Electromagnetic fields and DNA damage

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Abstract

A major concern of the adverse effects of exposure to non-ionizing electromagnetic field (EMF) is cancer induction. Since the majority of cancers are initiated by damage to a cell's genome, studies have been carried out to investigate the effects of electromagnetic fields on DNA and chromosomal structure. Additionally, DNA damage can lead to changes in cellular functions and cell death. Single cell gel electrophoresis, also known as the 'comet assay', has been widely used in EMF research to determine DNA damage, reflected as single-strand breaks, double-strand breaks, and crosslinks. Studies have also been carried out to investigate chromosomal conformational changes and micronucleus formation in cells after exposure to EMF. This review describes the comet assay and its utility to qualitatively and quantitatively assess DNA damage, reviews studies that have investigated DNA strand breaks and other changes in DNA structure, and then discusses important lessons learned from our work in this area.
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1. The comet assay for measurement of DNA strand breaks

DNA is continuously damaged by endogenous and exogenous factors and then repaired by DNA repair enzymes. Any imbalance in damage and repair and mistakes in repair result in accumulation of DNA damage. Eventually, this will lead to cell death, aging, or cancer. There are several types of DNA lesions. The common ones that can be detected easily are DNA strand breaks and DNA crosslinks. Strand breaks in DNA are produced by endogenous factors, such as free radicals generated by mitochondrial respiration and metabolism, and by exogenous agents, including UV, ionizing and non-ionizing radiation, and chemicals.

There are two types of DNA strand breaks: single- and double-strand breaks. DNA single-strand breaks include frank breaks and alkali labile sites, such as base modification, deamination, depurination, and alkylation. These are the most commonly assessed lesions of DNA. DNA double-strand breaks are very critical for cells and usually they are lethal. DNA strand breaks have been correlated with cell death [1–5], aging [6–8] and cancer [9–13].

Several techniques have been developed to analyze single- and double-strand breaks. Most commonly used is microgel electrophoresis, also called the 'comet assay' or 'single cell gel electrophoresis'. This technique involves mixing cells with agarose, making microgels on a microscope slide, lysing cells in the microgels with salts and detergents, removing proteins from DNA by using proteinase K, unwinding/equilibrating and electrophoresing DNA (under highly alkaline condition for assessment of single-strand breaks or under neutral condition for assessment of DNA double-strand breaks), fixing the DNA, visualizing the DNA with a fluorescent dye, and then analyzing migration patterns of DNA from individual cells with an image analysis system.

The comet assay is a very sensitive method of detecting single- and double-strand breaks if specific criteria are met. Critical criteria include the following. Cells from tissue culture or laboratory animals should be handled with care to minimize DNA damage, for instance, by avoiding light and high temperature. When working with animals exposed to EMF *in vivo*, it is better to anesthetize the animals with CO_2 before harvesting tissues for assay. Antioxidants

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such as albumin and sucrose, or spin-trap molecules such as α-phenyl-*tert*-butyl nitrone (PBN), should be added during dispersion of tissues into single cells. Cells should be lysed at 0–4 °C to minimize DNA damage by endonucleases. Additionally, antioxidants such as tris and glutathione, and chelators such as EDTA, should be used in the lysing solution. High concentrations of dimethylsulfoxide (DMSO) should be avoided due to its chromatin condensing effect. Treatment with proteinase K (PK; lyophilized DNAse-free proteinase-K from Amresco is ideal) at a concentration of 0.5–1 mg/ml (depending upon cell type and number of cells in the microgel) should be used for 1–2 h at 37 °C to reveal all possible strand breaks which otherwise may go undetected due to DNA–protein crosslinks. Longer times in PK will lead to loss of smaller pieces of DNA by diffusion. Glass slides should be chosen based on which high resolution agarose (3:1 high resolution agarose from Amresco is ideal) will stick well to the slide and on the ability of the specimen to be visualized without excessive fluorescence background. Choice of an electrophoresis unit is important to minimize slide-to-slide variation in DNA migration pattern. A unit with uniform electric field and buffer recirculation should be used. Electrophoresis buffers should have antioxidants and chelators such as DMSO and EDTA. DNA diffusion should be minimized during the neutralization step by rapidly precipitating the DNA. Staining should employ a sensitive fluorescent dye, such as the intercalating fluorescent labeling dye YOYO-1. A cell-selection criteria for analysis should be set before the experiment, such as not analyzing cells with too much damage, although, the number of such cells should be recorded.

