

Oxidative stress-induced biological damage by low-level EMFs: mechanism of free radical pair electron spin-polarization and biochemical amplification

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Abstract

Low-level electromagnetic field (EMF) interactions with organisms are based on the physics and chemistry of electron spin shifting of the transient radical pair and triplet state molecules formed by homolytic bond splitting within cells, and on the biochemistry of non-linear dynamic processes as they are related to the biological amplification of the EMF-induced initial effect. These processes, alone or in combination, could induce biochemical signal transduction interaction pathways by which weak EMFs can cause organism dysfunction and disease. EMF effects originate for the most part in the geminate recombination processes where free radical pairs are created. No recombination permitting electron spin shifting can result from local EMF effects on unpaired electrons if both free radicals are tethered by interactions with macromolecules or supramolecular biological structures at the right separation distance. Any field-induced change in the concentration of the free radicals that survive recombination may alter the rates of their subsequent reactions. These effects can become quite pronounced and harmful for man by existing dynamic, non-linear biological mechanisms that amplify the biochemical effects of small changes in radical concentrations, especially those of oxygen-centered free radicals responsible for the creation of genotoxic oxidative stress. This synergistic mechanism is supported by experimental evidence from vast EMF exposure studies on various biological systems (human/animal cell cultures, whole animals, and even plants) covering static magnetic, extra low frequency and radiofrequency fields (SMF, ELF and RF, respectively); SMF (as low as 0.05 W/m²), ELF 3-195 Hz (as low as 10 μT) and RF 400 MHz-300 GHz (as low as 0.2 W/m² and SAR 0.016 W/kg). In brief, EMF exposure has been shown to cause high oxidative stress-induced biological damage, manifested by a substantial increase of peroxidized lipids, oxidized proteins and fragmented/nicked DNA. Substantial decrease has been also documented in the antioxidant defense mechanisms, i.e., in the activity of crucial antioxidant enzymes and in the concentration of endogenous antioxidants. Exogenous antioxidants and inhibitors of certain ROS/RNS-producing enzymes reversed all these effects, which is another strong evidence for the causative relation between oxidative stress and EMF exposure. EMF-induced oxidative stress

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has been also shown *in vitro* by the increase of reactive oxygen/nitrogen species (ROS/RNS) indirectly assessed by non-specific assays. New quantitative and specific *in vivo* ROS assays are proposed for the conclusive verification of the oxidative stress mechanism, as well as specific quantitative indicators of biological damage that can be used for the reassessment of the EMF exposure limits. The present report offers a combined free radical pair/oxidative stress mechanism in order to explain how EMFs can cause disease in man. Moreover, it offers a scientifically solid background mechanism for the experimental design of epidemiological studies, while it extends its conclusions to the redefinition of safer EMF exposure limits for the public.

Key words: disease, EMF, oxidative stress, free radicals, radical pair mechanism

“Are there biological effects? The engineers and the physicists say absolutely not. Their view in general of what living systems consist of, is that the cells are little plastic bags filled with minestrone soup. And you can then, with that sort of a concept, calculate the field strength and the frequencies you would need to produce an effect on the minestrone soup. And this is exactly the concept that was employed after it became apparent that radar systems could heat up the human body. The physicists that were involved in answering the question: Are there effects? And at what level do they occur? And what would be a safe level? Basically, they followed a basic precept, which was to consider a spherical cow; a circular oval object filled with conducting solution and composed of a skin that is transparent to the radio frequency waves that microwave generators produce. And on that basis, they asked: How much does it take to heat this up? Where does the cow’s temperature start to rise? And that number was calculated and confirmed in actual procedures in the lab using the spherical cow concept. They said, “OK, that’s the number at which you are going to start heating people. Let’s say that’s not such a good idea and we’ll set a level ten times lower as the safe level”...“I have no doubt in my mind that at the present time the greatest polluting element in the earth’s environment is the proliferation of electromagnetic fields.”

Robert O. Becker, M.D., author of the books *The Body Electric* and *Cross Currents: The Perils of Electropollution* (interview: www.emrnetwork.org/pdfs/becker.pdf, accessed on June 2, 2010)

Introduction

Several non-thermal mechanisms have been proposed to explain the effect of low level EMFs (ELFs and RFs; extremely low frequency and radiofrequency fields, respectively) and static magnetic fields (SMFs) on biological systems and man. They involve e.g. induction of electric currents by acceleration of ions, resonant interactions involving driving vibrations or orbital transitions in biomolecules¹, direct interactions of EMFs with moving electrons within DNA², and forced vibrations of free ions of the cellular surface that distort the gating of electro-sensitive channels on the plasma membrane. Another proposed mechanism of action is that EMFs increase free radical activity. This mechanism is supported by experimental evidence and is based on sound physics and chemistry principles³⁻⁷.

The free radical mechanism presumes that EMFs must interact with the biological system via their electric and/or magnetic component. External electric fields, especially the low intensity ones, are strongly attenuated by polar organic molecules such as those composing the human body, thus, they become insignificant compared to external magnetic fields. On the other hand, since the magnetic field is essentially unchanged it

is a more likely source of biological effects. This has been supported by epidemiological studies with magnetic fields stronger than about $0.4 \mu\text{T}$ (superimposed on the geomagnetic field)⁸, and by direct biological and biochemical evidence from studies e.g. with fields $\sim 100 \mu\text{T}$ on murine fibroblast-derived 3T3-L1 preadipocytes and on rat brain cells (causing free radical induced increase of oxidative stress and significant DNA fragmentation, respectively)^{9,10}.

The effects of low-level electromagnetic radiation (ELF and RF) on a biological system can be explained by the free radical pair mechanism. This involves the recombination of short-lived species, such as reactive free radicals, whose importance in biology and disease is well established. It has been known that magnetic fields influence a certain class of chemical reactions that involve short-lived free radical intermediates through kinetic processes in an indirect manner⁴. Such chemical reactions occur widely within the body, and they maybe influenced by the magnetic field component of EMFs, which, unlike the electric field component, is not greatly attenuated inside the body and can affect the biochemistry within it.

In brief, the free radical pair mechanism requires the creation of free radicals in pairs with correlated electron spins^{3,6,11-14}. The thermal and enzyme reactions that produce free radicals in biological systems normally involve singlet states of the precursor molecules. The electrons in the chemical bond that breaks homolytically to form free radicals have antiparallel spins, as do the resulting free radicals themselves. Since the electron spins must be antiparallel to form a bond, the free radicals might be expected to recombine immediately. However, the energy released by the reaction causes them to separate rapidly so that relatively little instantaneous reaction occurs. Subsequently, the magnetic interactions of the electron spins with the nuclei of nearby hydrogen and nitrogen atoms modify the spin state of the radical pair, giving to it partially a triplet character. Therefore, EMFs stabilize free radicals in such a way as to permit their dispersion rather than their return to the ground state¹⁵. The effect of the field is indirect, and depends on the mixing of the singlet state and the existing three triplet sub-levels of the radical pair, two of whose energies are field-dependent. The prolonged lifetime of free radicals will increase the probability of radical-mediated biological damage, if the radicals involved are oxygen free radicals (such as superoxide and hydroxyl radicals) responsible for the development of oxidative stress¹⁶.

There is ample evidence that EMFs in their entire frequency spectrum induce increase of oxidative stress and oxygen free radicals in many experimental systems (including plants) and in man. Therefore, the free radical pair mechanism by working synergistically with the biological mechanism of oxidative stress provides the required coupling of EMFs to the chemistry of biological systems. Moreover, this combined mechanism overcomes the thermodynamic restrictions (imposed by EMFs non-ionizing energy), which say that the interaction energy of any electric or magnetic moment induced or possessed by an electromagnetic source (EMFs, geomagnetism) is negligible compared to the random thermal energy any biological system possesses at room temperature. This is the argument mainly physicists use to support their basic thesis that EMF effects on biological systems cannot occur at low field strengths, implying e.g. that they cannot affect the equilibrium in a chemical or biochemical system. However, this ignores the facts that biochemical and biological processes (a) rarely run at equilibrium, (b) are controlled by the kinetics of the chemical processes occurring within them⁵, and (c) they can result in amplification of the primary effect because they are non-linear and dynamic in nature, rendering these energetic arguments irrelevant.

The present report offers a new mechanism, which is a synthesis of the free radical pair and oxidative stress mechanisms, in order to explain how EMFs can cause disease in man. This mechanism is based on solid principles of physics and on ample experimental evidence, and thus it can be central for the experimental design of epidemiological studies as well. Moreover, this report extends its conclusions towards the introduction of additional new criteria for the redefinition of safer exposure limits for the public.

Free radical reactions

In order to understand the effect of a magnetic field on a radical reaction, its association with certain fundamental aspects of chemistry needs to be explored. These aspects concern the nature of the chemical bond formed by the sharing of two electrons between atoms or groups of atoms, and what happens after it is broken in the absence and presence of an external magnetic field. Electrons possess spin angular momentum, known as spin, a vector property normally represented by an arrow in magnitude and direction. When two of them interact, the spin of one can be oriented parallel or antiparallel to that of the other. In order for a bond to form, the two electrons must have opposite spins, the angular momenta of which then cancel so that the total angular momentum of a molecule containing paired electrons is zero. The resulting molecule is said to exist in a singlet electronic state, which is the normal lowest energy state of the vast majority of biological molecules. Molecules can also exist in higher energy states that can be singlet (S) or triplet (T) electronic state (also denoted by a superscript ‘1’ or ‘3’, respectively). In the latter state (T), the two electrons with parallel spins do not form a bond but inhabit different orbitals. In fig. 1 you can see a pictorial description of spin angular momentum of S and T states (fig. 1A) and the conversion of S to T state under the influence of local (different for each electron) magnetic field (figs. 1B, 1C).

If in a molecule being in its ground state a bond is broken in a homolytic biochemical reaction, one of the two electrons of the bond ends up on each of the two free radicals formed (denoted by a superscript dot to represent the single unpaired electron). As it is known, small free radicals, especially oxygen free radicals such as superoxide radical ($O_2^{\cdot-}$) and hydroxyl radicals (OH^{\cdot}), are characterized by extreme reactivity, and their normal reaction fate is to abstract atoms (e.g. hydrogen) from molecules, and to add to double bonds and to aromatic rings. They may also dissociate to expel a stable molecule such as carbon dioxide¹⁶. The common feature of all these processes is the production of secondary free radicals. Free radicals persist separated until they encounter other free radicals during diffusion to form another chemical bond, an overall process that typically takes place at the millisecond scale after radical formation (in normal viscosity solutions).

In order to appreciate the EMF effect, the chemical implications involved can be illustrated by the following photochemical example⁵ that proceeds via an excited triplet state and is relevant in a broad sense, for example, to the photosynthetic process.

