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Role of radical pairs and feedback in weak radio frequency field effects on biological systems

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ABSTRACT

Radio frequency electromagnetic fields (RF) have been shown to modify the concentrations of the radical O_2^-, H_2O_2 and cancer cell growth rates at exposure levels below those that cause significant heating. Reactive oxygen species (ROS) are both signaling molecules and species that can do damage, depending on timing, location and concentrations. We briefly look at some mechanisms by which electromagnetic fields can modify the concentrations of ROS and some of the feedback and repair processes that lead to variable biological effects. Of particular interest are the role of radical pairs and their spins, which have received considerable attention recently, and the role of feedback in biological systems, to which less attention has been paid.

1. Introduction

There have been substantial concerns for a long time over the possible effects of radio frequency electromagnetic fields at exposure levels below those that lead to significant increases in temperature. Part of the problem has been the lack of generally accepted mechanisms by which weak fields can lead to biological responses. An additional difficulty has been that observed changes at the whole body level often stem from not only perturbations due to the RF fields but compensating changes generated by the ubiquitous biological feedback and repair processes. In general when the external environment changes, an organism responds so as to keep the biological systems operating within their normal ranges. For example an increase in core temperature in humans leads to an increase in blood flow and sweating to bring the temperature back down to within the normal range. Further complicating attempts to identity when and under what circumstances adverse or positive health effects may result from exposures to RF fields, the biological responses are dependent on current state of the system and its past history. Additionally the effects may vary in time over the course of extended exposures.

We present here some theory about one way weak magnetic fields can modify the recombination rates of radical pairs, which in turn can lead to changes in the concentrations of O_2^-, H_2O_2, and other radicals. Additionally some limited experimental data is presented showing both increases and decreases in cancer cell growth rates. The literature contains reports of many other such changes including in Usselman et al. (2016) and Chavarriaga et al. (2016). We also present some theory describing feedback mechanisms and demonstrate that the observed inconsistencies in biological responses to electromagnetic fields are of the sort that can be described through the biological systems' inherent compensatory processes, whether mediated through the radical pair or some other mechanism.

2. Theory

2.1. Radical pairs

It has been known that magnetic fields can modify chemical reaction rates for a long time. Much of this work has been reviewed at length by Steiner and Ulrich (1989), and Grissom (1995). Reviews of dynamic spin chemistry by Nagakura et al. (1999) and by Hayashi (2004) present detailed descriptions of the theory for the conversion of singlet (S) to triplet (T) states for radical pairs and the resulting changes in radical concentrations as a function of magnetic field strength, orientation, and the viscosity of the medium. Additional detailed calculations and measurements of changes in radical concentration on the applications of weak magnetic fields have been carried out by the group at Oxford (Batchelor et al., 1993; Timmel et al., 1998; Timmel and Henbest, 2004; Rodgers et al., 2007).

Briefly, radicals are defined as molecules with an unpaired spin and thus a net magnetic moment (See Fig. 1). Most molecules have an even number of electrons in the outer orbit which are paired with opposite spins. However, when these molecules split into two fragments the resulting fragments have an odd number of electrons in the outer orbit

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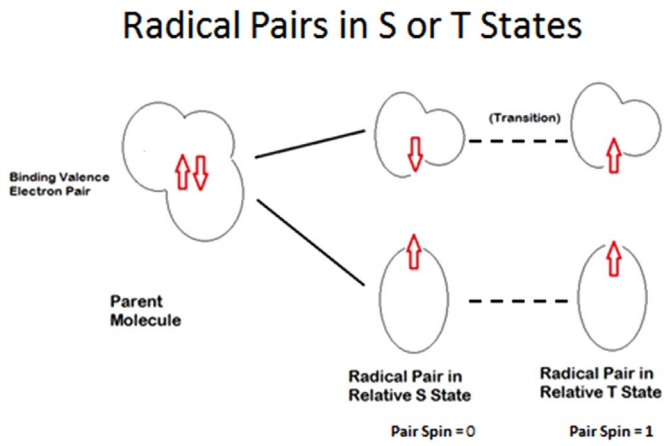


Fig. 1. A schematic diagram for a molecule which breaks into a pair or radicals which in turn may transition between S and T states with the spins aligned either antiparallel or parallel.

and an unpaired spin. Typically, they have different net magnetic moments. These fragments can typically recombine in 10^{-6} to 10^{-12} seconds depending on the viscosity of the medium and other parameters. A static magnetic field shifts the allowed energy states for the electrons differently in each fragment via the Zeeman Effect and changes the recombination rate. There are multiple energy levels corresponding to different electron orbits and the projections of the electron spins on the background static magnetic field (see Fig. 2) (Ramsey, 1956).