There are different versions of the comet assay that have been modified to meet the needs of specific applications and to improve sensitivity. Using the most basic form of the assay, one should be able to detect DNA strand breaks in human lymphocytes that were induced by 5 rad of gamma-ray [14,15].

2. Radiofrequency radiation (RFR) and DNA damage

In a series of publications, Lai and Singh [16–19] reported increases in single- and double-strand DNA breaks, as measured by the comet assay, in brain cells of rats exposed for 2 h to a 2450-MHz RFR at whole body specific absorption rate (SAR) between 0.6 and 1.2 W/kg. The effects were blocked by antioxidants, which suggested involvement of free radicals. At the same time, Sarkar et al. [20] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150, and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals. Their data also suggested breakage of DNA strands after RFR exposure. Phillips et al. [21] were the first to study the effects of two forms of cell cellular phone signals, known as TDMA and iDEN, on DNA damage in Molt-4 human lymphoblastoid cells using the comet assay. These cells were exposed to relatively low intensities of the fields (2.4–26 μW/g) for 2–21 h. They reported both increased and decreased DNA damage, depending on the type of signal studied, as well as the intensity and duration of exposure. They speculated that the fields may affect DNA repair in cells. Subsequently, different groups of researchers have also reported DNA damage in various types of cells after exposure to cell phone frequency fields. Diem et al. [22] exposed human fibroblasts and rat granulosa cells to cell phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; for 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h of exposure to different cell phone modulations in both cell types. The intermittent exposure schedule caused a significantly stronger effect than continuous exposure. Gandhi and Anita [23] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users. Markova et al. [24] reported that GSM signals affected chromatin conformation and γ-H2AX foci that co-localized in distinct foci with DNA double-strand breaks in human lymphocytes. The effect was found to be dependent on carrier frequency. Nikolova et al. [25] reported a low and transient increase in DNA double-strand breaks in mouse embryonic stem cells after acute exposure to a 1.7-GHz field. Lixia et al. [26] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 h of exposure to a 1.8-GHz field at 3 W/kg. Sun et al. [27] reported an increase in DNA single-strand breaks in human lens epithelial cells after 2 h of exposure to a 1.8-GHz field at SARs of 3 and 4 W/kg. DNA damage caused by the field at 4 W/kg was irreversible. Zhang et al. [28] reported that an 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 h of exposure. Aitken et al. [29] exposed mice to a 900-MHz RFR at a SAR of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as by alkaline and pulsed-field gel electrophoresis. Gel electrophoresis revealed no significant change in single- or double-strand breaks in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome and the nuclear β-globin locus. Changes in sperm cell genome after exposure to 2450-MHz microwaves have also been reported previously by Sarkar et al. [20]. Related to this are several publications that have reported decreased motility and changes in morphology in isolated sperm cells exposed to cell phone radiation [30], sperm cells from animals exposed to cell phone radiation [31], and cell phone users [32–34]. Some of these *in vivo* effects could be caused by hormonal changes [35,36].