The reaction of benzaldehyde (PhCHO, Ph = C_6H_5 , in tetrachloromethane solvent) is considered, under UV light exposure. Following UV absorption, the ground state singlet molecule is excited to an excited singlet electronic state, which then changes rapidly into an excited triplet state by intersystem crossing (ISC), that is, an isoenergetic non-irradiative transition between two electronic states having different spin multiplicities:

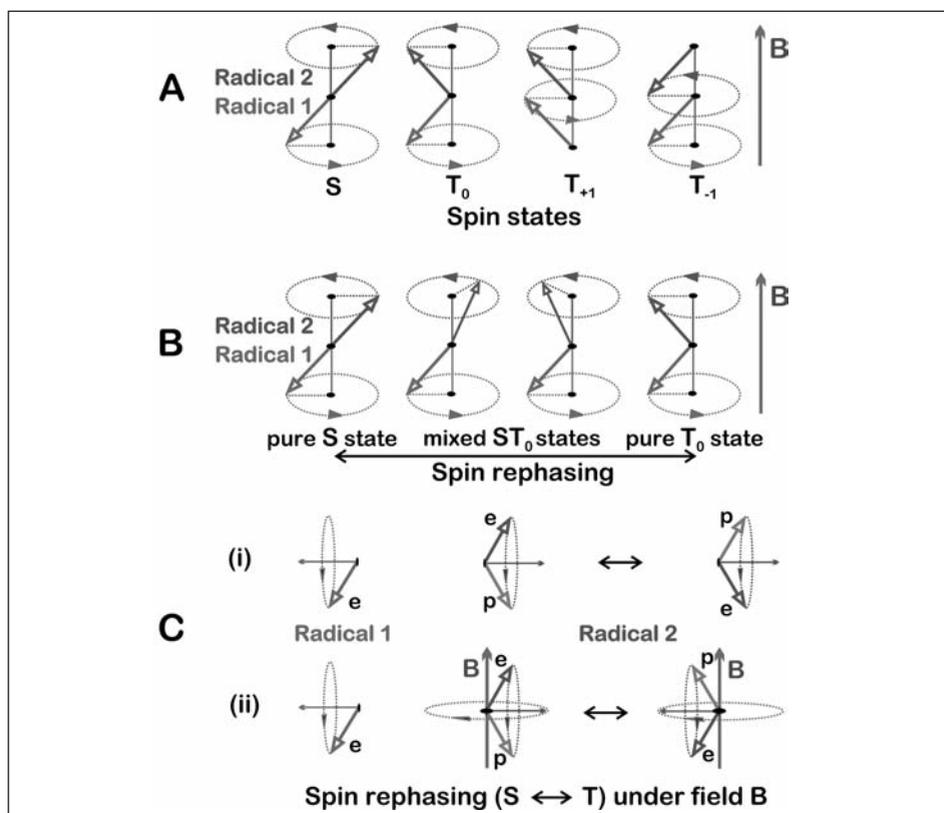


Fig. 1. A. Vector representation of the four electron spin states of the radical pair being in a magnetic field of magnitude B . The two arrows represent the intrinsic spin angular momenta of the two separate radicals. Spin state S- T_0 interconversion can occur by a simple change in the phase relationship of the two spins (see B). However, to convert electron spin S state to either of the other triplet states requires one spin to flip from one of its possible orientations to the other. Spin angular momenta can be resolved into three orthogonal components (not shown) and, as the diagram shows, the resultant component in the direction of the field is zero in the S and T_0 states, and non-zero for the others. T_0 differs from S in having a non-zero resultant perpendicular to the field in a reference frame rotating at the precessing frequency. B. The electrons precess about the magnetic field direction at different rates depending on the differing local magnetic fields at the electrons in the two radicals. This inevitably will cause an initially S state to transform into a T_0 . Between the two extremes, the radical pair shows mixed S and T_0 character. The diagram is drawn in a reference frame rotating at the precession rate of the electron of radical 1, and the electron of radical 2 is seen to move relative to it. C. Spin mixing in a radical pair concerns the relative orientations of two electron spins on separate radicals, which do not interact while the mixing occurs. That is, the one does not create a magnetic field at the other. This implies that in a radical pair, initially being in the singlet state, the evolution of the spin state of one radical is considered in relation to the others spin, whose direction is kept constant. (i) In zero field in a radical containing a single proton, the electron (e) and the proton (p) magnetic moments couple to give a resultant around which the electron and proton spins separately precess. This cannot change the direction of the electron spin completely with respect to the direction of the other. (ii) Application of a weak external field, however, establishes a local field in the radical with the coupled electron and proton magnetic moments, absent in the first case. While the electron and the proton continue to precess about their resultant, this in turn precesses about the field direction, and now the electron spin can become inverted with respect to the direction of the applied field, and to the second electron (of radical 1). Reference to (B), then, shows that an S-T conversion has been accomplished (adopted from elsewhere⁵)



The triplet state then abstracts a hydrogen atom from another molecule of benzaldehyde to form a geminate (i.e. born together) pair of free radicals, which may then combine to form a product known as the geminate cage product:



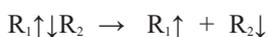
However, not all the free radicals produced react with their immediate partner free radicals because some diffuse from their initial region of formation into the surrounding medium, where they may undergo further reactions that form different products known as escape products. For example,



Various free radical reactions, therefore, continue to occur until two free radicals happen to diffuse together to form one of several possible radical combination products (additional escape products) until all free radicals are removed from the system.

Actually, an external EMF changes the probability by which the geminate free radicals recombine to form the cage product. In other words, the field alters the radical concentration and the overall escape product-to-cage product ratio^{5,15}. This experimentally established phenomenon is explained in more detail below.

The reaction mechanisms describing the spin involvement when a bond is broken in a homolytic process is based on the rule that the direction of the electron spin orientation is conserved after bond splitting. That is, the singlet molecule splits to a pair of free radicals (R_1 , R_2) the electron spins of which are antiparallel to each other at the time of formation. Both free radicals retain the same total angular momentum as the predecessor singlet molecule, and the so formed geminate radical is also in a singlet electronic state:



In the photochemical example, in particular, the geminate free radicals are formed from the reaction of the excited to the triplet state molecule. So, their electron spins would be parallel when they are formed. However, the free radicals that exist in organisms are created from molecules in singlet states that lead to singlet radical pairs. These free radicals can encounter a range of actual situations within cells. For instance, the free radicals might be produced in isotropic solution cytoplasmic regions and diffuse freely in relation to each other, and one radical may be immobilized by attachment to an enzyme surface with the partner radical able to diffuse around it (or both free radicals may be so attached), or localized within a membrane, at the time of their creation.

The fact that chemical bonds are formed between free radicals with electrons of opposite spin does not mean that the pair of singlet-correlated free radicals produced by homolytic bond splitting would quickly react to form the cage product. Some free radicals do not immediately recombine and because of the released energy they diffuse through their immediate environment. In other words, this is possible because biochemical reactions are not instantaneous but depend on overcoming a small activation free

energy, or satisfying steric requirements (i.e. a reaction may occur only if the free radicals approach each other in a certain direction). This is crucial for the effect of an EMF to manifest itself on a radical reaction, because it also depends on this rapid initial separation of the formed free radicals.

In terms of EMFs effect importance, the reactions between free radicals are differentiated by two types of reaction processes¹⁷: (1) Geminate processes, including those reactions that occur extremely rapidly as a result of encounter between pairs of free radicals created geminately with antiparallel spins from singlet precursors – they are said to involve the encounter of geminate pairs; (2) Diffusion-controlled processes, including those reactions (with large rate constants $\sim 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ in water) which occur on a longer timescale between two separately created free radicals wandering together and reacting one with the other – they are said to involve the encounter of freely diffusing pairs (F-pairs). Geminate pairs and F-pairs are strictly differentiated by the spin correlation existing at the instant of formation in the first case, and being established at the encounter in the second.

The probability of re-encounter of two geminate free radicals created together at the time origin falls rapidly with time, reaching about 10% of its initial value within about 100 ps in solutions of normal viscosity^{18,19}. However, field effects arise only 10-100 ns after radical creation.⁵ Therefore, if field effects are going to arise it is necessary to restrain the short-term diffusion of the free radicals formed in biological systems (e.g. by attachment on membrane, protein, enzyme surfaces, etc.), which has been shown experimentally with DNA and proteins (see section “the free radical pair mechanism”). This furthermore increases the re-encounter probability and increases the overall proportion of the initial radical pairs affected. This is true for F-pairs too, in which field effects also occur, but the overall effects on the chemistry involved tend to be smaller⁵.

If we consider that the half-life of superoxide radical is 1-100 ns²⁰, reaching up to 1 μs under certain conditions, it can be expected that this radical will experience external EMF effect as well. And this is very important for explaining the biological effects of EMFs, since superoxide radical is the central oxygen free radical responsible for the creation of high oxidative stress in organisms,¹⁶ as it will be explained in section 7 in more detail.

EMF effects originate from electron spin polarization

The effect of magnetic fields on free radical reactions primarily originates from the fact that the electron has a magnetic moment because it is electrically charged and has spin angular momentum. Therefore, the electron spin is the electron’s electromagnetic field angular momentum, making the electron nature’s smallest magnet. The electron spin magnetic moment is important in the interaction of atoms with external magnetic fields, in addition to the interaction between the magnetic field and the magnetic dipole moment associated with the electron’s orbital angular momentum (due to its rotation around the nucleus). Thus, free radical-involving chemical reactions are affected by the applied EMF because of its interaction with the magnetic moment of the electron.

The magnetic moment (its z-component) value associated with the electron spin has a magnitude equal to $\pm \frac{1}{2} g \mu_B$, where $\frac{1}{2}$ is the spin quantum number of the electron, g is an empirically defined constant (called gyromagnetic ratio, characteristic of the electron), and μ_B is the fundamental unit of quantum magnetism, the Bohr magneton.

The property is conveniently demonstrated in electron spin resonance (ESR) experiments where the free radicals are introduced into an applied field of magnitude B . ESR spectroscopy is based on measuring transitions between spin states of unpaired electrons by varying the applied magnetic field while irradiating the sample at microwave frequencies. However, in the absence of a field the free radicals that contain electrons of opposite spin are of equal energy, some electrons (very slightly greater than half) now align with the applied field and the others against it, and their energies differ. When the magnetic field reaches the point at which the energy difference between the two allowed orientations of the electron spin is equal to the microwave quantum ($h\nu$), a spectroscopically detectable resonance occurs at the resonant microwave frequency, ν , according to the relation $h\nu = g\mu_B B$, where h is Planck's constant; the experiment is usually performed by keeping the frequency constant and sweeping the field until a resonant absorption of energy is observed (fig. 2). Atoms and molecules with unpaired electrons (i.e. free radicals) are identified by their characteristic resonance spectra and by the so-called g value. The g value of a free electron is 2.0023, and thus, important biological radical species such as superoxide radical have a signature near the $g = 2$ region of the spectrum.

A free radical, however, does not exhibit a single field at which energy is absorbed (fig. 2). For example, the hydrogen atom (with a single electron) exhibits two resonance lines showing a characteristic splitting between them termed the hyperfine coupling constant, A_H . The methyl free radical, CH_3^\cdot (Me) exhibits a quartet spectrum with a different characteristic splitting with hyperfine coupling constant A_{Me} . For carbon-

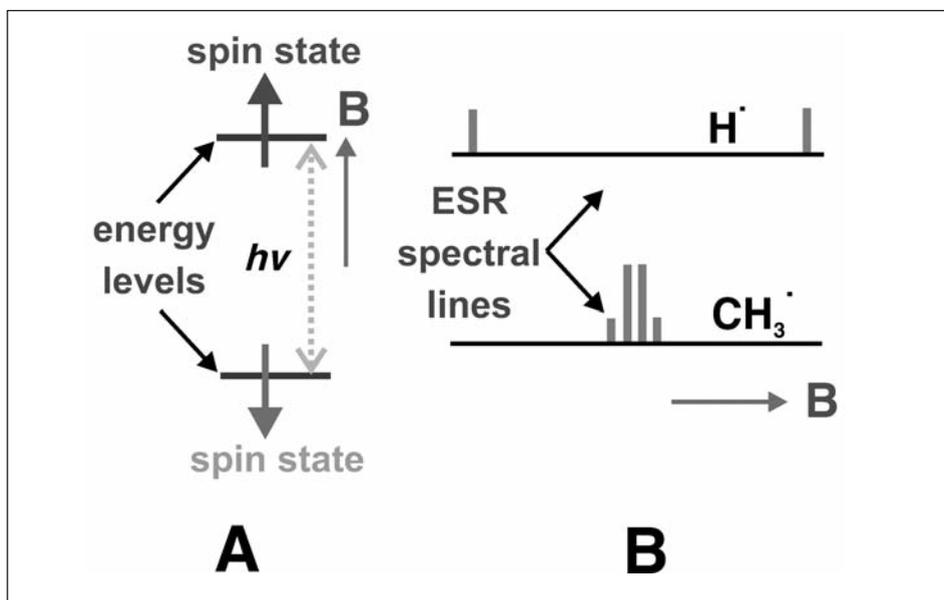


Fig. 2. A. The two spin states (antiparallel) of the electron acquire different energies in the presence of an applied field. By applying radiation at the correct frequency a spectroscopic transition can be induced between them, known as electron spin resonance (ESR) process. The magnetic moments of the electrons lie antiparallel to their spin angular momenta. **B.** Typical ESR spectra of the hydrogen atom and the methyl radical, exhibiting hyperfine structure due to coupling to the magnetic protons (adopted from elsewhere⁵)

centered free radicals, A varies from 0.01 to 3 mT, that is, it takes values even below the mean geomagnetic field (50 μ T). A is also, and more often, expressed in terms of equivalent frequency, with 1 mT corresponding to a frequency of 28.6 MHz.