Time varying electromagnetic fields can excite transition between energy levels when $\Delta E = hf$, where ΔE is the energy separation between states, h is Planck's constant and f is the frequency of the externally applied field. These transitions in turn can change the spin populations in the fragments so that they do or do not satisfy the requirements for conservation of energy and angular moment for recombination.

As the energy separation changes with the static magnetic fields, the frequency for exciting transitions also changes. It also changes the energy match between levels in each fragment and thus the energy barrier for recombination. The net result is that we can both increase and decrease the recombination rate for the radical fragments and the concentrations of radicals such as O_2^-, and derivative molecules such as H_2O_2 with both static and time varying magnetic fields (see Fig. 3). Note that it is also possible that the concentration of the antioxidant is either increased or decreased by the magnetic field, thus leading to changes in oxidative stress and H_2O_2 in the opposite directions. Hence, the biological system may respond in either direction, depending on overall conditions. The forgoing approach to theoretically possible changes in radical concentrations is reviewed in much more detail in Barnes and Greenebaum (2015, 2016). Detailed calculations for resonances at RF frequencies have been carried out by Woodward et al. (2001), and it is to be noted that there are many resonances throughout the radio frequency range. Direct measurements of these resonant frequencies are often masked by the strong RF absorption by the water content of most biological materials. There are other biological processes that may be affected by the magnetic fields as well as radical pairs. The magnetic fields can act on molecules that have magnetic dipole moments, including hemoglobin, many enzymes, and cryptochromes. Much more extensive reviews of a number of other possible mechanisms by which RF fields can modify biological systems include Binhi (2002), Binhi and Prato (2017a, 2017b) and Belyaev (2015). The review of mechanisms by Engstrom (2007) has a focus on radicals.

2.2. Biological feedback

A second method for modifying the concentrations of radicals and other signaling molecules such as H_2O_2 is though biological feedback. Feedback permeates all aspects of biological systems. It is at the heart of the way radicals and other signaling molecules, as well as endogenous electrical signals operate to control essentially every process of an organism. Halliwell and Gutteridge (2015) present a book-length review of the roles of radicals in biology and medicine. Feedback also operates in many other aspects of living things (Goldstein and Kopin, 2017). Radicals and other signaling molecules, depending on their role in a particular process, the state of the system and other signals and circumstances, can serve to stimulate or to suppress a subsequent process.