There also are studies reporting no significant effect of cell phone RFR exposure on DNA damage. After RFR-induced DNA damage was reported by Lai and Singh [16] using 2450-MHz microwaves and after the report of Phillips et al. [21] on cell phone radiation was published, Motorola funded a series of studies by Roti Roti and colleagues [37] at

Washington University to investigate DNA strand breaks in cells and animals exposed to RFR. None of the studies reported by this group found significant effects of RFR exposure on DNA damage [38–40]. However, a different version of the comet assay was used in these studies. More recently, four additional studies from the Roti-Roti laboratories also reported no significant effects on DNA damage in cells exposed to RFR. Li et al. [41] reported no significant change in DNA strand breaks in murine C3H10T1/2 fibroblasts after 2 h of exposure to 847.74- and 835.02-MHz fields at 3–5 W/kg. Hook et al. [42] showed that a 24-h exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA-modulated RFR did not significantly alter the level of DNA damage. Lagroye et al. [43,44] also reported no significant change in DNA strand breaks, protein–DNA crosslinks, and DNA–DNA crosslinks in cells exposed to 2450-MHz RFR.

From other laboratories, Vijayalaxmi et al. [45] reported no increase in DNA stand breaks in human lymphocytes exposed *in vitro* to 2450-MHz RFR at 2.135 W/kg for 2 h. Tice et al. [46] measured DNA single-strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed for 3 or 24 h at average SARs of 1.0–10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes. McNamee et al. [47–49] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 h to a 1.9-GHz field at SAR up to 10 W/kg. Zeni et al. [50] reported that a 2-h exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes. Sakuma et al. [51] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to cell phone radiation for 2 and 24 h. No significant changes in DNA strand breaks were observed up to a SAR of 800 mW/kg. Stronati et al. [52] showed that 24 h of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells. Verschaeve et al. [53] reported that long-term exposure (2 h/day, 5 days/week for 2 years) of rats to 900-MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

3. Extremely low frequency electromagnetic fields (ELF EMF) and DNA damage

To complete the picture, a few words on the effects of ELF EMF are required, since cell phones also emit these fields and they are another common form of non-ionizing EMF in our environment. Quite a number of studies have indicated that exposure to ELF EMF could lead to DNA damage [54–69]. In addition, two studies [70,71] have reported effects of ELF fields on DNA repair mechanisms. Free radicals and interaction with transitional metals (e.g., iron) [60,62,63,69] have also been implicated to play a role in the genotoxic effects observed after exposure to these fields.

4. Some considerations on the effects of EMF on DNA

From this brief literature survey, no consistent pattern of RFR exposure inducing changes in or damage to DNA in cells and organisms emerges. However, one can conclude that under certain conditions of exposure, RFR is genotoxic. Data available are mainly applicable only to radiation exposure that would be typical during cell phone use. Other than the study of Phillips et al. [21], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

Differences in experimental outcomes are expected since many factors could influence the outcome of experiments in EMF research. Any effect of EMF has to depend on the energy absorbed by a biological organism and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these factors have been discussed in a talk given by one us (HL) in Vienna, Austria in 1998 [72].

Radiation from cell phone transmission has very complex patterns, and signals vary with the type of transmission. Moreover, the technology is constantly changing. Research results from one types of transmission pattern may not be applicable to other types. Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [21], which demonstrated that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks after exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used poor exposure procedures with very limited documentation of exposure parameters, e.g., using an actual cell phone to expose cells and animals, thus rendering the data from these experiments as questionable.

Another source of influence on experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [73] and organisms [74,75] may not all respond similarly to EMF.

Comment about the comet assay also is required, since it was used in many of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay could be misleading. Another concern is that most of the comet assay studies were carried out by experimenters who had no prior experience with this technique and mistakes
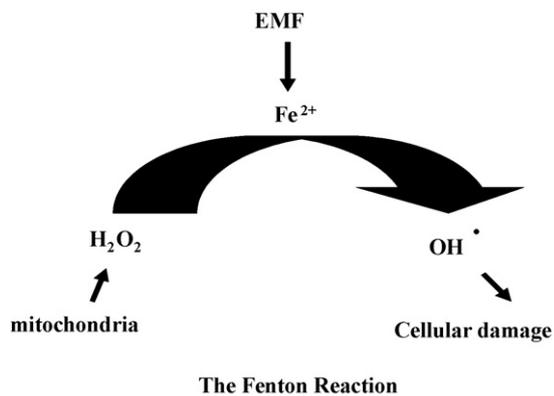
Fig. 1. A representation of the Fenton reaction and its role as a mediator in EMF-induced bioeffects.