The ESR-hyperfine coupling structure derives from the fact that protons are also spin $\frac{1}{2}$ species, and thus magnetic, as electrons also are. Specifically, it is due to spin coupling between protons and the singlet electron in the atom of the free radical, and is independent of the size of the applied field, B . This independence is important in understanding the effects of magnetic fields on radical reactions because it introduces the concept of individual local magnetic fields that electrons experience when exposed to external fields (fig. 1C), with both the magnetic parameters g and A signifying this. That is, the actual magnetic field experienced by the electron in the free radical is not the same with the applied field. Most importantly, the actual fields affect free radical-associated chemical reactions. The reason is that the actual field experienced by the electron of each of the two homolytically created free radicals is not the same to each other, and is not the same with the external field.

In understanding EMF effect, it should be also kept in mind that as the free radicals are created as a singlet-correlated pair in a homolytic reaction, they do not persist in this state. That is, the singlet state evolves in time into three triplet states, resulting in the so-called “ST mixing” (see vector model of this spin mixing in fig. 1B); S designates the singlet state of the radical pair, and T its triplet state. Spin evolution takes place because the electron on each radical experiences – in addition to the applied field – the local magnetic fields from nearby magnetic protons as modified by the applied field. In real systems spin state evolution occurs under the influence of many hyperfine couplings.

Radical pairs in S states can react if they encounter each other but not those in T states. Three quarters (i.e. the three T states) of the two electron spin states of the initial radical pairs are inhibited from reaction once this transformation has occurred⁵. The S–T change takes about 10-100 ns (as stated in section “free radical reactions”) when organic free radicals are involved, which is the period to allow field effects to develop, and free radicals, which then re-encounter, simply drift apart again. Because of the continuous nature of the ST mixing process, 10-100 ns later the radical pair could re-attain the singlet state but because the free radicals have become well separated the probability of re-encountering a second time and reacting is nearly zero.

Proteins containing heme as prosthetic group exhibit hyperfine coupling as well²¹. In particular, studies have shown that haemin exhibits a hyperfine structure; due to its iron ion existence in two angular momentum states ($S = 5/2$ and $1/2$). The applied magnetic field increases the occupation of the low-spin state²². Heme proteins are important biological molecules that catalyze radical reactions, and thus they can induce proton spin coupling dependent local field effects on the involved intermediate free radical substrates. Heme proteins are e.g. the important antioxidant enzymes catalases and peroxidases, the oxygen transporters hemoglobin and myoglobin, and all mitochondrial respiratory chain (and photosynthetic electron chain) cytochromes. Mitochondrial cytochromes include those responsible for formation of superoxide radical such as complex I and III (cytochrome bc_1 complex), functioning in conjunction with intermediately formed free radicals of FAD and coenzyme Q, respectively^{16, 23}.

In conclusion, external EMFs do not change the nature of the free radical reaction product. They only alter the ratio of free radicals that react in the geminate and escape processes, with consequent changes in the ratios of the amounts of cage and escape products. That is, a field may increase the number of escaping free radicals as it is sometimes

observed when free radicals are formed by a homolytic splitting of a singlet state molecule at very low field strengths, including those of the order of the geomagnetic field. Under these conditions, more free radicals survive the geminate period of reaction than at either higher or zero field⁵. This provides a possible mechanism for a field to affect biological processes, given the experimental observation that the increase of oxygen free radicals in organisms is harmful because it imposes to them increased oxidative stress. Although the formation of specific oxygen free radicals under EMF (ELF and RF) exposure has not yet been shown directly, their indirect presence (manifested as oxidative effects on crucial biological molecules such as lipids, DNA, and on the antioxidant defense) has been already documented experimentally (as shown in section “EMF-induced oxidative stress via the radical pair mechanism”).

The free radical pair mechanism

EMFs have measurable effects on the kinetics and yield of chemical reactions that use geminate radical pairs through their effect on the spin precession rates of unpaired electrons and consequent effects on the lifetime of radicals²⁴⁻²⁷. As stated previously, all free radical producing biological reactions yield their free radical products in singlet state pairs. Under the action of a local field, a free radical pair in S state at the instant of formation subsequently changes into T. This affects the probability of the reaction governed by the strict combination between free radicals of the S state only. The first stage lies in the spin-mixing process under the influence of the hyperfine interactions in the free radicals. Then, it should be taken into account the probability that the free radicals re-encounter when the pair is in a specific spin state, and the magnitude of the field effect depends intimately on the interplay between the rate processes of spin-mixing (fig. 1B) and molecular diffusion. It follows, that the lifetime of the free radical pair has a crucial effect on the magnitude of the field effect observed, particularly in the low-field region.

Spin state S–T conversion for organic free radicals lasts at least a few nanoseconds, which means that biological processes will be affected by small ELF fields if they involve long-lived radical pairs in which the free radicals remain in close proximity for about 100-1000 ns. Such time durations can exist inside cells since free radicals (such as the oxygen centered superoxide radical ion) may be formed in regions of high viscosity (e.g. in mitochondrial membrane bilayers) or of restricted motion (e.g. in or on cell walls, on enzymes, etc.). If two radicals are formed in a restricted biological site such as a lipid bilayer or a micelle, the possible spin evolution of this pair can follow two major processes: (1) reaction of the paired radicals with each other, and (2) their separation followed by reaction with other molecules present in the system. In many cases, this radical pair will have a triplet configuration (i.e., having parallel spins).

This configuration may result e.g. from the simple fact that random encounters lead to a triplet configuration 75% of the time and the rest by other means (e.g. via a photo-induced process)²⁸ as follows. Pairs of radicals in a triplet configuration cannot react with each other unless spin evolution (intersystem crossing; ISC) leads to a singlet state, where radical spins are adequate for product formation. That is, if radicals are generated in the triplet state they must move to the singlet state (spins antiparallel) before reacting. This interchange can occur as a result of local magnetic fields from nearby magnetic nuclei through the hyperfine interaction. Moderate EMFs can influence the kinetics of intersystem crossing (k_{isc}) through Zeeman-splitting of the triplet sub-levels and, as a

result, modify the partition between the radicals that react with their partner (within the radical pair) and those that separate and become available for alternative free-radical reactions. They actually remove the degeneracies of the triplet state sub-levels and can cause separation between triplet states greater than the hyperfine interaction, effectively preventing interchange of electrons and stopping up to two thirds of radical pairs reacting²⁶. These radicals that undergo escape or separation processes are those most likely to participate in reactions of relevance in the biological and health sciences (fig. 3). The fact that EMFs can modify free radical reactions implies that they should be also able to modify cellular processes¹⁵.

The individual free radical events (the lifetime of radical-radical encounters) take place in the ns to μ s time scale. Since at 60 Hz each field cycle takes 16.67 ms and a 900 MHz (GSM cell phone carrier frequency) takes 1.1 ns, one can anticipate that the radicals will “sense” SMF (static magnetic field)/ELF/RF during the short lifetime of the radical-radical encounters (radicals may have very long lifetimes but it is the lifetime of their encounters that is important for EMF interaction purposes). For example, the influence of 60-Hz magnetic fields on free radical reactions (using benzophenone as the source of pair radicals; ketyl and cyclohexadienyl radicals) can be quantitatively predicted from the knowledge of the effect of SMF on free radical behavior. Studies of radical reactions in micellar systems show that the behavior under a 60-Hz field is identical to that under a SMF at any given point in time. The following expression provides an empirical experimental data fit: % Escape = $30.4 + 28.4 (1 - e^{-0.00337 H})$, with 30.4 being the % escape at zero field and $H = 2^{1/2} H_{rms} |\sin \theta|$ (where H_{rms} the average 60 Hz-field magnitude, θ the field phase angle at the time radical generation takes place, and the use of the absolute value reflecting that radical behavior is independent of field polarity)^{15,29}.

Free radical confinement e.g. by proteins and DNA has been already shown experimentally with the benzophenone-derived pair radicals³⁰ mentioned above. Radical pairs derived by hydrogen abstraction of triplet benzophenone and some of its derivatives from bovine serum albumin, human serum albumin and calf thymus DNA are confined by proteins and DNA for a sufficiently long period of time for spin evolution to be

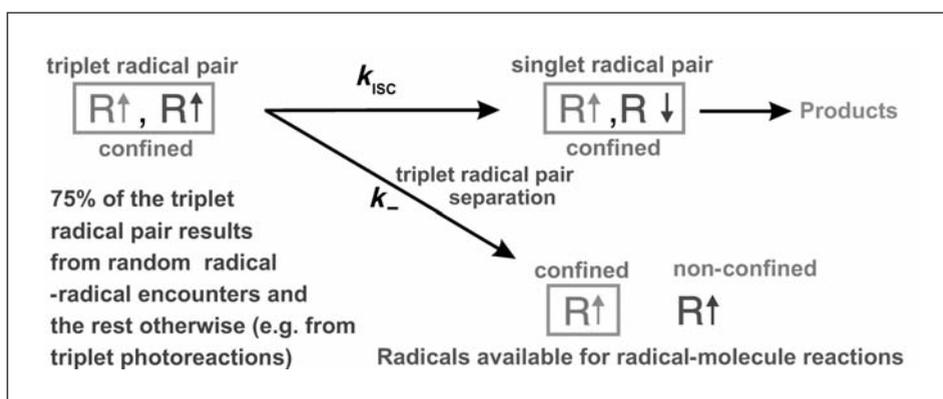


Fig. 3. A. EMF effects on paired spin radicals resulting from homolytic splitting. Under most circumstances, EMF will reduce intersystem crossing (k_{isc}), and, as a result, will increase the availability and steady-state concentration of free radicals (R). Boxes designate free radical confinement condition (adopted from elsewhere¹⁵)

affected by external EMFs. In proteins the radical pair retains its geminate character (i.e. remains confined) for about 0.5-1 μ s. For DNA, the magnetic field alters the radical reactivity only over times ≤ 50 ns, suggesting poor confinement, with electron transfer interactions maybe playing a rôle; timescale for these effects can be increased by promoting coulombic (positive-negative) attraction between DNA and the radical precursor³⁰.

Spin state S-T interconversion can be also affected by random, incoherent, "relaxation" processes (well known in isolated free radicals in ESR spectroscopy) from excited or otherwise perturbed spin states towards or into thermal equilibrium. Crucial free radicals in organisms for the development of oxidative stress, such as oxygen free radicals, although may have very short relaxation times not favoring direct EMF-effects, they become insignificant. The reason being that oxygen free radicals are very reactive, resulting in the formation of secondary carbon-centered free radicals within the geminate pair, with conservation of spin orientation. All these are more probable sources of field effects. Relaxation processes can cause either random spin flips (the so called spin-lattice relaxation process occurring with a characteristic time T_1) or change the relative phase of the components of the spins of the two electrons in the direction perpendicular to the field (with a characteristic time T_2). The former originates in fluctuating local fields (including RF-EMF's) inside the sample, and causes S and T_0 to $T_{\pm 1}$ interconversion, while the latter depends on static components of the local fields, and causes S to T_0 conversions. In normal solutions at room temperature, T_1 and T_2 are equal and of the order of a microsecond, and relaxation can usually be neglected. If, however, the free radical is restricted e.g. on a protein or in a biological membrane, T_2 can shorten considerably as a result of an increase in the rotational correlation time.

The spin-interconversion processes and rapid free radical reactions already described occur on a timescale of a few tens of ns. This means that free radical pairs see as static any field oscillating at a frequency of less than about 0.01 GHz (10^7 Hz). In particular, power mains (line) frequencies of 50-60 Hz are static on this timescale, as are the lower frequencies whose resonant effects in biological systems have been reported. Thus, magnetic field effects on radical recombination reactions remain independent of the frequency of the radiation until resonant effects are observed in the radio frequency (RF) region.^{5,31} The effects of resonant radiofrequency and microwave fields (EMF-RF) on chemical and biochemical systems observed in the presence of static fields of various magnitudes are well established. They depend upon exciting spectroscopic transitions between the singlet and triplet states of radical pairs and are fully consistent with the free radical pair mechanism^{4,17}.

