10 W/kg. The temperature was reported to be 37 ± 1 °C. They examined the leukocytes for DNA strand breaks using the single cell gel (SCG) assay. For all treatment conditions, they found no evidence of DNA strand breaks for any of the signals, at any SAR tested up to 10 W/kg, for either time point.

Malyapa et al. [1997a] exposed human U 87MG glioblastoma cells or mouse C3H 10T1/2 fibroblasts to a 2450 MHz signal, for 2, 4, and 24 h, at SARs of 0.7 and 1.9 W/kg. The temperature in the medium was reported to be 37 ± 0.3 °C. The technique used to detect DNA SSBs was the alkaline comet assay. The cells were assessed for DNA SSBs immediately after exposure for the above times. The cells were examined also at 4 h after the 2 h RF exposure. No DNA SSBs were detected at either time.

Malyapa et al. [1997b] exposed human U 87MG glioblastoma cells or mouse C3H 10T1/2 fibroblasts to 835.62 MHz, FMCW, and 847.74 MHz, CDMA signals for various times up to 24 h, at an SAR of 0.6 ± 0.3 W/kg. The temperature in the medium was reported to be 37 ± 0.3 °C. The technique used to detect DNA SSBs was the alkaline comet assay. The cells were...
examined for DNA SSBs after 2, 4, and 24 h of RF exposure. No DNA SSBs were detected at any of these times.

Li et al. [2001] exposed mouse C3H 10T1/2 fibroblasts, growing exponentially or in plateau phase, to 847.74 MHz, CDMA or 835.62 MHz, FDMA signals for 2, 4, and 24 h, at SARs of 3.2–5.1 W/kg. The temperature was reported to be 37.0 ± 0.3 °C. The technique used to detect the DNA SSBs was the alkaline comet assay. The cells were assessed for DNA SSBs immediately after exposure for the above times. The cells were examined also at 4 h after the 2 h RF exposure. No DNA SSBs were detected.

**DNA Exposed in Tissues in Animals**

Malyapa et al. [1998] attempted a near replication of the study by Lai and Singh [1995]. Male Sprague–Dawley rats were exposed to a 2450 MHz CW for 2 h at an SAR of 1.2 W/kg. No temperature information was provided, as was the case in the Lai and Singh articles [1995, 1996, 1997]. The technique used to detect the DNA SSBs was the alkaline comet assay. The cells were assessed for DNA SSBs immediately after the 2 h RF exposure and also at 4 h after the 2 h RF exposure. No DNA SSBs were detected.

The weight of the experimental scientific data does not support the observations of Lai and Singh [1995, 1996, 1997]. There is no reason to expect that the effect of a physical agent, if it directly damages the DNA, can only be seen in the brain exposed in vivo, and not in other cell types in vitro. If the agent were to indirectly damage the DNA through the induction of free radicals, there is no reason to explain why it does not happen during, but only after, the completion of a 2 h PW exposure, while it occurs during, but possibly not after, a CW exposure, since the same extent of damage appears to be present immediately and at 4 h after the CW exposure [Lai and Singh, 1995].

The overwhelming evidence demonstrates that RF does not induce DNA SSBs. If RF induced DNA DSBs, there would be evidence for SSBs using the alkaline assay, unless one suggested that every piece of broken double stranded DNA, from the smallest to the largest, was cross linked. This has not been demonstrated.

**Absence of Genetic Alterations After RF Exposure In Vitro or In Vivo**

**DNA repair.** Error-free repair of damaged DNA (and possibly repair allowing errors to remain) can occur in the cells of all persons, while some individuals with genetically inherited diseases have obvious defects in the DNA repair process. These diseases include, for example, Xeroderma pigmentosum and Ataxia telangiectasia. Individuals with these types of genetic disorders are often at increased risk for cancer induction by genotoxic agents, including sunlight exposure. In the normal situation, if error-free repair occurs and removes the DNA damage prior to DNA synthesis, a mutation would not be passed on to the daughter cells.

Different molecular events occur in different types of DNA repair. Among these are DNA repair synthesis, which can occur after base damage induced by a genotoxic agent, and DNA SSB and DSB rejoining. DNA repair synthesis is a slower process, taking as much as 20 h or longer depending on the amount of initial damage within the exposed cells. DNA SSB rejoining is very rapid, being close to complete within 2 h. DNA DSB rejoining takes longer [Foray et al., 1996] and may not always occur, leading to cell death. Other types of DNA repair include post replication repair and recombinational repair.