were made. For example, in the study by Lagroye et al. [43] to investigate the effect of PK digestion on DNA migration after RFR exposure, PK was added to a lysing solution containing the detergent Triton X-100, which would inactivate the enzyme. Our experience indicates that the comet assay is a very sensitive and requires great care to perform. Thus, different detection sensitivities could result in different laboratories, even if the same procedures are followed. One way to solve this problem of experimental variation is for each research team to report the sensitivity of their comet assay, e.g., the threshold of detecting strand breaks in human lymphocytes exposed to X-rays. This information has generally not been provided for EMF-genotoxicity studies. Interestingly, when such information was provided, a large range of sensitivities have been reported. Malyapa et al. [40] reported a detection level of 0.6 cGy of gamma radiation in human lymphocytes, whereas McNamee et al. [76] reported 10–50 cGy of X-irradiation in lymphocytes, which is much higher than the generally acceptable detection level of the comet assay [15].

A drawback in the interpretation and understanding of experimental data from bioelectromagnetics research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which EMF produces changes in DNA is unknown. Since the energy level associated with EMF exposure is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced biochemical changes in cells.

One possibility is that DNA is damaged by free radicals that are formed inside cells. Free radicals affect cells by damaging macromolecules, such as DNA, protein, and membrane lipids. Several reports have indicated that EMF enhances free radical activity in cells [18,19,61,62,77,78], particularly via the Fenton reaction [62]. The Fenton reaction is a process catalyzed by iron in which hydrogen peroxide, a product of oxidative respiration in the mitochondria, is converted into hydroxyl free radicals, which are very potent and cytotoxic molecules (Fig. 1).

It is interesting that ELF EMF has also been shown to cause DNA damage. Furthermore, free radicals have been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR. Effects via the Fenton reaction predict how a cell would respond to EMF. For instance:

(1) Cells that are metabolically active would be more susceptible to EMF, because more hydrogen peroxide is generated by mitochondria to fuel the reaction.
(2) Cells that have high level of intracellular free iron would be more vulnerable to EMF. Cancer cells and cells undergoing abnormal proliferation have higher concentrations of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF. Consequently, this suggests that EMF could potentially be used for the treatment of cancer and hyperplastic diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells. One consequence of this consideration is that epidemiological studies of cancer incidence in cell phone users may not show a risk at all or even a protection effect.
(3) Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high levels of iron. Special molecular pumps are present on nerve cell nuclear membranes to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capacity for DNA repair, and DNA breaks could easily accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues, particularly in the brain. These particles could enhance free radical activity in cells and thus increase the cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, the more likely consequences of DNA damage in nerve cells include changes in cellular functions and in cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double-strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cell, the glial cell, can become cancerous as a result of DNA damage. The question is whether the damaged cells

would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors—an increase, decrease, or no significant change in cancer risk could result from EMF exposure.

(4) On the other hand, cells with high amounts of antioxidants and antioxidative enzymes would be less susceptible to EMF. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

Additionally, the work of Blank and Soo [79] and Blank and Goodman [80] support the possibility that EMF exposure at low levels has a direct effect on electron transfer processes. Although the authors do not discuss their work in the context of EMF-induced DNA damage, the possibility exists that EMF exposure could produce oxidative damage to DNA.

5. Lessons learned

Whether or not EMF causes biological effects, let alone effects that are detrimental to human health and development, is a contentious issue. The literature in this area abounds with apparently contradictory studies, and as presented in this review, the literature specific to the effects of RFR exposure on DNA damage and repair in various biological systems is no exception. As a consequence of this controversy, there are several key issues that must be addressed—contrary data, weight of evidence, and data interpretation consistent with known science.