The free radical pair mechanism can explain free radical-induced damage in biological systems exposed to SMF, ELF and microwave frequencies. For example, tumor-promoting phorbol 12-myristate 13-acetate (PMA) - induced oxidative burst (producing reactive oxygen free radicals) in rat peritoneal neutrophils was further increased by exposure to 60Hz. This was attributed to the increase of the probability that a free radical pair will remain in the triplet configuration (by decreasing intersystem crossing), thus increasing the probability that two free radicals will escape without termination. Because fewer terminations of radical pairs occur, the overall concentration of radicals increases, and a potentiation of free-radical induced effects in biological systems may be expected, with both time varying and static magnetic fields participating in such interactions³². In relation to RF effects, in Fe^{2+} -treated rat lymphocytes exposed to continuous 930 MHz (carrier of cellular phone emitted signals) an increase of reactive oxygen species (ROS)

was documented³³. This was attributed to RF-induced rate increase of free radical reactions taking place in the presence of Fe²⁺ (Haber-Weiss/Fenton reaction, see section “EMF-induced oxidative stress via the radical pair mechanism”), where both geminate and freely-diffusing free radical pairs are produced⁵ by the unpaired electrons containing substrates/products Fe²⁺, Fe³⁺, O₂^{•-} and H₂O₂.

EMF dependence of enzymatic reactions via radical pair recombination

The free radical pair mechanism could also function synergistically and in parallel with an EMF-induced decrease of the natural antioxidant defenses. These depend on the overall cell metabolism controlled by numerous biochemical reactions, especially those involving reactive oxygen species (ROS) such as O₂^{•-}, OH[•] and H₂O₂, and reactive nitrogen species (RNS) such as nitric oxide radical (NO[•]), peroxyxynitrite (ONOO⁻) and nitrite ion (NO₂⁻). Chemical reactions are sensitive to external magnetic fields and biochemical reactions are expected to be sensitive as well. In optimized chemical systems, the change in chemical reaction rate is typically less than 50%^{7,34-36}. On the basis of these EMF effects, six criteria have been proposed for a magnetic field to affect an enzyme reaction^{14,37}: (1) one step in the reaction mechanism should involve a catalytically competent radical pair enzyme-substrate complex; (2) the free radicals that constitute the pair must be “weakly coupled”, that is, being apart by at least 0.6 nm; (3) there must be a mechanism for the interconversion of singlet (antiparallel electron spins) and triplet (parallel electron spins) states of the radical pair; (4) the radical pair must live long enough to allow significant S–T interconversion to take place; (5) the rate of the enzyme reaction must be sensitive to the concentration of the radical pair; and (6) the reaction steps that precede the formation of the enzyme–substrate complex must be reversible such that the commitment to catalysis is low.

EMFs can affect typical Michaelis-Menten biochemical reaction kinetics scheme based on a developed model³⁸ that involves an intermediate enzyme-substrate complex where a spin-correlated radical pair state exists. This model calculates the enzyme reaction rate explicitly by combining chemical kinetics with magnetic field-dependent spin kinetics that takes into account pair radical recombination probability (radical pair mechanism). The size of the magnetic field effect depends on relations between different rate constants, such as 1) the ratio between radical pair-lifetime and the rate of magnetic field-sensitive intersystem crossing induced by the hyperfine interaction, and 2) the chemical rate constants of the enzyme reaction cycle. An amplification factor, derived from the specific relations between the rate constants, accounts for the fact that although the magnetic field-induced change in radical pair recombination probability is very small, the effect on the enzyme reaction rate is considerably larger, for example, by a factor of 1 to 100³⁸. Model simulations enable a qualitative comparison with recent experimental studies reporting magnetic field effects on coenzyme B₁₂-dependent ethanolamine ammonia lyase (coB₁₂-EAL) *in vitro* activity that revealed a reduction in V_{max}/K_M at low flux densities and a return to the zero-field rate or an increase at high flux densities³⁹. The kinetic parameter V_{max}/K_m (where K_m is the Michaelis constant) for the coB₁₂-EAL was decreased 25 percent by a static magnetic field near 0.1 T with unlabeled ethanolamine and decreased 60% near 0.15 T with perdeuterated ethanolamine. This effect is likely caused by a magnetic field-induced change in intersystem crossing rates between the singlet and triplet spin states in the [cob(II)alamin:5'-deoxyadenosyl

radical] spin-correlated radical pair.⁴⁰ The magnetic field dependent step in coB_{12} -EAL is radical pair recombination.³⁹ The documented increase in the lifetime of free radicals by EMFs leads to elevated free radical concentrations for extended periods of time^{32,39}.

Organisms contain many enzymes that use free radicals or other paramagnetic molecules as reaction centers, intermediates, substrates or products. A typical magnetic-field sensitive biochemical reaction is the reduction of hydrogen peroxide by the plant enzyme horseradish peroxidase (HRP). Changes in catalytic rates of up to 30% were found for fields up to 0.3 T^{41-45} . Another example of EMF-sensitive enzyme, mammalian this time, is the rat cerebellum free radical nitric oxide (NO) synthase, which exhibited a statistically significant increase (11.2%) in activity when exposed to pulsed DC magnetic field (0.1 mT, for 1 hr)⁴⁶. Important enzymes with paramagnetic reaction centers (and thus prone to external EMF effect) are those containing iron-sulfur reaction centers (most frequently, Fe_2S_2 , Fe_3S_4 , and Fe_4S_4 clusters). They are found in all life forms, with typical example the mitochondrial Krebs cycle mammalian aconitase and the complexes I, II and III of the mitochondrial electron transport chain. These modular clusters undergo oxidation-reduction reactions, may be inserted or removed from proteins, can influence protein structure by preferential side chain ligation, and can be interconverted. They are involved in electron transfer, act as catalytic centers and sensors of iron, dioxygen and free radicals such as $\text{O}_2^{\cdot-}$ and NO^{\cdot} , and their most common oxidation states are paramagnetic via electron spin-dependent delocalization that arises in delocalized mixed-valence systems^{47,48}. Moreover, mobile phone emission was shown to interfere with electron transfer processes that take place during enzymic reactions catalyzed by oxidases and peroxidases. These reactions proceed by generating free radical intermediate compounds, which are paramagnetic species sensitive to electromagnetic fields. Microwaves emitted by a dual band mobile phone (915-1822 MHz) altered the steady-state transition complex formed by these enzymes⁴⁹.

The most promising candidates for EMF-induced oxidative stress effects are mammal (and man) membrane bound heme-enzymes such as the mitochondrial cytochrome *c* oxidase (i.e. Complex IV)³⁷ and complexes I, III, both of which can produce $\text{O}_2^{\cdot-}$ by a single electron leaking to dioxygen. There are also enzymes that catalyze reactions that produce ROS ($\text{O}_2^{\cdot-}$, OH^{\cdot} , H_2O_2), such as the $\text{O}_2^{\cdot-}/\text{H}_2\text{O}_2$ -forming xanthine oxidase⁵⁰, the $\text{O}_2^{\cdot-}$ -forming NAD(P)H oxidase¹⁶ and possibly cyclooxygenases/lipoxygenases. In addition, there are enzymes involved directly/indirectly in RNS formation (NO^{\cdot} , ONOO^- , NO_2^{\cdot}), such as the NO^{\cdot} synthase⁵¹; peroxyinitrite (ONOO^-), in particular, is a powerful biological oxidant that can be generated by $\text{O}_2^{\cdot-}$ and NO^{\cdot} ¹⁶.

On the level of organism (and man) enzymatic antioxidant mechanisms, superoxide dismutase (SOD) - both cytoplasmic (CuZnSOD) and mitochondrial (MnSOD) - is another enzyme candidate for positive EMF effect via the pair radical mechanism. This important antioxidant enzyme catalyzes the dismutation (and thus neutralization) of two superoxide free radicals into O_2 and H_2O_2 ⁵². Having already stated that the half-life of superoxide radical is near 100 ns^{20} , an expected EMF-induced spin rephasing on superoxide radicals (experiencing different local fields due to their attachment to different biological molecules, or to SOD active site not in an identical way⁵³) may not allow their spontaneous or SOD-mediated reaction with each other, respectively, to form O_2 and H_2O_2 . In either case, EMFs may allow time for superoxide radicals to damage (directly and indirectly) important biological molecules (and DNA), and this may result in increased oxidative stress¹⁶. Moreover, ESR experiments have shown hyperfine coupling due to the presence of hydroxyl radical in the active site of CuZnSOD in the presence of

its natural product hydrogen peroxide, suggesting the possibility of SOD reaction reversal, and thus reformation of superoxide radical (from O_2 and H_2O_2). Another possible SOD reaction outcome would be the formation of a copper-bound hydroxyl radical⁵⁴. These finely tuned radical involving reactions of SOD could be possibly affected by EMFs, making the antioxidant enzyme act as an oxidant.

Amplification of EMF-induced effects on biological systems via the free radical pair mechanism

Biological effects from low strength EMFs are strongly dependent on the lifetime of the free radical pair, and consequently on the parameters affecting diffusion in the location where the pair is formed. Free radicals have been observed experimentally to escape recombination in the geminate cage in the presence of a very low (non-thermal) electromagnetic field and diffuse into the surroundings with possible harmful oxidative effects, and 30% is suggested to be possible¹⁵. If we assume the lowest reported case of 1% increase in non-recombined free radicals⁵, it can be suggested that it is very small to be harmful for the body's sophisticated antioxidant defense mechanisms under normal conditions. However, even these very low levels of escaped free radicals can become biologically harmful if the free radical pair mechanism functions synergistically with amplification biological mechanisms (e.g., EMF-induced signal transduction pathways, high free iron, etc.) and environmental stimuli (e.g., pollution factors) that would amplify the biological effect resulting from the EMF-induced small increase of free radical concentration. That is, EMFs can provoke a disproportionate biological response via biological amplification/induction of small chemical effects. Such oxidative stress-related amplification phenomena have been already documented experimentally (see section "EMF-induced oxidative stress via the radical pair mechanism").

Increased free radical concentrations in biological systems from weak EMF exposure may be quite harmful. In metabolic signal transduction chain reactions a single radical may result in the production of thousands of product molecules; biological reactions sometimes involve high gain non-linear amplifiers; and autocatalytic reactions, with chemical feedback steps, show non-linear responses to changes in reactant concentrations. In a physiological context, the small increases in radical concentrations that might arise from EMF effects should be seen in the light of antioxidant protection mechanisms against free radical attack. It is barely conceivable that biological systems in general are so finely balanced that a small change in radical concentration might have a direct effect. However in the presence of an efficient amplification mechanism, the situation can change, as if a field is applied to a system in which the defense mechanism is already severely challenged.

Amplification mechanisms depend on non-linear dynamic phenomena, which are necessary prerequisites for the creation, stabilization, and maintenance of specific states of order and function. Rhythmic phenomena are of fundamental importance for specific dynamic states of order and function in biology. The creation and stabilization of periodic states within biological systems is based on non-linear internal processes. They allow for the occurrence of temporal, spatial, or spatio-temporal structures within the system, with most prominent examples non-linear oscillations, exhibiting a regular (periodic or quasiperiodic) or an irregular (chaotic) motion. Non-linear dynamics (nonlinear equations of motion) create these regular and irregular states via self-organizing stochastic processes³.

Stochastic amplification can be exercised by cells/organisms through noise-induced bistability with oscillations, where the external noise may induce a bistable oscillatory (dynamic switching) behavior that is both quantitatively and qualitatively different from what is predicted or possible deterministically. The noise required to produce these distinct properties can itself be caused externally (e.g. by EMFs) and internally (by biochemical stimulants), making it feasible for biological systems of sufficient complexity to generate such behavior internally. This dynamics then induces stochastic amplification of signal transduction, gene expression, GTPase cycles, mitogen-activated protein kinase (MAPK) cascades, glucose mobilization, cell division/apoptosis, checkpoint control, actin treadmilling, membrane transport etc., and of metabolism in general. The main evolutionary design objectives to select for these cycles and cycle cascades are considered to be the need for switch-like elements that convert graded increases in an input to a more binary output and the demand for signal amplification, which may be necessary because the primary messengers are often present in extremely low concentrations⁵⁵.

Living organisms exhibit natural electrical oscillations as well, seen over a wide range of metabolizing systems, from primitive bacteria to man, with such coherent excitations associated with cell membrane⁵⁶ and thus with normal cell metabolism, cell-cell communication and organism function as a whole. Natural oscillations can be related e.g. to the interfacial membrane transport of hydrogen ions, to the low-frequency collective motion in biomacromolecules, to internal oscillations or photo-dissociation of solitons in alpha-helix protein molecules, to excitation of spin states in molecules or in intermediate complexes⁵⁶. The oscillation frequencies extend from the sub-Hz to the microwave (10^{10} - 10^{12} Hz) frequency region³ and can create ELF/RF-induced resonances in biological materials. For example, neurons of the basolateral amygdaloidal complex exhibit intrinsic oscillations⁵⁷, and CA3 neurons exhibit coherence and stochastic resonance in the 4–8 Hz range⁵⁸.