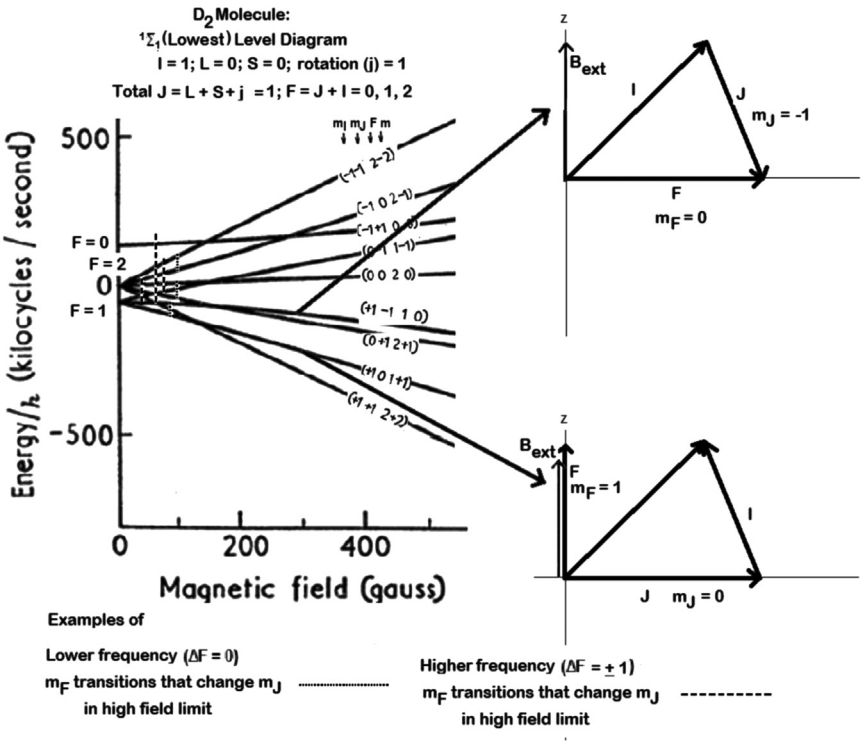


Fig. 2. An energy level diagram for D_2 showing the Zeeman Effect and a vector diagram for the sum of the nuclear and electronic magnetic moments. I is the nuclear spin quantum number, S is the electronic spin, L is the electronic orbital angular momentum, J is the total electronic angular momentum, and F = I + J is the total molecular angular momentum, applicable only at low magnetic field. The projection of each angular momentum along the direction of the applied magnetic field is given by m_I, m_S, m_L, etc.

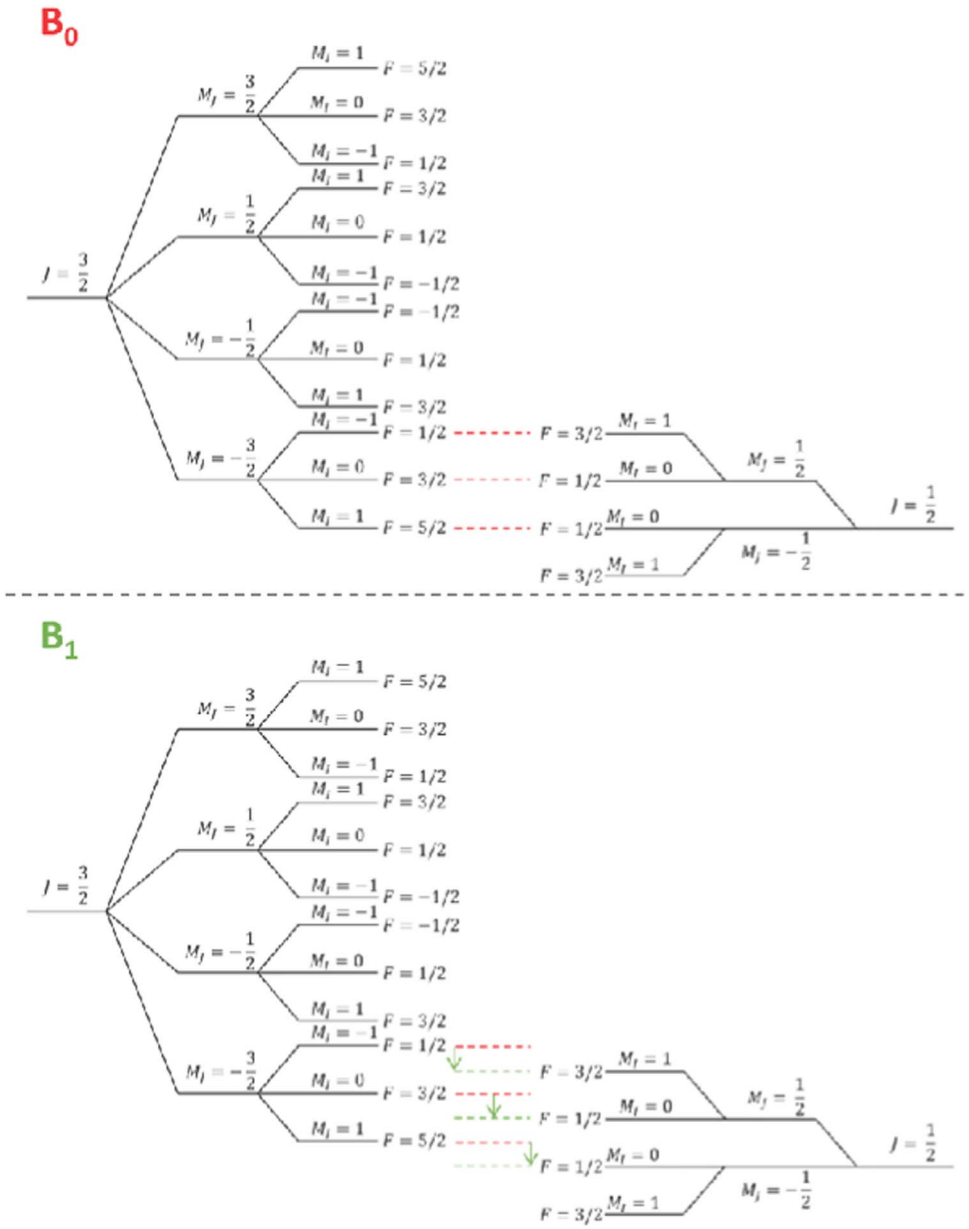


Fig. 3. An energy level diagram for two radicals. For B_o the energy levels line up for several different states of the two radicals and they can recombine rapidly. For B_1 the states do not line up and there is an energy barrier for recombination. Additionally for recombination the angular momentum transitions must be allowed.