Consider that EMF does not share the familiar and comforting physical properties of chemical agents. EMF cannot be seen, tasted, smelled, or felt (except at high intensities). It is relevant, therefore, to ask, in what ways do scientists respond to data, especially if that data are contrary to their scientific beliefs or inconsistent with long-held hypotheses? Often such data are ignored, simply because it contradict what is accepted as conventional wisdom. Careful evaluation and interpretation of data may be difficult, because technologies used to expose biological systems to EMF and methodologies used to assess dosimetry generally are outside the experience of most biomedical scientists. Additionally, it is often difficult to assess differences in methodologies between studies, one or more of which were intended to replicate an original investigation. For instance, Malyapa et al. [40] reported what they claimed to be a replication of the work of Lai and Singh [16]. There were, however, significant differences

in the comet analyses used by each group. Lai and Singh precipitated DNA in agarose so that low levels of DNA damage could be detected. Malyapa et al. did not. Lai and Singh treated their samples with PK to digest proteins bound to DNA, thus allowing DNA to move toward the positive pole during electrophoresis (unlike DNA, most proteins are negatively charged, and if they are not removed they will drag the DNA toward the negative pole). The Malyapa et al. study did not use PK. There were other methodological differences as well. Such is also the case in the study of Hook et al. [42], which attempted to replicate the work of Phillips et al. [21]. The latter group used a PK treatment in their comet assay, while the former group did not.

While credibility is enhanced when one can relate data to personal knowledge and scientific beliefs, it has not yet been determined how RFR couples with biological systems or by what mechanisms effects are produced. Even carefully designed and well executed RFR exposure studies may be summarily dismissed as methodologically unsound, or the data may be interpreted as invalid because of inconsistencies with what one believes to be correct. The quintessential example is the belief that exposure to RFR can produce no effects that are not related to the ability of RFR to produce heat, that is, to raise the temperature of biological systems [81,82]. Nonetheless, there are many examples of biological effects resulting from low-level (athermal) RFR exposure [83,84]. Consider here the work of Mashevich et al. [85]. This group exposed human peripheral blood lymphocytes to an 830-MHz signal for 72 h and at different average SARs (SAR, 1.6–8.8 W/kg). Temperatures ranged from 34.5 to 38.5 °C. This group observed an increase in chromosome 17 aneuploidy that varied linearly with SAR. Temperature elevation alone in the range of 34.5–38.5 °C did not produce this genotoxic effect, although significant aneuploidy was observed at higher temperatures of 40–41 °C. The authors conclude that the genotoxic effect of the radiofrequency signal used is elicited through a non-thermal pathway.

Also consider one aspect of the work of Phillips et al. [21]. In that study, DNA damage was found to vary in direction; that is, under some conditions of signal characteristics, signal intensity, and time of exposure, DNA damage increased as compared with concurrent unexposed controls, while under other conditions DNA damage decreased as compared with controls. The dual nature of Phillips et al.'s [21] results will be discussed later. For now consider the relationship of these results to other investigations. Adey et al. [86] performed an *in vivo* study to determine if rats treated *in utero* with the carcinogen ethylnitrosourea (ENU) and exposed to an 836.55-MHz field with North American Digital Cellular modulation (referred to as a TDMA field) would develop increased numbers of central system tumors. This group reported that rather than seeing an increase in tumor incidence in RFR-exposed rats, there was instead a decrease in tumor incidence. Moreover, rats that received no ENU but which were exposed to the TDMA signal also showed a decrease in the number of spontaneous tumors as compared

with animals exposed to neither ENU nor the TDMA signal. This group postulated that their results may be mechanistically similar to the work of another group. Stammberger et al. [87] had previously reported that rats treated *in utero* with ENU and then exposed to low doses of X-irradiation exhibited significantly reduced incidences of brain tumors in adult life. Stammberger and colleagues [87] hypothesized that low-level X-irradiation produced DNA damage that then induced the repair enzyme 0^6-alkylguanine-DNA alkyltransferase (AT). Numerous groups have since reported that X-irradiation does indeed induce AT activity (e.g., [88,89]). In this context, it is significant that Phillips et al. [21] found that cells exposed *in vitro* to a TDMA signal identical to that used in the study of Adey et al. [86] produced a decrease in DNA damage under specific conditions of intensity and time of exposure (lower intensity, longer time; higher intensity, shorter time). These results raise the intriguing possibility that the decrease in tumor incidence in the study of Adey et al. [86] and the decrease in DNA damage in the study of Phillips et al. [21] both may have been the result of induction of AT activity resulting from DNA damage produced by exposure to the TDMA signal. This remains to be investigated.