The only investigation of whether or not RF exposures can induce DNA repair synthesis was reported by Meltz et al. [1987]. This repair process, examined by measurement of repair replication in pre-existing DNA strands, would be expected to occur after damage to the bases in the DNA. By implication, the absence of the induction of repair synthesis would suggest that the RF exposure did not damage the bases in DNA.

Meltz et al. [1987] exposed MRC-5 normal human diploid fibroblasts to several different signals, including 350, 850, and 1200 MHz, continuous and pulsed wave (350 MHz, 5000 pps, 10 μs pulse width; 850 MHz, 5000 pps, 10 or 100 μs pulse width; 1.2 GHz, 80000 pps, 3 μs pulse width). The SARs ranged from 0.39 to 4.5 W/kg (5 and 10 mW/cm²) and depended on the frequency. They examined whether or not radio labeling due to repair synthesis of parental (pre-existing) DNA occurred during the 3 h RF exposure. The DNA repair studies were purposefully performed where the temperature of the medium was either at 37 or 39 °C. The higher temperature was used to test the hypothesis that it would “pre-stress” the cells. The results demonstrated an absence of induced DNA repair synthesis for all of exposure conditions examined. Therefore, there is no indication that repairable base damage occurred as a result of the RF exposures.

**Does RF exposure result in the inhibition of DNA repair synthesis?** Individuals with genetically inherited defects in the DNA repair process in their cells, e.g., Xeroderma pigmentosum, can inherently be at increased risk for sunlight induced skin cancer. It was therefore hypothesized that if RFs could interfere with the repair of DNA, by analogy this could increase the risk of an adverse effect. This possibility was tested by first exposing the cells acutely to UV-C radiation to
damage the DNA, and then monitoring the subsequent DNA repair synthesis. For this study [Meltz et al., 1987], the MRC-5 normal human diploid fibroblast cells were exposed to UVC (21 J/m²). Over the subsequent 1, 2, and 3 h of repair labeling, the cells were then exposed to either 350, 850, or 1200 MHz pulsed wave signals at SARs ranging from 0.39 to 4.5 W/kg (1, 5, and 10 mW/cm²). This DNA repair study was also performed at temperatures of 37 and 39 °C. Just as there was no induction of DNA repair synthesis by RFs, there was no evidence that the RF exposures at the different frequencies examined interfered with three of the enzymatic steps of the DNA repair synthesis process: recognition of the damage to the DNA, nicking of the DNA, or repair synthesis. A weakness of this study was that statistical analysis was not performed. The authors attempted to deal with this by replicating experiments where there was evidence of a change in the repair; in doing so they also replicated some of the exposure conditions indicating an absence of an effect.

SCE induction. The SCE assay is used as a sensitive indicator for the genotoxic action of some agents, such as UV light and some chemical mutagens. The exact mechanism for formation of SCEs is still unknown; it may have something to do with damage to the process of DNA replication, rather than to direct genotoxic action of the agent on chromosomes. In any event, SCE results in small pieces of the adjoining arms of chromosomes being exchanged between those arms. The occurrence of SCEs could potentially lead to adverse genetic consequences over the lifetime of the cell. It should be noted that SCE induction is not typically observed after ionizing radiation exposures, although such exposures are clearly genotoxic.

An extensive series of investigations involving RF exposures alone and RF exposures during simultaneous treatments with different genotoxic chemicals, were carried out by Ciaravino et al. [1987, 1991]. The procedures were similar to the assay procedures in use at that time in the Gene-Tox program of the U.S. EPA. The studies involved replicate exposure flasks and replicate experiments. The chemicals were selected because they were known to interact with the DNA of cells via different mechanisms.

Ciaravino et al. [1987] exposed CHO cells to a 2450 MHz pulsed wave signal at an SAR of 33.8 W/kg for 2 h. The temperature was observed to increase during the 2 h exposure from 37 to 39.2 °C. There was no evidence for the induction of SCEs by the RF exposure. As a further challenge, it was hypothesized that the RF exposure could alter the extent of SCEs that would be induced by a simultaneous exposure of the cells to the RF and the genotoxic chemical mitomycin C (MMC) at a concentration of 1 × 10⁻⁸ M. Because the RF exposure resulted in an increase in the temperature of the medium, simultaneous water bath and chemical treatment temperature controls were performed. There was no difference between the RF/chemical induction of SCEs and the chemical induction of SCEs, indicating that the RFs (as electromagnetic fields) did not alter the chemically induced induction of SCEs.