Crucial to successfully maintaining the organism is maintaining a proper balance between stimulation and suppression. A signal to stimulate is said to provide positive feedback; one to suppress, negative feedback.

Typically increases in radical concentrations and other reactive oxygen species also signal increases in the generation of antioxidants. These antioxidants in turn reduce the concentrations of the reactive oxygen species (ROS). The generation of the antioxidants takes time after the increase in the concentrations of the radicals or other molecules such as H_2O_2, and there is a further time delay before this feedback process brings the concentration of the oxidative stressor back to normal range.

The effects of this time delay can be seen if we model the system as a simple electrical circuit with a feedback amplifier and a time delay in the feedback, as shown in Fig. 4.[1] This modeling method has been

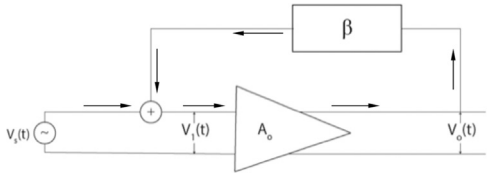


Fig. 4. A simple operational amplifier with gain Ao and a time delay τ in the feedback circuit β.

widely-used for many years to characterize a wide variety of things, ranging from robots to various aspects of biological systems. In this instance, the amplifier gain A_o and feedback coefficient β account for the net multiplicative effect of the signaling molecule, which can be positive (stimulative) or negative (suppressive); and we introduce a time delay in the feedback circuit to account for the time between generating the signal and generating the outcome, each of which being represented by voltages. The behavior of the circuit, which represents

[1] Note: A detailed description of an operational amplifier is available in many electronics text books including Sedra and Smith (2014).

the behavior of the signaling system, is modeled by a simple set of equations.

We can set the input of the amplifier $V_1(t)$ equal to the sum of the input voltage $V_s(t)$ and the feedback β from the output voltage at (t-τ) so that

$$V_1(t) = V_s(t) + \beta V_o(t-\tau) \quad (1)$$

and

$$\frac{V_o(t)}{V_s(t)} = A_o + \frac{\beta A_o V_o(t-\tau)}{V_s(t)} \quad (2)$$

where

$$V_o(t) = A_o V_1(t) \quad (3)$$

Here $V_o(t)$ is the output voltage at time t, $V_s(t)$ is the input signal at time t, β is the feedback coefficient, A_o is the gain of the amplifier and $V_o(t-\tau)$ is the output voltage at a time τ seconds earlier than t. If we apply a step function input to an amplifier with negative gain A_o and content feedback with a time delay τ we get an output voltage that decays exponentially in steps with intervals of τ. If we assume that the input signal is given by $V_s = V_{in}\cos(\omega t)$ and $V_o \cos(\omega t - \theta)$ where $\theta = \omega\tau$, the steady state equation can be rewritten as

$$A_f = \frac{V_o(t)}{V_s(t)} = \frac{A_o}{1 - \beta A_o \frac{\cos(\omega t - \theta)}{\cos \omega t}} = \frac{A_o}{1 - \beta A_o(\cos\theta + \tan \omega t \sin\theta)} \quad (4)$$

From this equation it is easy to see that that the sign of the feedback changes as the phase angle θ changes. The term $(\tan \omega t \sin\theta)$ varies from zero to infinity with ωt so that our overall gain, A_f, oscillates between zero and $A_f = \frac{A_o}{1-\beta A_o \cos\theta}$ in time with ωt. When , the system breaks into oscillation with no externally applied signal. As θ is frequency dependent the response of our amplifier system is also frequency dependent. If we examine the system at times when $t = 2n\pi$ the term $\beta A_o \cos(\omega\tau)$ changes sign with frequency and A_f will increase or decrease from the value for a system with zero time delay with changes in frequency. A more realistic model that describes the effects of time delays in the control of the frequency in biochemical oscillators is presented by Novak and Tyson (2008). An additional example for the modeling of a more complex system is given by O'Clock (2016) and points out the some of the problems in modeling a cell system with multiple feedback loops and nonlinearities.