Because the issue of RFR-induced bioeffects is contentious, and because the issue is tried in courtrooms and various public forums, a term heard frequently is weight of evidence. This term generally is used to describe a method by which all scientific evidence related to a causal hypothesis is considered and evaluated. This process is used extensively in matters of regulation, policy, and the law, and it provides a means of weighing results across different modalities of evidence. When considering the effects of RFR exposure on DNA damage and repair, modalities of evidence include studies of cells and tissues from laboratory animals exposed *in vivo* to RFR, studies of cells from humans exposed to RFR *in vivo*, and studies of cells exposed *in vitro* to RFR. While weight of evidence is gaining favor with regulators [90], its application by scientists to decide matters of science is often of questionable value. One of the reasons for this is that there generally is no discussion or characterization of what weight of evidence actually means in the context in which it is used. Additionally, the distinction between weight of evidence and strength of evidence often is lacking or not defined, and differences in methodologies between investigators are not considered. Consequently, weight of evidence generally amounts to what Krimsky [90] refers to as a "seat-of-the-pants qualitative assessment." Krimsky points out that according to this view, weight of evidence is "a vague term that scientists use when they apply implicit, qualitative, and/or subjective criteria to evaluate a body of evidence." Such is the case in the reviews by Juutilainen and Lang [91] and Verschaeve and Maes [92]. There is little emphasis on a critical analysis of similarities and differences in biological systems used, exposure regimens, data produced, and investigator's interpretations and conclusions. Rather, there is greater emphasis on the number of publications either finding or not finding an effect of RFR exposure on some endpoint.

To some investigators, weight of evidence does indeed refer to the balance (or imbalance) between the number of studies producing apparently opposing results, without regard to critical experimental variables. While understanding the role these variables play in determining experimental outcome could provide remarkable insights into defining mechanisms by which RFR produced biological effects, few seem interested in or willing to delve deeply into the science.

A final lesson can be derived from a statement made by Gos et al. [93] referring to the work of Phillips et al. [21]. Gos and colleagues state, "The results in the latter study (Phillips et al., 1998) are puzzling and difficult to interpret, as no consistent increase or decrease in signal in the comet assay at various SARs or times of exposure was identified." This statement is pointed out because studies of the biological effects of exposure to electromagnetic fields at any frequency are often viewed as outside of or distinct from what many refer to as *mainstream science*. However, what has been perceived as an inconsistent effect is indeed consistent with the observations of bimodal effects reported in hundreds of peer-reviewed publications. These bimodal effects may be dependent on concentration of an agent, time of incubation with an agent, or some other parameter relating to the state of the system under investigation. For instance, treatment of B cells for a short time (30 min) with the protein kinase C activator phorbol 12,13-dibutyrate increased proliferative responses to anti-immunoglobulin antibody, whereas treatment for a longer period of time ($\geq$3 h) suppressed proliferation [94]. In a study of κ-opioid agonists on locomotor activity in mice, Kuzmin et al. [95] reported that higher, analgesic doses of κ-agonists reduced rearing, motility, and locomotion in non-habituated mice. In contrast, lower, subanalgesic doses increased motor activity in a time-dependent manner. Dierov et al. [96] observed a bimodal effect of all-trans-retinoic acid (RA) on cell cycle progression in lymphoid cells that was temporally related to the length of exposure to RA. A final example is found in the work of Rosenstein et al. [97]. This group found that the activity of melatonin on depolarization-induced calcium influx by hypothalamic synaptosomes from rats sacrificed late evening (2000 h) depended on melatonin preincubation time. A short preincubation time (10 min) stimulated uptake, while a longer preincubation (30 min) inhibited calcium uptake. These effects were also dependent on the time of day when the rats were sacrificed. Effects were maximal at 2000 h, minimal at 2400 h, and intermediate at 400 h. At 1000 h, only inhibitory effects of melatonin on calcium uptake were observed. These examples point out that what appears to be inconsistency may instead be real events related to and determined by the agents involved and the state of the biological system under investigation. The results of Phillips et al. [21] may be the result of signal modulation, signal intensity, time of exposure, or state of the cells. The results may indicate a bimodal effect, or they may, as the investigators suggest, represent time- and signal-dependant changes in the balance between damage and repair because of direct or indirect effects of RFR exposure on repair mechanisms.