The interactions of the internal self-oscillating non-equilibrium biological states with external EMFs can result in many state transitions such as synchronization, sub- and super-harmonic resonances, an extreme frequency and intensity sensitivity, very sharp resonances, continuous and discontinuous frequency and amplitude changes, etc.³ This has been shown experimentally, more than two decades ago, by the effect of (1) microwave frequency and intensity on cellular response and (2) by ELF on signal transduction events in cells. In the first experiment, in single yeast cells synchronized in G1-phase and exposed to 41.7 GHz, over three growth cycles, at 0.01 W/m², 10 μ W/m², and 0.05 μ W/m² the growth rate was reduced up to 20%. These radiation intensity values correspond to a mean electric field of 1.9 V/m, 61 mV/m, and 4.3 mV/m, and to a mean specific absorption rate (SAR) of 40 mW/kg, 0.04 mW/kg, and 0.2 μ W/kg, respectively (fig. 4). The effects showed a strong dependence on frequency in a resonant-like fashion even at drastically reduced intensity^{3, 59, 60}. In the second experiment, Ca²⁺ transduction (transport across the cell membrane) was studied on rat thymic lymphocytes exposed to a (non thermal) 60-Hz sinusoidal magnetic field. It was found that after the addition of an activator (the mitogenic plant lectin concanavalin A) of the membrane-mediated signal transduction cascade in these cells, the field stimulated the Ca²⁺ uptake on the average up to 170%⁶¹. However, when a 3-Hz square-wave magnetic field was used in similar experiments the Ca²⁺ uptake by mitogen-activated lymphocytes was reduced by 45-70%^{62, 63}. The results demonstrate that cellular signal transduction pathways can be measurably influenced by non-thermal ELF field intensities. Additionally, these findings

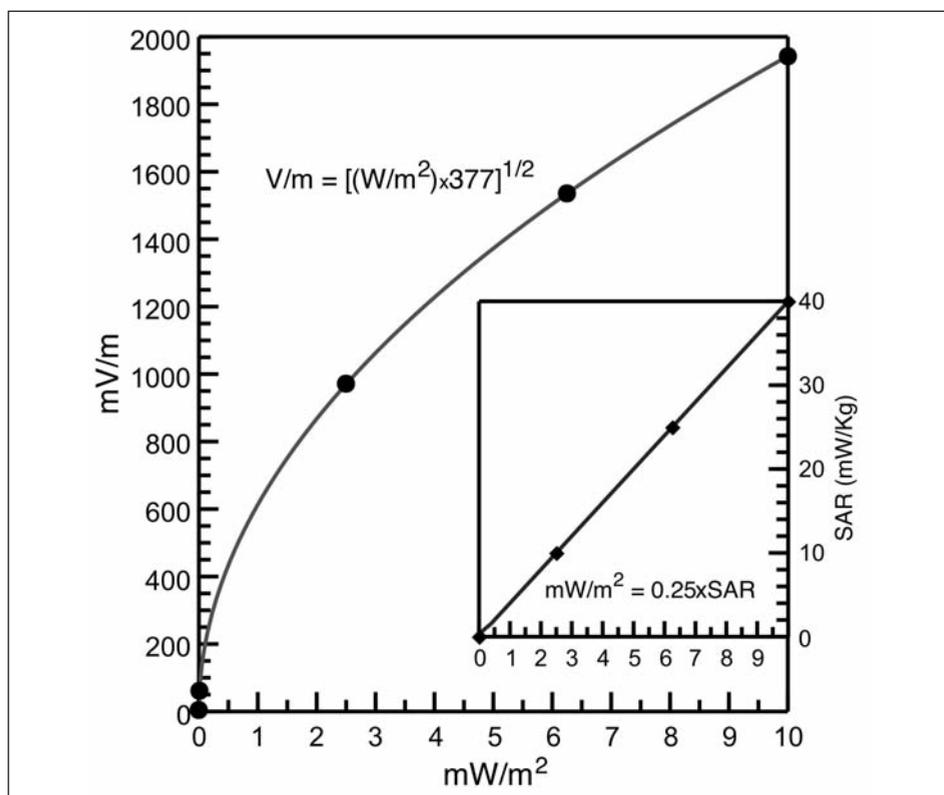


Fig. 4. Relationship among the main three unit expressions of EMF exposure limits. It is based on the formula $W/m^2 = (V/m)^2/377$, where 377 is the field resistance of air (in Ohms). The mean electric field (in V/m) is a square root function of the energy flux density (in W/m^2 , and so is the mean magnetic field [$A/m = [(W/m^2)/377]^{1/2}$]. Insert shows that SAR (in mW/Kg) is analogous to the radiation energy flux density (in mW/m^2), as pertaining to single yeast cell exposure at 41.7 GHz.³ Radiation energy flux density (and SAR) represents EMF biological exposure more accurately than the mean electric field component of EMF (normally used for expressing EMF radiation exposure limits) since electric and magnetic fields do not form separately in RF (higher than 300 MHz). This inadequacy is demonstrated by the following example: For a 250-fold exposure increase from 0.01 to 2.5 mW/m^2 , the corresponding exposure increase in mV/m (from 61 to 955) is only 16 fold. Radiation exposure misrepresentation using V/m gets even worse at lower exposure values

also show that biological parameters (i.e., the activation status) can be as important as physical EMF exposure parameters (i.e., intensity, frequency) in triggering field effects.

Sharp resonances found in the yeast experiments and the field influence on Ca^{2+} -mediated signal transduction events are two typical examples for illustrating the general idea of EMF coupling to a non-linear biological (e.g., membrane) oscillator, which in turn is coupled directly or via a chemical pathway to the internal oscillator. This process uncovers the potential of cells to amplify weak external stimuli and thus the ability to actively enhance the signal-to-noise ratio (e.g., even of EMFs modestly increased concentrations of free radicals) of received low-energy signals. EMF interactions have been studied primarily with the plasma membrane and membrane-mediated signal transduction processes. In any such interaction the primary excitation localized, e.g., some-

where on the membrane, must be translated into some persistent biochemical change in order to generate a downstream cellular effect. In some cases the sensitivity reaches the basic physical limit. For example, the ability of (1) photoreceptors to detect single photons, (2) hair cells to sense tiny displacements in the order of only a few Angstrom, or (3) cells of the olfactory system to sense only one or a few molecules is proof of the surprising ability of some specialized cell types to respond to extremely weak signal inputs in the presence of biological noise. Molecular studies of membrane signaling processes have shown, for example, that the involved cells can use mechanisms such as intracellular second-messenger (e.g., Ca^{2+} , cAMP, cGMP) cascades, positive feedback, and non-linear membrane channel-gating³.

Weak EMFs may be received and processed by cells in a manner reminiscent of sensory transduction by two ways: (1) Primary biological receptors may also act as primary EMF receptors and (2) enzymatic steps in the cellular transduction/amplification pathways may be sensitive to EMFs, even in cells which are not considered specialized sensory cells (e.g., cells of the immune, nervous, or musculo-skeletal system). There is evidence that cytochrome *P-450* and cytochrome-catalyzed reactions, which involve transient radical pairs, can be affected by weak magnetic fields^{4, 64}. This free radical pair/amplification synergism explains the ability of animals, and in particular birds, to sense the Earth's magnetic field as a source of navigational information during migration^{65, 66}. For example, when robins were exposed to vertically aligned broadband (0.1-10 MHz) and single-frequency (7 MHz) oscillating EMF of magnitude only 85 and 470 nT, respectively, the birds were disoriented⁶⁷. The suggested radical pair biochemical magnetoreceptor is located in the bird's retina, and an extraordinarily efficient process involving the visual transduction pathway amplifies the primary response to the geomagnetic field. This, together with the increasingly recognized importance of oxygen free radicals and nitric oxide in cellular regulation and signaling¹⁶, points towards a sensible EMF interaction mechanism based on electron spin-mediated field effects.

The free radical pair mechanism can also explain the hypothesis that magnetic nanoparticles, found in many organisms, mediate EMF-induced DNA damage which could result in increased risk of childhood leukaemia and other cancers. The naturally occurring magnetic field generated by a magnetic nanoparticle within a cell is calculated to be in the range of about 1-200 mT, which exceeds the level of the natural geomagnetic field by orders of magnitude. It has been shown that magnetic nanoparticles can increase the rate of free radical formation by a few percent, in the course of an idealized radical-pair reaction in a cell, and a mechanism has been proposed to explain how weak alternating magnetic fields, of the order of 0.4 μT , could cause an increase in the rate of leukaemia via mT fields produced around superparamagnetic nanoparticles in hematopoietic stem cells⁶⁸.

EMF-induced oxidative stress via the free radical pair mechanism

EMF (RF-ELF) and SMF effect via the free radical pair mechanism enhanced or not by amplification/signal transduction biochemical processes, can be exhibited by two plausible biological mechanisms involving free radicals. The first involves increased reactive oxygen and nitrogen species (ROS and RNS, respectively) and genetic damage as a response to EMF exposure. The second involves increased ROS and genetic damage because of an induced decrease of natural free radical scavenger levels, that is, decreased

antioxidant defense. With either mechanism, the net result is creation of oxidative stress. As it will be documented in the following chapter, oxidative stress has been developed in various biological experimental systems after low-level exposure to both ELF and RF, which suggests that the free radical mechanism presented above holds true for the entire EMF spectrum and SMF.

Metabolic processes that generate oxidants and antioxidants can be influenced by environmental factors, such as EMFs. Increased EMF exposure can modify the activity of the organism by reactive oxygen species leading to oxidative stress. It is well established that free radicals can interact with DNA resulting in single strand breaks. DNA damage could become a site of mutation, a key step to carcinogenesis. Furthermore, different cell types react differently to the same stimulus, because of their cell type specific redox status. On the other hand, modulation of antioxidants by ELF-EMF can lower the intracellular defense activity promoting the development of DNA damage. It has also been demonstrated that low levels of reactive oxygen species trigger intracellular signals that involve the transcription of genes and lead to responses including cell proliferation and apoptosis⁶⁹.

Oxidative stress is caused by an imbalance between the production of ROS/RNS and the biological system's ability to readily neutralize the ROS/RNS molecular components and/or easily repair the resulting damage. The most biologically destructive feature of oxidative stress is its concurrence with the production of highly oxidative oxygen and nitrogen species which are composed of both free radicals and peroxides (Table 1)^{16, 70-72}. The less reactive of these can be converted to highly reactive free radicals by redox reactions with transition metals (Fe and Cu, constituents of proteins) and biological redox cyclers such as quinones.⁷³ ROS and RNS are continuously generated under normal conditions. If their levels are not kept low by antioxidant mechanisms, they are capable of attacking lipids, nucleic acids and proteins, resulting in various degrees of oxidative damage¹⁶.

1. Reactive oxygen and nitrogen species

The term reactive oxygen species (ROS) has been used to refer to all species of oxygen that are more reactive than oxygen in its ground (O_2) or triplet (3O_2) state. These are, dioxygen in its two excited state singlet forms (1O_2), and the partially reduced forms of oxygen (i.e., superoxide radical ion and its protonated form $O_2^{\cdot-}$ and HO_2^{\cdot} , respectively), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). Superoxide radical is the most important ROS component and central element of oxidative stress because it is usually formed first in cells and it is the main source of other important ROS components (Table 1). Specifically, it is generated from molecular oxygen being reduced by a single electron. The next ROS in series is hydrogen peroxide, formed by superoxide radical capturing an electron from another superoxide radical molecule (dismutation reaction). Finally, the very potent hydroxyl radical is formed from hydrogen peroxide that captures an electron from another superoxide radical molecule or from free ferrous (Fe^{2+}) and cuprous (Cu^{1+}) ions (released e.g. from proteins oxidatively modified under abnormal conditions). Another important ROS component is singlet oxygen (1O_2), which can result from the reaction between two peroxide radicals resulting from the oxidative attack of cell membrane lipids by ROS or by UV-excitation of molecular oxygen.