Ciaravino et al. [1991] repeated the study a second time, again with multiple replicate flasks per exposure condition and again with repeated experiments and with the same 2450 MHz pulsed wave signal with the SAR at 33.8 W/kg and temperatures increasing from 37 to 39.7 ± 0.2 °C during the 2 h exposure. The RF exposure again did not induce SCEs. In this series of studies, the genotoxic chemical Adriamycin, which interacts with DNA in a different manner than MMC, was used to look for a synergistic (or other) effect. The CHO cells were incubated with Adriamycin at two different concentrations, 7.75 × 10⁻⁷ or 1 × 10⁻⁶ M during the RF exposure. Again, there was no difference between the RF/chemical induction of SCEs and the water bath/chemical induction of SCEs.

Maes et al. [1993] subsequently explored the induction of SCEs by exposure of human blood lymphocytes to a 2450 MHz signal. The exposure time was 30 or 120 min; the SAR was reported as being 75 W/kg. The temperature was reported as being 36 °C, which is suspect considering the high SAR reported. While the result was negative for SCE induction, the methodology must be challenged. It appears from the description in the methods section that a metal syringe needle was immersed in the blood sample during the RF exposure. This could have led to artifacts and is not an acceptable procedure. The authors also indicated that the temperature uniformity in the sample during the exposure was “guaranteed”; this is simply not a scientifically acceptable statement.

In a series of studies, Maes et al. [1996, 1997, 2000, 2001] explored the possible interaction of RF at different frequencies and modulations and MMC. The authors did not perform a simultaneous exposure in these studies; the cells were first exposed to the RF signal and then subsequently treated with MMC. The SARs were lower than those reported by Ciaravino et al. [1987, 1991]; it is not clear that the SARs were uniform or properly determined. In the first study [Maes et al., 1996], human blood was exposed to a 954 MHz, GSM signal 5 cm from a base station antenna, with a calculated SAR of 1.5 W/kg. The exposure duration was for 2 h. Immediately after RF exposure, cells were stimulated to divide by addition of PHA in the pre-
sence or absence of MMC. The temperature was said to be 17 °C during the RF exposure. The authors reported that the RF exposure alone did not induce SCEs at this frequency. They did report that those cells that were exposed to RF and then treated with MMC showed an increase in SCEs compared to chemical treatment alone. In the second study [Maes et al., 1997], the cells were exposed to a 935.2 MHz signal at an SAR of 0.3–0.4 W/kg for 2 h. The temperature in the medium is not clear. This signal also did not induce SCEs. In the third study [Maes et al., 2000], the cells were exposed to a 455.7 MHz signal at an SAR of 6.5 W/kg for 2 h. The temperature was reported to be 17 ± 1 °C, but it is not clear that this was the measured sample temperature during the exposure. This signal did not induce SCEs. In the fourth study [Maes et al., 2001], the cells were exposed to a 900 MHz, GSM signal at SARs of 2 and 3.5 W/kg for 2 h. The temperature in the medium was not stated. This signal also did not induce SCEs.

After first reporting an interactive effect between the RF exposure and the subsequent MMC treatment [Maes et al., 1996], the interactive effect in the second article [Maes et al., 1997] was considered to be weak, the interactive effect was described as inconsistent in the third article [Maes et al., 2000], and then the interactive effect was reported to be absent in the fourth article [Maes et al., 2001].

In summary, the evidence for a broad range of different RF frequencies and modulations by different laboratories is that RF exposure does not induce SCEs. It also now appears that RF exposure does not alter the extent of chemically induced SCEs compared to temperature controls.

Does RF exposure induce gross chromosomal aberrations or increase chromosome aberrations caused by genotoxic chemicals? Many of the early studies reporting that RF exposures caused chromosome damage or aberrations are problematic in terms of the exposure system used, the temperature measurement or control, the lack of an adequate description of the methods used, and/or the analysis of the data. Many articles describing chromosome aberration induction and RF exposure can be found by the reader in both the IEEE and WHO databases.

One of the most thorough series of studies examining the question of whether RF exposure can cause chromosome aberrations was reported by Kerbacher et al. [1990]; protocols similar to those employed at the time in Gene-Tox program of the U.S. EPA were followed. Replicate treatment flasks were included in each experiment, and experiments were always repeated. CHO cells were exposed to a 2450 MHz pulsed wave signal at an SAR of 33.8 W/kg. The temperature increased from 37 to 40 °C during the 2 h RF exposure. A wide range of different types of chromosome aberrations was scored. No evidence was found that RF caused an increase in chromosome aberration frequency in the cells above that in simultaneous water bath temperature controls.

To further stress the cells in an effort to detect a potentially adverse RF effect, the cells were simultaneously treated with RF and the genotoxic anticancer agent Adriamycin or the genotoxic anticancer agent MMC in independent experiments. As expected, the chemical agents alone caused an increase in chromosome aberrations in the cells. When the RF exposures were performed during the chemical treatment, with the temperature in the medium increasing because of the high SAR, no change was observed relative to the similarly chemically treated and non-RF exposed, but temperature controlled (water bath heated) cells.