If our externally varying magnetic field is sinusoidal it can be shown that the sign of the feedback varies as cos θ where $\theta = \omega\tau$ and ω is the angular frequency of the applied signal and τ is the time delay in the feedback loop. The time delay is a function of many variables including the distance between the source of the ROS and the source of the antioxidant. Thus the net result is that the concentrations of the ROS can either increase or decrease with change in the frequency and amplitude of the externally applied magnetic fields. The feedback mechanism's parameters, the effective gain or time delay, may be changed through other mechanisms as well as that of the radical pairs. The parameter changes could result from a change in ROS or counterpart reactive nitrogen species (RNS) or from some other regulatory or signaling molecule.

3. Experimental results at RF

3.1. ROS and other radicals

A substantial number of experimental results show weak magnetic fields can change the concentrations of ROS and modify biological systems. A clear cut demonstration that RF field can modify radical concentrations in solution is described in the paper by Woodward et al. (2001). In this paper they present measurements across the spectrum (1–80 MHz) of the effect of a weak (500 μT) radio frequency magnetic field on the electron-hole recombination of radical ion pairs in solution. Distinct spectra are observed for the pyrene anion/dimethylaniline cation radical pair in which one or both of the radicals are perdeuterated. The observed spectra show resonances in the 5 MHz, 30 MHz, 45 MHz and 50 MHz regions, indicating changes in the chemical reaction rates of up to approximately 25%. Additional resonances are calculated up to frequencies up to 150 MHz.

Usselman et al. (2014) have shown that for rat pulmonary arterial smooth muscle cells, enhanced cell proliferation was observed with continuous applied 45 μT static magnetic field (SMF) and 7 MHz, 10 μT RMS magnetic fields compared with the control group with only 45 μT SMF. The RF magnetic fields enhanced cellular proliferation by up to 40% on day two in proportion to the SMF control group, and at three days, it led to a decrease of 45% in O^{*2-} and an increase in H_2O_2 of 50%. Note that the calculated SAR is estimated to be approximately 0.12 W/kg. In other results, Castello et al. (2014) have shown that the exposure of HT1080 fibrosarcoma cells to 45 μT SMFs oriented vertical to the plane of growth or to SMFs combined with weak 5 and 10 MHz RF magnetic fields of 10 μT RMS perpendicular to the static field inhibits the growth rate. Cell numbers were reduced up to 30% on day two for the cells exposed to the combination of SMF and a 10 MHz RF magnetic field compared with the SMF control cells. In addition, cells exposed to 10 MHz magnetic fields for 8 h increased H_2O_2 production by 55% (Castello et al., 2014). The results demonstrate an overall magnetic field-induced biological effect that shows elevated H_2O_2 levels with accompanying decrease in cellular growth rates. These effects are time dependent and different cells can respond in opposite directions. Both the forgoing results are believed to occur through the interaction of the RF fields with hyperfine transitions between energy level associated with the generation or absorption of the radicals in the cells.

3.2. Feedback processes

The recent review by Goldstein and Kopin (2017) indicates the ubiquity of feedback through a great many examples of feedback processes in biology and medicine. It is difficult to study directly the effects of RF on the feedback parameters in the equations. However, the overall effects of RF on input and output have been studied. Included in these are the examples above by Usselman et al. (2014) and Castello et al. (2014) of how RF, the input signal of the feedback process, can affect ROS levels. In addition, He et al. (2016) found that 120 μW/cm^2 900 MHz RF induced more protein and also mRNA levels of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme which plays an important role in the repair of damaged DNA, than a 1.5 Gy dose of gamma rays. The feedback process can be interfered in these experiments and many others as well. For example, Maskey et al. (2013) found effects of mobile phone RF on hippocampal immunoreactivity of various calcium binding proteins that were blocked by ginseng.

4. Some possible implications

Change in the concentrations of H_2O_2 have been shown to both increase and decrease the growth rates of cells. See Fig. 5. (Halliwell and Gutteridge, 2011) As changes in magnetic fields have been shown to both increase and decrease both concentrations of H_2O_2 and cell growth rates there is a correlation that warrants farther investigation.

It has also been shown that extended excess concentrations of reactive oxygen species, ROS, are associated with cancer, aging and Alzheimer's (Droge, 2002). During exercise concentrations of ROS can increase by 10–15 times those at rest. However, these elevated concentrations return to their normal resting levels. However, extended ROS elevations can lead to a resetting of the base line and this is when damage is believed to occur. See Fig. 6. There is a pathway from H_2O_2 to cancer via damage to DNA, lipids and proteins by a direct chemical attack, depletion of ATP and NAD^+, activation of poly (ADP-ribose) synthetase i.e PARP (Nilson et al., 2017).