6. Summary

Exposure of laboratory animals *in vivo* and of cultured cells *in vitro* to various radiofrequency signals has produced changes in DNA damage in some investigations and not in others. That many of the studies on both sides of this issue have been done well is encouraging from a scientific perspective. RFR exposure does indeed appear to affect DNA damage and repair, and the total body of available data contains clues as to conditions producing effects and methodologies to detect them. This view is in contrast to that of those who believe that studies unable to replicate the work of others are more credible than the original studies, that studies showing no effects cancel studies showing an effect, or that studies showing effects are not credible simply because we do not understand how those effects might occur. Some may be tempted to apply incorrectly the teachings of Sir Karl Popper, one of the great science philosophers of the 20th century. Popper proposed that many examples may lend support to an hypothesis, while only one negative instance is required to refute it [98]. While this holds most strongly for logical subjects, such as mathematics, it does not hold well for more complex biological phenomena that are influenced by stochastic factors. Each study to investigate RFR-induced DNA damage must be evaluated on its own merits, and then studies that both show effects and do not show effects must be carefully evaluated to define the relationship of experimental variables to experimental outcomes and to assess the value of experimental methodologies to detect and measure these outcomes (see Section 2).

The lack of a causal or proven mechanism(s) to explain RFR-induced effects on DNA damage and repair does not decrease the credibility of studies in the scientific literature that report effects of RFR exposure, because there are several plausible mechanisms of action that can account for the observed effects. The relationship between cigarette smoking and lung cancer was accepted long before a mechanism was established. This, however, occurred on the strength of epidemiologic data [99]. Fortunately, relevant epidemiologic data relating long-term cell phone use (>10 years) to central nervous system tumors are beginning to appear [84,100–102], and these data point to an increased risk of acoustic neuroma, glioma and parotid gland tumors.

One plausible mechanism for RFR-induced DNA damage is free radical damage. After finding that two free radical scavengers (melatonin and N-tert-butyl-α-phenylnitrone) prevent RFR-induced DNA damage in rat brain cells, Lai and Singh [62] hypothesized that this damage resulted from free radical generation. Subsequently, other reports appeared that also suggested free radical formation as a result of RFR exposure [103–105]. Additionally, some investigators have reported that non-thermal exposure to RFR alters protein structure and function [106–109]. Scientists are familiar with molecules interacting with proteins through lock-and-key or induced-fit mechanisms. It is accepted that such interactions provide energy to change protein conformation and protein function. Indeed, discussions of these principles are presented in introductory biology and biochemistry courses. Perhaps then it is possible that RFR exposure, in a manner similar to that of chemical agents, provides sufficient energy to alter the structure of proteins involved in DNA repair mechanisms to the extent that their function also is changed. This has not yet been investigated.

When scientists maintain their beliefs in the face of contrary data, two diametrically opposed situations may result. On the one hand, data are seen as either right or wrong and there is no discussion to resolve disparities. On the other hand, and as Francis Crick [110] has pointed out, scientists who hold theoretically opposed positions may engage in fruitful debate to enhance understanding of underlying principles and advance science in general. While the latter certainly is preferable, there are external factors involving economics and politics that keep this from happening. It is time to acknowledge this and embark on the path of fruitful discussion. Great scientific discoveries await.

Acknowledgment

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