Vijayalaxmi et al. [1997a, 2001a,b] performed three independent studies examining the possible induction of chromosome aberrations in human peripheral lymphocytes due to RF exposure. In the first study, Vijayalaxmi et al. [1997a] exposed freshly isolated human blood to a 2450 MHz continuous wave signal at a mean SAR of 12.5 ± 0.1 W/kg for a total of 90 min. The exposure was intermittent, i.e., a repeated sequence of 30 min on, 30 min off. The temperature increased during each 30 min RF-on interval, decreasing during each following 30 min RF-off period. The temperature never returned to 37 °C, but never exceeded 39 °C. No evidence of induction of chromosome aberrations in the lymphocytes due to the RF exposure was observed.

In the second study, Vijayalaxmi et al. [2001a] exposed freshly isolated, diluted human blood to an 847.74 MHz CDMA signal at an SAR of 4.9 or 5.5 W/kg for 24 h. The temperature was reported to be 37 ± 0.3 °C. Again, no evidence of induction of chromosome aberrations due to the RF exposure was observed. In the third study, Vijayalaxmi et al. [2001b] exposed freshly isolated human blood to an 835.62 MHz FDMA signal at a mean SAR of 4.4 or 5.0 W/kg for 24 h. The temperature was reported to be 37 ± 0.3 °C. Again, there was no evidence for the induction of chromosome aberrations.

Maes et al. [1997] exposed human blood samples to a 935.2 MHz GSM/CDMA signal at a reported SAR of 0.3–0.4 W/kg for 2 h. No temperature was reported. No evidence of the induction of chromosome aberrations was observed. Maes et al. [2000] then exposed human blood samples to a 455.7 MHz signal at an SAR of 6.5 W/kg for 2 h. The temperature was reported to be 17 ± 1 °C. Again, no evidence for the induction of chromosome aberrations was observed. In a more
recent article, Maes et al. [2001] reported on the exposure of human blood samples to a 900 MHz GSM signal at SARs ranging from 0.4 to 10 W/kg for 2 h. No temperature was reported. As in the two previous studies, no evidence of the induction of chromosome aberrations in the lymphocytes was observed.

As mentioned above, a series of articles by Garaj-Vrhovac et al. [1990, 1991, 1992] reported chromosomal damage after exposure of mammalian cells to RF. All of the studies were said to use the exposure system described in the first article [Garaj-Vrhovac et al., 1990]. After careful examination of the description of the methods used in all three articles, the following can be stated: (a) there is no information about the container that the cells were in during the RF exposures, (b) it is not at all clear that the cells were in medium during the exposures nor was the volume of any medium stated, (c) the distance between the antenna horn and the “sample” was not provided, (d) the “controlled temperature conditions” referred to may be the room temperature, rather than the “sample” temperature, (e) the temperature was not measured during the exposure, (f) the use of a surface probe to measure the temperature after the exposure suggests that the temperature measured was at the surface of the membrane holding the cells, and (g) the “sample” that was placed on the table surface for the RF exposure may have been a wet membrane containing the cells. Obviously, several of these items are speculative, since the term “sample” is never defined in the articles. Since guesswork is required to attempt to understand how the experiments were performed, they cannot be depended upon for making scientific judgements.

The conclusion from the above studies is that RF exposures at several different frequencies and modulations for exposure times ranging from 90 min to 2 h at SAR levels that did not increase the average medium temperature in which the cells were exposed, did not induce chromosome aberrations. There is also evidence at SAR levels where a temperature increase did occur that the RF exposures did not induce chromosome aberrations. Further, there is evidence that an RF exposure at a high SAR, where the temperature of the medium was increased, did not alter the frequency of mutagen-induced chromosomal aberrations beyond that occurring due to mutagen treatment in the appropriate water bath temperature controls.

**Induction of micronuclei.** As an alternative to the time consuming task of examining metaphase spreads microscopically to detect gross structural chromosome aberrations, investigators have employed detection of micronucleus (MN) formation as a surrogate of chromosome aberration induction. The MN assay is advantageous because it is much more rapid, costs much less, does not require the skills of a trained cytogeneticist, and can allow for many cells to be screened using automated techniques. A MN, however, can be formed via two different known mechanisms. A MN can be a small encapsulated piece of a chromosome, an acentric fragment, which is a piece of a chromosome lacking a centromere, that lags behind in the cytoplasm of a cell at the time of cell division. It can also appear in the cytoplasm of differentiating blood cells that loose their nuclei as they differentiate. Alternatively, it can be an encapsulated whole chromosome that was not properly “distributed” into the daughter nucleus at the time of cell division. If the latter is the case, the presence of the MN may not truly represent a chromosome damaging (genotoxic/mutagenic) event; it may instead be evidence of the disruption by the treatment of the mitotic machinery of the cell.