The data from the Interphone study is a possible place to start to see

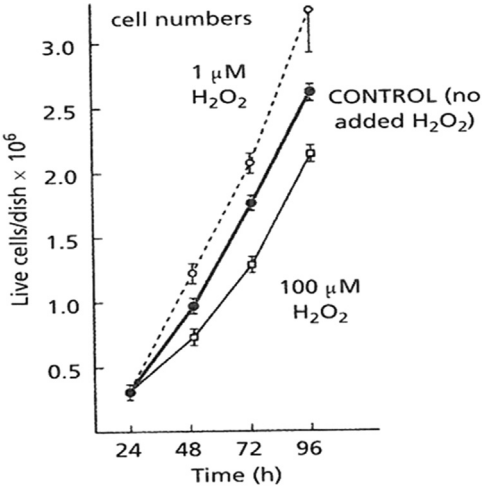


Fig. 5. The effect of H_2O_2 on the proliferation of baby-hamster kidney fibroblast cells. Monolayer cultures were treated with 1 μM H_2O_2, which stimulated cell division, or 100 μM H_2O_2, which inhibited it. Data from Radic. Res., 29, 121, 1994, by courtesy of the late Professor Roy Burdon and Harwood Academic Press. For a more recent example see Zhou et al. (2011).

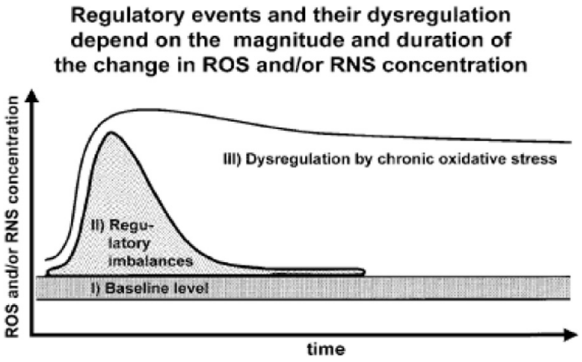


Fig. 6. Concentration levels of ROS as function of time under normal conditions and with extended and by dysregulation (Droge, 2002).

what the implications might be as the result of exposures to radio frequencies. The Interphone study results show no overall elevated odds ratio (OR) for brain tumors was observed 5–10 years after first phone use. However, in the 10th decile of recalled cumulative call time, > 1640 h, the OR was 1.40 (95% CI 1.03–1.89) for glioma, and 1.15 (95% CI 0.81–1.62) for meningioma (Cardis et al., 2010). There are some implausible values of reported use in this group. There are other studies that show increased odds ratios for exposures to RF and low frequencies in the range from 1.5 to 2.

The combined number of new cases of brain and other nervous system cancers in the US for men and women per year in 2014 was estimated to be 8.4 new cases per 100,000 per year. These rates are age-adjusted and based on 2010–2014 cases and deaths. (https://nccd.cdc.gov/uscs/braincancersbytumortype.aspx).

If we use the data from the interphone study we can estimate that this number would increase by about a factor of 1.4 to about 11.8/100,000 for brain tumors among the heaviest cell phone users. This number might be compared to the number of traffic fatalities of 10.92 per 100,000 population per year in the US in 2015 (NHTSA, https://crashstats.nhtsa.dot.gov/Api/Public/ViewPublication/812376, Accessed 26 December 2017). It is very clear that much more complete data is need before the conditions where exposures to electromagnetic fields leads to or does not lead to increases in the incidence of cancers will be understood. This includes knowing the magnitude, frequency, pulse repetition rates, and the length of the exposures. Exposures that lead to damage may well be required for years. Additionally the biology may well vary from person to person and be vary for a given person with time and state of health.

5. Conclusions

There are now both theoretical reasons and experimental results that show that weak magnetic fields can modify radical concentrations in biological systems. Both increases and decreases in the concentrations of these radicals are expected to be observed as a function of the amplitude, frequency and length of exposures for externally applied electric and magnetic fields. Additionally as these radicals are signaling molecules that can modulate the concentration of antioxidants and other molecules. The net result can be a feedback system containing a time delay which in turn can lead to either amplification or inhibition of the radical concentrations.

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