ROS, like superoxide radical, are produced by various sources; e.g., from electron leaking mitochondria, and from biochemical reactions catalyzed by the enzymes

Table 1 - Reactive oxygen/nitrogen species and their contribution to oxidative stress

ROS and RNS	Formation and function
$O_2^{\cdot-}$ (superoxide free radical anion)	One-electron reduction state of O_2 : it is formed in many autoxidation and redox cycling reactions, and by electron leaking in the mitochondrial respiratory chain. It can release reactive Fe^{2+} from proteins with iron-sulfur centers and from the iron storage protein ferritin. Two moles of it dismutate to form H_2O_2 spontaneously or by enzymatic catalysis (via the antioxidant enzyme superoxide dismutase). Moreover, it is a precursor for the metal-catalyzed hydroxyl radical formation via the Haber-Weiss/Fenton reaction.
H_2O_2 (hydrogen peroxide)	Two-electron reduction state of O_2 : it is formed by the dismutation of 2 moles $O_2^{\cdot-}$, and by the direct reduction of O_2 . It can easily diffuse across cell membranes. OH^{\cdot} (hydroxyl free radical) Three-electron reduction state of O_2 : it is formed by the Haber-Weiss/Fenton reaction and from decomposition of peroxynitrite. It is highly reactive and can attack most cellular components indiscriminately.
RO^{\cdot} and ROO^{\cdot} (mainly lipid alkoxy and peroxy free radicals)	Mostly lipid peroxidation process-associated oxygen centered organic radicals, produced by free radical addition to double bonds or after hydrogen abstraction from lipids.
$ROOH$ (mainly lipid hydroperoxides)	It is formed by radical reactions with important cellular components such as membrane phospholipids (known as lipid peroxidation process).
$HOCl$ (hypochlorous acid)	Reaction product of myeloperoxidase-catalyzed oxidation of H_2O_2 . Highly reactive and easily diffusible across cell membranes. It damages proteins by readily oxidizing thiol and amino groups.
NO^{\cdot} (nitric oxide free radical)	Formed enzymically by nitric oxide synthase via five-electron oxidation of L-arginine. It is a powerful biological oxidant.
$ONOO^{\cdot}$ (peroxynitrite)	Product of the reaction between $O_2^{\cdot-} + NO^{\cdot}$. Highly reactive (as hypochlorous acid) and easily diffusible across cell membranes. In its protonated form (i.e. peroxynitrous acid) can undergo homolytic splitting to form the highly reactive hydroxyl free radical (and nitrogen dioxide).

xanthine oxidase, NAD(P)H oxidases, cyclooxygenases and cytochromes *P-450* (fig. 5). Hydrogen peroxide is produced by a wide variety of enzymes including several oxidases (e.g. glucose oxidase)¹⁶. Certain organic compounds can also produce ROS. The most important are the quinones which can redox cycle with their conjugate semiquinones and hydroquinones, and in some cases catalyze the production of $O_2^{\cdot-}$ from O_2 or H_2O_2 from $O_2^{\cdot-}$. Cells possess efficient antioxidant defense systems, composed mainly of antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidase (GPx) and catalase (CAT), which can scavenge the oxygen free radicals excessive for cellular metabolism, and make their level relatively stable under physiological conditions (fig. 6). ROS physiological concentrations are under the control of the main antioxidant enzymes working in collaboration with auxiliary antioxidant enzymes such as peroxiredoxins and sulfiredoxin, and with other enzymes having secondary antioxidant role such as paraoxonase, glutathione-S transferases, and aldehyde dehydrogenases¹⁶.

Transition metals such as iron, copper, cobalt and vanadium, freed from their enzyme hosts after oxidative attack, are capable of redox cycling (accepting and donating in cycle single electrons). This cyclic process catalyzes reactions that produce ROS, with

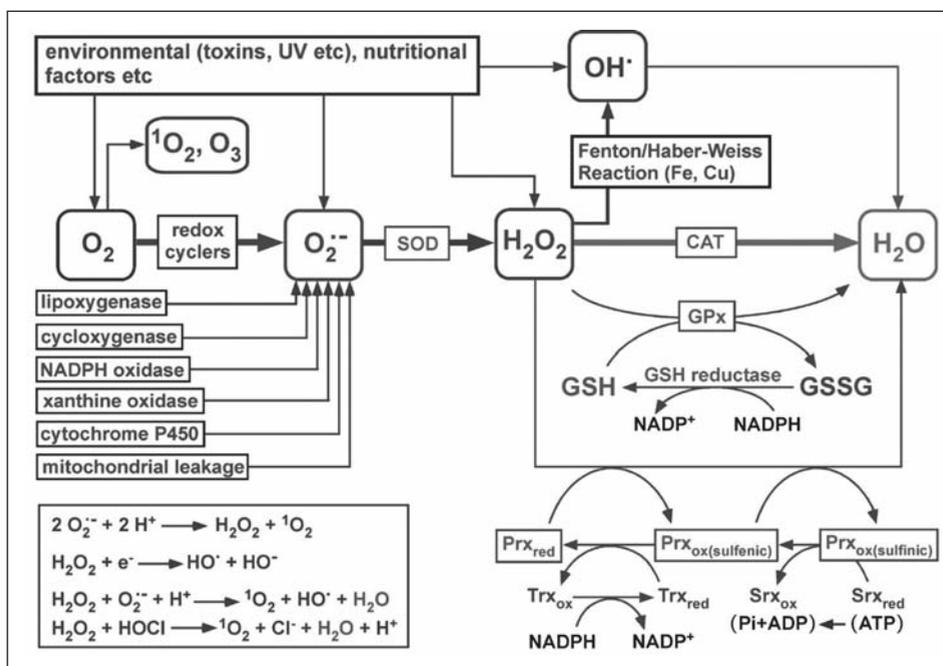
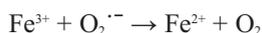


Fig. 5. Mechanism of ROS production and enzymatic antioxidant protection: ROS are produced either by atmospheric molecular oxygen excitation into ozone and singlet oxygen (O_3 , 1O_2 , respectively) or by reduction into superoxide radical, hydroxyl radical and hydrogen peroxide ($O_2^{\bullet-}$, OH^\bullet , H_2O_2 , respectively). Species O_3 , 1O_2 , $O_2^{\bullet-}$, OH^\bullet and H_2O_2 are most reactive. Superoxide radical can be generated enzymically and non-enzymically, and can react with another superoxide radical as well as with other radicals, while H_2O_2 reacts with the iron sulfur centers and cysteines of certain proteins. However, both superoxide and hydrogen peroxide can spontaneously form singlet oxygen and hydroxyl radicals, which are much more reactive. The main reactions for 1O_2 , $O_2^{\bullet-}$, and OH^\bullet are shown. Superoxide is dismutated by superoxide dismutases (SOD), and H_2O_2 is decomposed by catalase (CAT), peroxidases (such as glutathione peroxidase, GPx), and by peroxiredoxins (Prx). The thiol group of a sensitive cysteine (Cys) in Prx is oxidized to a Cys-sulfenic acid (Prx_{ox}) and is reduced by reduced thioredoxin (Trx_{red}). The Cys-sulfenic acid in Prx_{ox} can be further oxidized by H_2O_2 to Cys-sulfinic acid, which is reduced back to Cys-sulfenic acid by the reduced sulfiredoxin ($Sr_{x_{red}}$) and ATP. *iation exposure misrepresentation using V/m gets even worst at lower exposure values*

most important the Haber-Weiss/Fenton reaction that forms OH^\bullet from Fe^{2+} and H_2O_2 . The OH^\bullet then can oxidatively modify amino acids (e.g., attack phenylalanine to form *meta*- and *ortho*-tyrosine), carbohydrates, initiate lipid peroxidation, and oxidize nucleobases. Most enzymes that produce reactive oxygen species contain one of these metals. The presence of such metals in biological systems in free form (not complexed in a protein or in a metal complex) can significantly increase the level of oxidative stress.

The Haber-Weiss/Fenton reaction is catalyzed mainly by free iron (as well as by copper)^{16,74,75}, with the first step of the catalytic cycle involving reduction of ferric to ferrous ion:



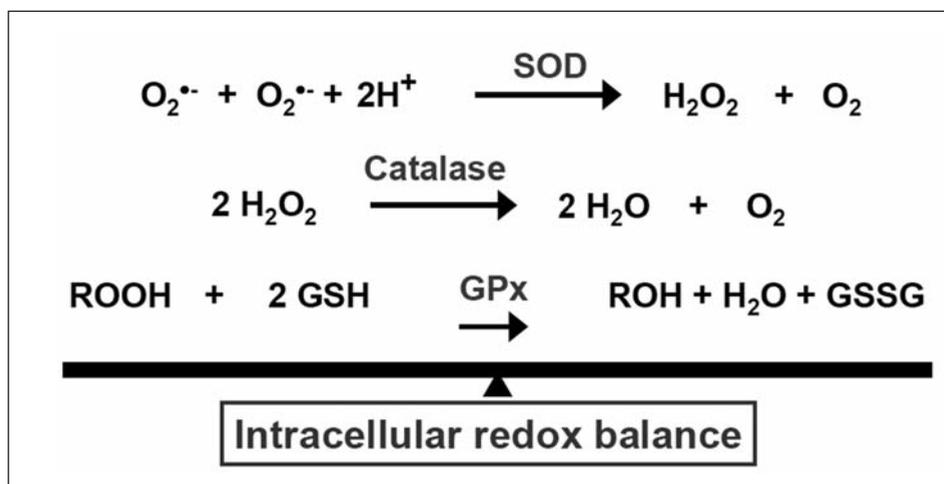
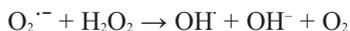


Fig. 6. Antioxidant enzymes mainly maintain reactive oxygen species-regulated intracellular redox balance. Superoxide dismutase (SOD) converts superoxide radical to hydrogen peroxide, which, in turn, is neutralized to molecular oxygen by catalase (CSAT). Hydrogen peroxide and other toxic biological hydroperoxides (ROOH) such as lipid hydroperoxides (byproduct of lipid peroxidation) are also neutralized by glutathione (GSH) peroxidase (GPx) and are converted to alcohols (ROH). The resulting oxidized glutathione (GSSG) is converted back to GSH by the enzyme glutathione reductase at the expense of NADPH (not shown)

The second step is the Fenton reaction⁷⁶,

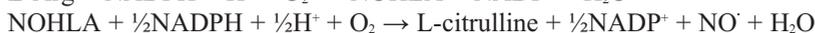
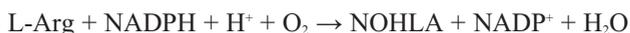


and the net reaction is



This metal-catalyzed reaction can occur in cells and is therefore a possible source for increased oxidative stress and genotoxicity.

High oxidative stress can also result from increased reactive nitrogen species (RNS) such as nitric oxide radical (NO[·]), peroxynitrite (ONOO⁻) and nitrite ion (NO₂⁻); especially peroxynitrite, is a powerful oxidant and nitrating agent. Because of its oxidizing properties, peroxynitrite can damage a wide array of molecules in cells, including DNA⁷⁷ and proteins. With proteins in particular, it is involved in nitration of tyrosine residues. Dysfunction of proteins due to nitration has been related to several cardiovascular diseases, including autoimmune myocarditis, hypertension, and heart failure⁷⁸. Nitric oxide (NO) is a central molecular component of RNS. It is produced by nitric oxide synthase via five-electron oxidation of a guanidino nitrogen of L-arginine (L-Arg) to L-citrulline, which occurs by the following two successive monooxygenation reactions producing intermediate N^ω-hydroxy-L-arginine (NOHLA):



Formation of peroxynitrite *in vivo* has been ascribed to the reaction of the free radical

superoxide with the free radical nitric oxide⁷⁹, with the latter formed by nitric oxide synthase⁵¹:



The resultant pairing of these two free radicals results in the formation of peroxy-nitrite, which is not a free radical but a powerful oxidant. Conversion of NO[·] (by O₂^{·-}) to the toxic ONOO⁻ undermines NO[·] antioxidant rôle in regulating lipid peroxidation induced by ROS⁸⁰. NO in lipid reactions is important since (a) it significantly concentrates in lipophilic cell compartments, thus enhancing its ability to regulate oxidant-induced membrane lipid oxidation, and (b) it reacts with LO[·] and LOO[·]⁸¹.