Attention to the ability of RF to induce micronuclei was accentuated after an abstract of research performed by the Integrated Laboratory Systems (ILS) group under contract to Wireless Technology Research LLC (WTR) was presented at the 21st Annual Meeting of the Bioelectromagnetics Society [Hook et al., 1999]. The abstract reported that at wireless frequencies and modulations using SARs of 5 and/or 10 W/kg after a 24 h, but not a shorter exposure time, an increase in MN in cytochalasins B (CB) induced binucleate cells was observed. The studies were recently published [Tice et al., 2002]. The investigators exposed human blood leukocytes and lymphocytes to 837 MHz (analog), 837 MHz TDMA, 837 MHz CDMA, and 1909.8 MHz PCS signals for 3 or 24 h, at average SARs of 1–10 W/kg. The temperature was reported to be 37 ± 1 °C. There was no evidence for induction of MN by any of these signals, even at SARs of 10 W/kg, when the exposures were of 3 h duration. For all of the signals, when the exposures were for 24 h at 10 W/kg, significant increases in MN compared to sham irradiated controls were observed. When the SAR was lowered to 5 W/kg, significant increases were observed only for the analog and TDMA RF signals.

A second positive report of MN induction, qualified by the authors, is from an in vivo study by Vijayalaxmi et al. [1997b, 1998]. These authors examined micronuclei induction in cells from the bone marrow and peripheral blood of cancer prone mice exposed to a continuous wave 2450 MHz signal for 20 h/day, 7 days/week, for 18 months. The exposures were part of a study to determine whether the RF resulted in tumor formation in the mice [Frei et al., 1998b]. The initial conclusion that was published [Vijayalaxmi et al., 1997b] was that a statistically
significant increase in MN was not observed in the cells from the exposed animals, compared to those from the sham exposed animals. After a mathematical error was discovered in the statistical analysis, a correction was submitted and published [Vijayalaxmi et al., 1998], indicating that there was a statistically significant increase in the MN in both cell types, but not in the animals found to have tumors. The correction unfortunately had a typographical error in the number—the increase was said to be 1 in 200 polychromatic erythrocytes. Another correction appeared in a later issue of the Journal, reporting that the increase was actually 1 in 2000 PCEs. The authors pointed out that while the increase was statistically significant, they did not consider the extremely small numerical change to be biologically significant, especially in light of the extensive RF exposure and the observation that there was no statistically significant increase in tumors in the RF exposed animals.

In addition to the above, d’Ambrosio et al. [2002] recently reported that RF exposure of diluted blood exposed in vitro to a 1.748 GHz GMSK modulated signal for 15 min at a reported maximum SAR of approximately 5 W/kg, resulted in the induction of micronuclei. The exposure system consisted of two coax-to-wave guide adapters connected end to end; a flask with cells was somehow supported within the resulting cavity. Based on the SAR determination method described, the SAR measured was for the flask, the supporting structure (unspecified) of the flask, the media in the flask, which were said to have slightly varying volumes (not reported), and the cells. If this average value is correct, it is possible, contrary to the statements of the authors, that the SAR in the medium in the flask where the cells were located was considerably higher than 5 W/kg. The heterogeneity/homogeneity of the E field in the flask, the resulting SAR, and any possible thermal hot spots are not known or reported. The authors used a second method to make heating and cooling measurements in nine positions in the flask and to calculate SARs, which differed at some points by more than 50%. It is not clear how the temperature measurements were made within the metal of the presumably closed system.

These and other technical matters of the exposure system need to be addressed. The authors also used only one flask of cells for each exposure condition for each donor; there were no replicate exposure flasks and no repeated experiments with any donor. The authors did not perform sham exposure controls, but used as a surrogate incubator controls; the exposed and nonexposed cells were handled in different ways. If one compares the average percentage of binucleate cells with MN from the first seven donors to the next nine donors, the average values of the former are 20% higher than the latter. In scoring the MN, the numbers of binucleated cells scored were different for every donor and even different for the RF exposed versus incubator control samples for each of the donors. For all of these reasons, the results must be considered questionable.

At the time of the first of the positive reports, mentioned above, of MN induction by RF exposure of mammalian cells, Vijayalaxmi et al. [1997a] reported the absence of the induction of MN after exposure of human blood in vitro to a continuous wave 2450 MHz signal at a mean SAR of 12.5 W/kg for 90 min (intermittent on and off). Subsequently, in an attempt to examine the response reported by Hook et al. [1999], Vijayalaxmi et al. [2001a] determined that exposure of diluted human blood in vitro to a 847.74 MHz CDMA signal at mean SARs of 4.9 or 5.5 W/kg for 24 h, the same exposure time as used by Hook et al. [1999] and Tice et al. [2002], did not result in the induction of MN. An additional study by Vijayalaxmi et al. [2001b] also reported that after exposure in vitro of diluted human blood to a 835.62 MHz FDMA signal at SARs of 4.4 or 5.0 W/kg for 24 h, no increase in micronuclei was observed.

Bisht et al. [2002] reported on the induction of micronuclei after exposure in vitro of C3H 10T1/2 mouse fibroblast cells to either an 835.62 MHz FDMA or 847.74 MHz CDMA signal. The SARs for the FDMA signal were 3.2 or 5.1 W/kg, and for the CDMA signal 3.2 or 4.8 W/kg. The exposure times were for 3, 8, 16, or 24 h, and the temperature reported was 37 °C. The authors reported the absence of the induction of micronuclei by the different exposures.

McNamee et al. [2002a,b] reported on the induction of micronuclei after exposure of human leukocytes in diluted human blood from five subjects to a 1.9 GHz CW signal [McNamee et al. 2002a] at 0.0, 0.1, 0.26, 0.92, 2.4, and 10 W/kg for a 2 h exposure period. The temperature was reported to be 37 ± 0.5 °C. The authors repeated the experimental design, but used a 1.9 GHz PW signal [McNamee et al. 2002b]. The authors reported the absence of induction of micronuclei in all cases.

One additional study, involving examination of the induction of micronuclei as a result of a 24 h RF exposure in vivo, was reported by Vijayalaxmi et al. [2001c]. Male Sprague–Dawley rats were exposed to a continuous wave 2450 MHz signal at a whole body average SAR of 12 W/kg for 24 h. Polychromatic erythrocytes from the bone marrow and peripheral blood of the rats were examined for MN induction. No evidence for MN induction was found.

Based on the above information, the induction of micronuclei by RF exposures, even for continuous 24 h
exposures at moderate to high SARs, is not established at the biological level, as contrasted with the experimental level. This statement is meant to convey confidence that the Tice et al. [2002] study does describe observed induction of MN, but is also meant to convey that it may not be a real biological phenomena. The weight of the evidence appears to indicate that RF exposure does not result in the induction of micronuclei. As of the time of final preparation of this review, a formal investigation of this result is underway. The study design was not available, and so it would be important to determine if the study examines the in vitro issues of not only increased temperature at high SAR regions in the exposure vessels, but also the possible effects of local pH change and local hypoxia for cells which have settled upon each other over a 24 h period, if conical tubes are used.

**Phenotypic mutation.** The detection of phenotypic mutations by selection has been one of the most important genotoxic assays, since for such a mutation to be observed, the daughter cells must remain viable and also proliferate clonally. In contrast, those cells with gross observable structural abnormalities (chromosome aberrations) or unrepared DNA DSBs are likely to die. These latter cells therefore cannot become cancer-like cells. A phenotypic mutation, since it appears in all of the daughter cells of the initially damaged cell, results in an observable and inheritable change in all of the daughter cells. This could be a change in morphological appearance, a change in a membrane property, a change in an enzyme activity, etc. It also could lead to a molecular change resulting in unregulated cell growth, a cancer-like change. One phenotypic mutation may not lead to cancer or cancer in a specific organ. Sometimes, multiple mutations and aberrations in specific locations in cellular DNA are required. In addition, if damage occurs in the DNA of a nonproliferating cell, that damage may never be expressed as a phenotypic mutation.

Although the induction of phenotypic mutations have been investigated in lower organisms in the past, it is unfortunate that there are almost no published studies in more recent times of whether or not RF at different frequencies and modulations can cause phenotypic mutations. Very detailed work in this area has been reported by Melzt et al. [1989, 1990]. In both independently performed series of investigations, L5178Y mouse leukemic cells were exposed to a 2450 MHz pulsed wave signal for 4 h. The mean SARs [30 W/kg in Meltz et al., 1989; 40 W/kg in Meltz et al., 1990] were such that the temperature in the medium increased above the initial temperature of 37 °C during the 4 h exposure period. The medium temperature increased to, but did not exceed 38.9 °C. The endpoint examined was the induction of forward mutations at the thymidine kinase (TK+/−) locus. This assay is used regularly in examining the mutagenic potential of drugs and chemicals for regulatory purposes. The authors reported that these RF exposures, at SAR levels considerably above the RF exposure guidelines published by international organizations [ICNIRP, 1998; IEEE, 1999], did not increase the mutation frequency compared to water bath temperature controls performed simultaneously.