2. Oxidative stress-induced biological damage upon EMF exposure via the Haber-Weiss/Fenton reaction

In order for the Haber-Weiss/Fenton reaction to take place, and thus cause serious biological damage, it requires the presence in cells and biological fluids of free transition metals, such as iron (Fe) and copper (Cu), together with organic peroxides (e.g. hydrogen peroxide, lipid hydroperoxides)¹⁶. Metabolically active cells require high respiration rates, which, in turn, create high electron flux via the mitochondrial electron transport chain. This results in an increase of electron leaks (mainly from coenzyme Q-cycling in Complex III) to molecular oxygen and the formation of superoxide radical. The Haber-Weiss/Fenton reaction can take place in organisms *in vivo* because Fe can be released from [Fe-S]-containing enzyme centers upon superoxide radical and peroxy-nitrite attack. For instance, an important enzyme that could leak iron from its [Fe-S] cluster upon such attack is the mitochondrial aconitase⁸²⁻⁸⁷. Candidates for the Haber-Weiss/Fenton reaction are cells undergoing abnormal proliferation, having high concentration of free (labile) iron and being under ROS/RNS-associated redox signaling control such as cancer cells⁸⁸. Another iron source comes from superparamagnetic iron-particles (magnetites) in body tissues, particularly in the brain⁸⁹. Such example is the dopamine and 6-hydroxydopamine-mediated free iron release from ferritin magnetic nanoparticles, which may lead to substantial lipid peroxidation (via the Haber-Weiss/Fenton reaction) of the substantia nigra in the brain, and explains the pathogenesis of fever-induced Parkinson's disease⁹⁰. In general, metal ions such as Zn, Fe and Cu are known to participate in neurobiological processes, and major neurodegenerative disorders such as Alzheimer's and Parkinson's diseases are characterized by elevated tissue Fe and miscompartmentalization of Cu and Zn⁹¹. Such high iron situations could enhance free radical activity in cells and cellular-damaging effects that could be amplified by EMF exposure. There is ample experimental evidence supporting this hypothesis throughout the entire electromagnetic spectrum, steady magnetic fields (SMF), ELF's and RF's, and in the presence of free iron it is attributed to EMF-induced rate increase of the free radical-forming Haber-Weiss/Fenton reactions, where both geminate and freely-diffusing free radical pairs are produced since the involved reaction substrates/products Fe²⁺, Fe³⁺, O₂^{·-} and H₂O₂ possess unpaired electrons^{5,33}. Oxidative stress-inducing biological damage (e.g. involved in carcinogenic and neuro-generative) upon EMF exposure can also result by other metals such as heavy metals⁹².

SMF/ELF effects: The involvement of copper in Haber-Weiss/Fenton reaction-induced lipid peroxidation was shown indirectly in an *in vivo* study involving steel

workers working from 3-10 years and more than 10 years at processing shops in the presence of a heater where they were exposed to 50 Hz (1.3 mT). Lipid peroxidation was increased by 28% and 56%, respectively, accompanied by decreased ceruloplasmin levels (by 41% and 54%, respectively)⁹³, suggesting that the released copper due to decrease of ceruloplasmin contributes in the increased generation of free radicals. In Fe²⁺-pre-treated rat lymphocytes exposed to 50 Hz (20, 40, 200 μ T, for 1 hr) ROS levels (measured non-specifically with fluorescent dichlorofluorescein diacetate) were increased by 14%⁹⁴, and in a similar study with isolated rat-liver microsomes simultaneously Fe²⁺-treated and exposed to a SMF (5 mT, for 40 min) lipid peroxidation was increased up to 12%⁹⁵. In another experiment, intact erythrocytes incubated with Fe²⁺/ascorbate mixture and exposed also to SMF (0.5 mT) induced a 20% decrease in hexokinase activity and a 100% increase in methaemoglobin production⁹⁶. The involvement of the Haber-Weiss/Fenton reaction was also shown in rat peripheral blood lymphocytes exposed to 50 Hz (7 mT, for 3 hrs) with/without pre-treatment with melatonin and ferrous chloride. DNA damage was significantly increased by 690% in lymphocytes only after simultaneous exposure to ELF and treatment with iron, while treatment with antioxidant melatonin prior to ELF exposure reduced the amount of damaged cells in a concentration-dependent manner, clearly implying the involvement of ELF-amplified levels of free radicals in DNA damage⁹⁷. Similar effect was documented in rat (Wistar male albino) lymphocytes exposed to SMF or 50 Hz (7 mT, for 3 hrs), which caused increase in the number DNA damaged cells (by 20% or 15%, respectively) only when incubated with FeCl₂, and this was attributed to the substantial increase of ROS generated by Fe²⁺ via the Haber-Weiss/Fenton reaction⁹⁸. Moreover, in a study involving SMF exposure alone, rat peripheral blood lymphocytes pre-treated with FeCl₂ exhibited increased lipid peroxidation (by 152%), which was further amplified by an extra 23% when the cells were simultaneously treated with FeCl₂ and exposed to SMF (7 mT, for 3 hrs). In addition, simultaneous SMF/iron treatment caused a significant increase in apoptotic and necrotic cells (by 83% and 50%, respectively), accompanied by a decrease in cell viability (by 27%). All these effects were attributed to the Haber-Weiss/Fenton reaction mechanism⁹⁹.

RF effects: The Haber-Weiss/Fenton reaction-associated effect with RFs are very limited to a study that showed induction of ROS formation induced by the frequency carrier of signals emitted by a typical cellular phone. In Fe²⁺-treated rat (Wistar male albino) lymphocytes exposed to 930 MHz (continuous wave, at 5 W/m² corresponding to SAR 1.5 W/kg, for 5 and 15 min), a 16% increase of ROS (measured non-specifically by dichlorofluorescein diacetate) was observed³³.

3. EMF exposure amplifies oxidative stress-related metabolic processes by extracellular stimulants and signal transduction pathways

It has been already hypothesized that EMFs may provoke disproportionate oxidative stress response by amplification of their primary oxidative stress-inducing free radical effect. There is ample experimental evidence that this amplification phenomenon can be provoked by extracellular stimulants (e.g. environmental pollutants) as well as by non-linear intracellular processes (e.g. signal transduction pathways), with the latter being under the influence of oxidative stress. Oxidative stress has been known to affect directly enzymes participating in signal transduction pathways, especially those involved in Ca²⁺ homeostasis. For example, oxidative damage in the membrane enzymes Na⁺/K⁺-

ATPases and Ca²⁺-ATPases containing functional –SH groups (thus, vulnerable to oxidative attack by ROS/RNS) can disturb Ca²⁺ homeostasis, resulting in its intracellular accumulation. This, then, can lead to phospholipase and protease activation and Ca²⁺ accumulation in mitochondria, events that contribute to cell metabolism disturbance and eventually to cell death¹⁶.

ELF effects: We have already presented experimental evidence showing that increased intracellular free iron levels can amplify the initial increase in ROS formation (via the Haber-Weiss/Fenton reaction) upon ELF exposure^{33,94-100}. This phenomenon is observed by other ROS stimulants besides iron. For example, the combination of 60-Hz exposure (1.2 mT, for 3 hrs) and the oxidant *t*-butyl-hydroperoxide (an organic lipid hydroperoxide analogue) increased ROS (non-specifically measured by chemiluminescence) by 40% in mouse brain homogenates¹⁰¹, suggesting that ELF could deteriorate the antioxidant defense system via the Haber-Weiss/Fenton reaction, where lipid hydroperoxides in the presence of transition metals form cancer-promoting alkoxy free radicals¹⁰². The combination of 50 Hz-field exposure (40 μT, for 1 hr) and *in vitro* UVA irradiation (photochemical/free radical reaction inducing non-ionizing radiation) on rat lymphocytes caused the oxidative deterioration of DNA attributed to the oxidative stress-radical pair mechanism¹⁰³. The synergistic effect of 60-Hz exposure (0.1 mT, real time exposure) and of the ROS and tumour promoter phorbol 12-myristate 13-acetate (PMA) on rat peritoneal neutrophils increased by 12.4% their oxidative burst (H₂O₂ production, non-specifically detected by the 2',7'-dichlorofluorescein fluorescent probe)³². The same ROS stimulant (PMA), when combined with 60-Hz exposure (22 mT, for up to 10 min), induced in human neutrophils (PMN) a 26.5% increase of superoxide radical production (measured *in vitro* in cell culture by the SOD-inhibited reduction of ferricytochrome *c*) and a 53% increase of β-glucuronidase release (controlled by intracellular signaling)¹⁰⁴.

The association of signal transduction pathways with ELF effects was also shown by the following Ca²⁺ uptake studies, although the experimental approaches were not designed to investigate their relation with oxidative stress. Rat thymic lymphocytes exposed to 60-Hz (sinusoidal magnetic field, 1 mV/cm, for 1 hr) showed Ca²⁺ uptake increase by 2.7 fold after the addition of the activator concanavalin A (mitogenic plant lectin), and this stimulation of Ca²⁺ metabolism was attributed to a membrane-mediated signal transduction cascade in these cells⁶¹. The relation of calcium uptake and its metabolism with apoptosis (indirectly with oxidative stress) has been also shown in mouse lymphocytes¹⁰⁵.

Many other experiments with ELFs (3-60 Hz, 0.02-22 mT) have documented various signal transduction-associated biochemical effects (e.g. 50-100% synthesis increase in *c-myc* and 30-50% increase in uridine uptake in HL-60 cells, 8% increase in cell cycle progression of phytohemagglutinin-activated human peripheral blood lymphocytes, etc), which are related to induced membrane-mediated Ca²⁺ signaling processes in cells of the immune system¹⁰⁶. Another ROS-dependent signal transduction pathway affected by ELF is the Na⁺-dependent choline uptake in brain cells of the central cholinergic systems. In this study, rats (male Sprague-Dawley) exposed to 60 Hz (up to 1 mT, for 45 min) showed a ~50% decrease in Na⁺-dependent, high-affinity choline uptake (HACU) (at ≥0.75 mT) in the frontal cortex and hippocampus brain synaptosomes. Pre-treating the animals with the narcotic antagonist naltrexone blocked such ELF effects. Given the fact that activity and subcellular trafficking of the Na⁺-coupled choline transporter is regulated acutely by peroxynitrite¹⁰⁷, naltrexone blocking effect can be attributed to its antioxidant action. It reduces inducible nitric oxide synthase activity (thus

decreases the formation of the free radical NO^\cdot and peroxyxynitrite, its reaction product with $\text{O}_2^{\cdot-}$ in neuronal cells and oligodendrocytes¹⁰⁸. In humans, changes in cholinergic activity of the brain can lead to various neurological and psychiatric disorders, such as Alzheimer's disease¹⁰⁹.

ELFs can even induce ROS/RNS-controlled cell proliferation signal transduction pathways in animals and plants. This was shown in primary chick embryo fibroblast (CEF) cultures and in *Spirodela oligorrhiza* (a small aquatic plant, commonly known as Duckweed) exposed to 100 Hz (0.7 mT, for 24 hrs), where enhanced cell proliferation was observed. To demonstrate that free radicals may induce enhanced CEF proliferation, cells were exposed to the ROS production-inducing ascorbate/ Fe^{2+} system, which enhanced the rate of cell proliferation by 6% compared with control cells. In the absence of radical scavengers, cell proliferation was enhanced by 33% compared to the sham exposed cells, while in the presence of the antioxidant enzymes CAT and SOD, and of vitamin E, the enhancement of cell proliferation was reduced by 79, 67, and 82%, respectively, compared with their sham exposed cells^{110,111}. In another study with HL-60 cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts exposed to 50 Hz (0.50, 0.75 and 1.0 mT, for 3–72 hrs) there was a 30% increase in cell proliferation of all cell types after 72-hr exposure to 1 mT, as well as 25% increase of the percentage of cells in the S phase for Rat-1 cells after 12-hr exposure. These effects were prevented by pre-treatment of cells with vitamin E, suggesting that free radical reactions were involved in this signal transduction-regulated amplification phenomenon¹¹².