The authors were the first to undertake a more demanding hypothesis: that an RF exposure could increase the mutation frequency induced in the L5178Y TK± cells when they were treated simultaneously with the RF exposure and known genotoxic and mutagenic chemicals. The hypothesis was that the RF exposure could alter the mutation frequency by altering one or more of the steps involved in “fixing” the mutation in the cells. These steps could include uptake of the chemical because of a membrane effect; transport of the chemical across the cytoplasm into the nucleus; metabolism of the chemical to a reactive state, if this was required; altering the availability of sites on the DNA, thereby altering the extent of the damage; effecting any error prone repair occurring as a result of the damage; or affecting the subsequent fixation of the damage by affecting the subsequent DNA synthesis and cell proliferation. If any of these steps were altered during or because of the simultaneous chemical and RF exposure, there could be an increase or a decrease in the chemically induced mutation frequency. In addition, two different chemical mutagens were examined, MMC [Meltz et al., 1989] and proflavin [Meltz et al., 1990]. The chemicals act in different ways: the MMC can break DNA strands and induce cross links; the proflavin intercalates between the DNA strands. Again, the authors reported that the RF exposures did not induce phenotypic mutations or alter the extent of the increase in mutation frequency produced in the cells by either of the genotoxic chemicals MMC or proflavin, compared to water bath temperature controls performed simultaneously.

**Molecular mutation, in vivo exposure.** A recent study by Takahashi et al. [2002] investigated the mutagenic activity of RF exposure using an in vivo model system. “Big Blue Mice” were exposed to a 1 GHz TDMA signal for 90 min/day, 5 days/week, for 4 weeks. The SARs were reported to be a whole body average of 0.27 W/kg with a brain average SAR of 2 W/kg. Exposure at a brain average SAR of 0.67 W/kg was also performed. The method by which the SARs were determined was not reported. The authors looked for mutation at the molecular level, i.e., for independent
mutations of the lacI transgene in the brains of the exposed animals. No statistically significant evidence of mutation was found.

**Transformation Events After RF Exposure In Vitro**

Cellular transformation has been used to assess the potential ability of an agent to cause cancer-like changes in cells after different treatments. The assay is more time consuming, more labor intensive, more costly, and more difficult to analyze than most of the mutation assays. The transformation assay has been performed with only a limited number of cell systems; cell line selection is often required. If transformation is observed in vitro, additional in vivo tests are still required; confirmation that the resulting transformed cells are able to form tumors in animals is needed. The cell transformation assay can be used as a model system to investigate the ability of different agents to promote the initiating activity of another agent, as well as to investigate whether the agent is itself an initiating agent and not a complete transforming agent. The latter requires treatment of the cells, after the first agent treatment, with a known promoting chemical to cause the transformation. Still a third approach would be to see if two agents together caused transformation, while neither could by itself; they then would be “cocarcinogens.”

All of these approaches were employed in investigations by Balcer-Kubiczek and Harrison in a series of three articles. In all three articles, Balcer-Kubiczek and Harrison [1985, 1989, 1991] performed 24-h exposures of C3H 10T1/2 fibroblast cells to 2450 MHz RF, pulsed wave, at a reported SAR of 4.4 W/kg. The maximum medium temperature was reported to be 37.2°C. In all three studies, the authors reported that there was no evidence for transformation upon RF exposure alone.

In the first article [Balcer-Kubiczek and Harrison, 1985], an increase in transformation frequency was observed if the cells were exposed for 6 h to RF, then acutely to 1.5 Gy of ionizing radiation, followed by 18 h of additional RF exposure, before trypsinizing the cells and plating them for colony formation and focus formation in medium with the tumor promoter TPA. Unfortunately in this experiment [Balcer-Kubiczek and Harrison, 1985], the authors did not report a needed control, i.e., what happens if the cells are exposed to RF and the tumor promoter without the X-rays. The authors reported that the RF was not a cocarcinogen; the transforming efficiency was not increased in combined treatments with X-rays or benzo(a)pyrene with no TPA present.

In the second article [Balcer-Kubiczek and Harrison, 1989], for the same RF exposure parameters and the same cells, in addition to reporting the absence of an effect by RF alone, the authors reexamined the interaction of RF, X-rays, and tumor promoter. They state that “on a statistical basis, the presence of the additional component due to microwaves in combined treatments with X-rays and tumor promoter cannot be demonstrated with the present protocols” (p. 535). They did report in this article that if a tumor promoter was present after exposure, there was a significant increase in transformed colonies. They concluded (p. 534) “thus, in the experiments reported here, microwaves appear to act as an initiator in a two-stage transformation assay.” This conclusion, however, is not supported by the weight of evidence indicating that RF is not genotoxic.

In the third article [Balcer-Kubiczek and Harrison, 1991], the authors exposed the C3H/10T1/2 cells to 26 different exposure conditions. Among them were RF alone at different SARs: 0.1, 1, or 4.4 W/kg, RF at different SARs + TPA, X-rays alone at different doses, X-rays at different doses + TPA, or combinations of RF at different SARs and X-rays at different doses in different sequences, with and without TPA. The data reported an increase in transforming frequency with increasing SAR, but only when TPA was added. Unfortunately in this and the second article, there was only one exposed cell sample for each exposure condition; the statistics appear to be for the assay plates prepared from each of these single exposed samples. The trend is suggestive of an effect. However, as noted previously, the hypothesis of RF being an initiator must be challenged.

In a more recent investigation, Roti Roti et al. [2001] examined the ability of an 847.74 MHz, FDMA RF signal, or an 847.74 MHz CDMA signal to transform mouse C3H 10T1/2 fibroblasts. Multiple (18) independent flasks were exposed to each exposure condition for 7 days at an SAR of 0.6 ± 0.3 W/kg. The medium temperature was 37°C. No evidence of transformation by the RF exposures was reported. In an additional experiment, cells were exposed to X-rays, and then exposed to the RF signals. No effects of the RF exposures on transformation by the X-rays were observed. These authors did not investigate the interaction of RF with TPA.

**Tumor Promotion, In Vivo**

Two recent studies provide an in vivo correlation to the in vitro studies indicating that RF does not have promotional activity. The first [Adey et al., 1999] looked for the induction of tumors in Fischer 344 rats due to a chronic RF exposure with an 836.55 MHz NADC signal. Promotion was examined using a nitro-
sourea to induce tumors in the CNS. The exposures were for up to 24 months, beginning prior to birth, 2 h/day, 4 consecutive days/week. No increase in the incidence of nitrosourea induced tumors was reported due to the RF exposure. A second study by Adey et al. [2000], using the same animal model and approach, examined the effects of an 836.66 FM signal. This investigation also revealed the absence of an increase in the incidence of nitrosourea induced tumors due to the RF exposure.

CONCLUSIONS

A number of important conclusions can be drawn from the discussion of all of the studies described above.

1. There is extensive evidence that RF exposures at different frequencies, at SAR levels that do not result in exposing cells at elevated temperatures over time, are not toxic. This is the case for both in vitro and in vivo exposures, both acute (short term) and chronic (long term).

2. There is an abundance of evidence that RF exposures at various frequencies and modulations at SAR levels that do not result in exposing cells at elevated temperatures over time, do not cause a wide range of different types of genotoxic damage. The measures of genotoxic damage that are absent after RF exposures, by the weight of evidence, include the induction of DNA SSBs or DSBs, the induction of chromosomal aberrations, and the induction of SCEs.

3. Limited evidence is available indicating the absence of induction of phenotypic mutations by RF exposure and the inability of RF exposure to interfere with DNA repair synthesis after the DNA is damaged by another agent (UV). There is no evidence contradicting either observation.

4. There is some evidence indicating that RF exposure does not interact synergistically with several different chemical mutagenic agents. The evidence which initially appeared to contradict this was not reproduced over time in the same laboratory that reported it.

5. There is limited evidence that RF exposure, using some exposure systems, results in the induction of micronuclei; considerable other evidence exists that this does not occur. The induction of micronuclei is not consistent with the demonstrated absence of chromosome aberrations and DNA strand breaks. This matter is under further formal investigation.

6. There is limited evidence that RF exposure does not result in cancer-like changes of cells, as measured by the technique of in vitro cell transformation. There is no evidence contradicting this observation.

7. There is limited evidence that RF exposure is not a cocarcinogen from studies involving either X-ray exposure or treatment with the chemical carcinogen benzo(a)pyrene.

Most of these conclusions are based on studies where the temperature of the biological sample was reported not to increase above the physiological temperature of 37 °C for both short and prolonged exposure times. Some studies did involve elevated temperatures due to the RF exposure or water bath heating. Clearly, the results discussed challenge the statement that studies of RF bioeffects at “athermal” (or “nonthermal” or “normothermal”) conditions have not been performed. Many such studies have been performed. The weight of evidence, as stated above, indicates an absence of toxic or genotoxic effects of low level exposures to RF electromagnetic fields.

REFERENCES


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