SMF effects: Oxidative amplification was shown in the following experiment combining the effects of environmental and chemical factors with steady magnetic field (SMF) exposure. Combined SMF exposure (25-150 mT) and UVA (>300 nm) irradiation of the non-steroidal anti-inflammatory agent ketoprofen (KP) and erythrocytes, significantly speeded up the time required for cell photo-hemolysis via the oxidative stress-inducing radical pair mechanism¹¹³. This mechanism involves the initial generation of a triplet radical pair derived from the reaction of triplet state KP [or 3-ethylbenzophenone (3-EtBP)/UVA, the main photoproduct of KP which has the same chromophore as KP] with erythrocyte component(s) probably lipids. The applied SMF increased the concentration and/or lifetime of free radicals that escape from the radical pair so that the critical radical concentration needed to initiate membrane damage (lipid peroxidation) and the caused cell lysis is reached sooner. Free radical spin-trapping studies with the trap 2,6-dibromo-1-nitrosobenzene-4-sulfonate confirmed that the application of the external SMF increased the concentration of radicals released during the photolysis of either KP or 3-EtBP dissolved in media such as sodium dodecyl sulfate micelles. In another study, the combination of the potent chemical pollutant CCl_4 (injected to mice) and SMF exposure (at 4.7 T, for 3-48 hrs,) caused an increase of lipid peroxidation in liver and in glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activities, thus enhancing hepatotoxicity¹¹⁴. SMFs can also induce signal transduction pathways such as the one regulating melatonin secretion¹¹⁵. This was shown by the decrease (21.7%) of pineal *N*-acetyltransferase activity (the rate limiting enzyme in melatonin production) and by the decrease in pineal and serum melatonin levels (by 8.7% and 43.5%, respectively) in rats exposed (during the night) at pulsed DC MF (turned on and off at 1-s intervals with a rise/fall time constant of 5 ms, ranging from 50 to 500 μT , with the bulk of the studies being conducted using a 100 μT). Because of melatonin's known direct free radical scavenging action, the drop in serum melatonin could be explained by an increased uptake of melatonin by tissues that were experi-

encing increased levels of free radicals (developed via the pair radical mechanism) as a consequence of SMF exposure¹¹⁶. SMFs can even induce the signal transduction pathways leading to apoptosis (ROS/RNS-controlled)¹¹⁷. This was shown in female rats where SMF exposure (128 mT, for 10 days, 1 hr/day) induced apoptosis via increase of free radical levels and resulted in a 30% decrease of thymus relative weight¹¹⁸.

RF effects: Amplification of the RF-induced free radical effect was shown in a study where human umbilical cord blood-derived monocytes and lymphocytes were exposed to 1800 MHz [continuous wave, or intermittent GSM-DTX (hearing only, 5 min on/5 min off) and GSM-Talk (34% speaking and 66% hearing), at SAR 2.0 W/kg, for 30 or 45 min], with PMA (ROS-inducing stimulant)-pre-treated cells used as ROS production (positive) control. After continuous or intermittent exposure to the GSM-DTX signal (for 45 min), the human monocytes displayed a significant increase (by 12%) of ROS production (non-specifically detected by dihydrorhodamine 123 fluorescence) due to the synergistic effect of PMA-induced/amplified ROS and RF-increased lifetime of free radicals¹¹⁹. The synergistic induction of signal transduction pathways by RFs was shown in a study with rats (Wistar, 35 day-old) exposed to 2450 MHz (0.34 mW/cm² corresponding to SAR 0.1 W/kg, for up to 35 days, 2 hrs/day). A significant increase in Ca²⁺ efflux (by 82% after 20 min and by 118% after 35 days), and in ornithine decarboxylase activity (by 247%) was observed in the exposed group as compared to the control. Correspondingly, a significant decrease in the Ca²⁺-dependent protein kinase activity (by 57%) was observed. These results indicate that RFs at 2450 MHz affect the membrane bound enzymes that are associated with signaling transduction pathways regulating cell proliferation and differentiation¹²⁰, with both of these important biological processes being controlled by ROS/RNS¹²¹. In another study, rats (adult male albino) were exposed (for 30 min/day, for 7 days, at speech or standby position) to a commercially available cellular telephone of the GSM 900 type (900 MHz, 2 W peak power, average power density 0.02 mW/cm²) caused massive exocytosis in Merkel (epidermal) cells¹²². It was concluded that Merkel cells could detect this RF by showing an exocytotic activity via signal transduction pathways, resulting in discharge of their granules that lead the changes. Oxygen free radicals are involved in this process since it has been shown that exocytosis in HL-60 cells can be induced by 4-hydroxynonenal, a well known oxidant product of the ROS-caused lipid peroxidation process¹²³.

4. EMFs invoke oxidative stress-induced DNA damage and cell apoptosis/necrosis

EMFs can cause biological damage via oxidative stress (i.e. via ROS/RNS)-induced DNA damage¹²⁴. This is mainly done by the ROS formed via the Haber-Weiss/Fenton reaction, especially by the extremely reactive hydroxyl radical¹²⁵.

SMF/ELF effects: SMF/ELF exposure-induced DNA damage has been related with the Fe²⁺-associated Haber-Weiss/Fenton reaction by studies showing increase of DNA strand breaks in rat brain cells (acutely exposed to 60 Hz, 0.5 mT, for 2 hrs)¹⁰⁰, by the 15%-20 % increase of rat lymphocytes with damaged DNA (when exposed to SMF or 50 Hz, 7 mT, for 3 hrs)⁹⁸, by the 690% increase of damaged DNA in rat peripheral blood lymphocytes (exposed also to 50 Hz, 7 mT, for 3 hrs)⁹⁷, and by the increase of apoptotic and necrotic cells (83% and 50%, respectively) also in rat peripheral blood lymphocytes, accompanied by a 27% decrease in cell viability (after SMF exposure, 7 mT, for 3 hrs)⁹⁹.

In another study (also using rat brain cells), increase of DNA strand breaks by a field dose-dependent (0.1, 0.25, and 0.5 mT, for 2 hrs) was documented, although not tested

for relation with oxidative stress. However, increase of DNA strand breaks in cells (including human cells) exposed to ELF has been associated with oxidative stress in a number of studies, since this genotoxic ELF effect was shown to be partly inhibited by free radical scavengers. Specifically, this effect concurred by the increase of ROS in three different cell experimental systems: in macrophages from murine bone marrow after exposure to 50 Hz field (0.5 -1.5 mT, for 45 min)¹²⁶, and in monocytes derived from umbilical cord blood and human monocytic leukaemia cell line, after exposure of both cell types to 50 Hz (1 mT, for 45 min)¹²⁷.

Indirect evidence for ROS involvement in ELF-induced genotoxicity and cell apoptosis/necrosis comes from a series of studies. For example, in rats exposed to 60 Hz (0.01-0.25 mT, for 2-48 hrs) brain cells showed oxidative stress-induced increases in DNA single/double strand breaks and also cell apoptosis/necrosis, since these effects were blocked by pre-treating the animals with the free radical scavengers melatonin, *N*-tert-butyl- α -phenylnitron and Trolox (a vitamin E analogue)^{10,128,129}. In another study with HL-60 cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts exposed to 50 Hz (0.50, 0.75 and 1.0 mT, for 3-72 hrs), there was a dose-dependent increase in DNA damage (as strand breaks and 8-hydroxy-2'-deoxyguanosine formation). This effect was attributed to ELF-induced oxidative stress because it was cancelled by pre-treating cells with the antioxidant vitamin E¹¹².

Genotoxic effect (oxidative deterioration of DNA) was induced in rat lymphocytes by simultaneous UVA irradiation and exposure to 50 Hz (40 μ T, for 1 hr)¹⁰³, which was explained by the oxidative stress-radical pair mechanism. ELFs can provoke long-term genotoxic effects as it was shown in a study with rats exposed to 50 Hz (0.97 mT, 3 hrs/day) for 50 and 100 days. In particular, rat plasma showed an exposure time-correlated increase in damaged DNA (8-hydroxy-2'-deoxyguanosine formation) by 45% and 53%, respectively, suggesting the involvement of the oxidative stress mechanism via ELF-induced prolongation of free radical lifetime¹³⁰.

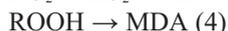
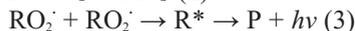
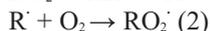
SMF exposure-associated DNA damage was observed in *Drosophila melanogaster* larvae (2- to 3-day old) exposed to a continuous magnetic field (5 T, for 24 hrs), where a significant enhancement of somatic recombination frequency was shown. This effect was suppressed by supplement of vitamin E and suggests that it is ROS/RNS-induced and exerted possibly by prolonging the lifetime of the involved free radicals¹³¹. SMF's also induced apoptosis in exposed (to 128 mT, for 10 days, 1 hr/day) female rats, which resulted in a 30% decrease of thymus relative weight¹¹⁸.

RF effects: In two studies with rats exposed to 2450 MHz (1.2 W/kg SAR, for 2 hrs), pulsed (2 μ s width, 500 pulses/s) or continuous, a substantial increase in DNA single-strand breaks was found in brain cells at 4-hr post-exposure^{132,133}. In view of the fact that DNA damage is mainly done by the ROS formed via the Haber-Weiss/Fenton reaction¹²⁵, the outcome from these studies can be attributed to the oxidative stress mechanism.

5. EMFs induce lipid peroxidation via the pair free radical mechanism

Activation of lipid peroxidation processes, irrespective of the inducer, may lead to destructive changes in the cells, which are associated with the accumulation of lipid peroxidation products (e.g. lipid hydroperoxides and aldehydes such as malondialdehyde and 4-hydroxynonenal) that are able to inactivate membrane enzymes, disturb protein-lipid interactions in membranes, form intermolecular cross-links, change viscosity of the lipid fraction, and prevent formation of enzyme-substrate complex¹⁶.

Free radical electron spin and EMF effects in biological systems are the privilege of membrane phospholipids¹³⁴, mainly because their peroxidation develops as a sequence of reactions involving free radicals¹⁶. The main chemical transformations characterizing the magneto-sensitive stages and changes in lipid peroxidation, resulting to the formation of the toxic malondialdehyde (MDA) accumulation, are described by the following reaction scheme¹³⁵:



Acceleration of free radical generation in the presence of EMFs should lead to an increase in accumulation of lipid hydroperoxides (ROOH) and MDA. This was experimentally confirmed within the temperature interval of 20-25°C¹³⁵. Competition for RO_2^{\cdot} in equations (1) and (3) depends on the initial spin state of generated radical pair (RO_2^{\cdot} , RO_2^{\cdot}) and the way of disproportionation of radicals. At the initial T state, EMFs accelerate recombination, i.e., inducing $T_{+1,0}$ -S-transitions. At the initial S-state, the sequence of events is reversed. In the last case, EMFs induce S- $T_{+1,0}$ -transitions. Temperature-dependent structural reconstructions are determined by changes in the spatial arrangement of long chains of fatty acids and polar groups contained in phospholipids. Apparently, this determines the mobility of RO_2^{\cdot} and consequently, the lifespan of the excited states and free radical pairs. Lipid peroxidation induced by EMF exposure has been documented by studies on man and various experimental systems including plants.

ELF/SMF effects: In steel workers working either from 3-10 years or more than 10 years at processing shops in the presence of a heater where they were exposed to 50 Hz (1.3 mT), lipid peroxidation was increased by 28% or 56%, respectively, and this effect was associated with the release of copper (and its participation to the Haber-Weiss/Fenton reaction mechanism¹⁶) because of a concomitant ceruloplasmin decrease by 41% or 54%, respectively⁹³.

Exposure of adult guinea pig to intermittent 50 Hz (for 4 days, 2 hrs on/2 hrs off/2 hrs on) resulted in increased plasma lipid peroxidation by 340%¹³⁶. This effect has been previously documented by Seyhan and Canseven (2006) in a cumulative report on studies with guinea pigs exposed to 50 Hz (1-3 mT, for 5 days, 4 or 8 hr/day), where lipid peroxidation increased in kidney (mainly after 4-hr exposure up to 2 mT) in response to ELF-induced increased oxidative stress¹³⁷. Lipid peroxidation levels were also increased in murine squamous cell carcinoma line (AT478) exposed to 50 Hz, and this effect was abolished after combined treatment with the natural antioxidant melatonin and ELF exposure¹³⁸. Similar effect was observed in 3T3-L1 preadipocytes (from murine 3T3 fibroblasts) exposed to 180-195 Hz (120 μ T, for 2 days, 36 min/day), where lipid peroxidation in the culture media increased by 22% after 24-hr exposure, and decreased to the control level after 48-hr exposure⁹. In two studies with rats fed/not fed with $ZnSO_4$ and exposed to 50 Hz (at 5 μ T, for 6 months, 5 min/day), lipid peroxidation in plasma and brain tissue was increased by 64% and 120%, respectively¹³⁹, and also increased in plasma, testicle and kidney,¹⁴⁰ while Zn administration caused a significant decrease of lipid peroxidation in all tissues. Given the known physiological function of Zn as antioxidant and metal constituent of the antioxidant enzyme CuZnSOD^{16,141}, these

oxidative effects can be explained by the RF-induced oxidative stress mechanism. In another study with rats exposed to 50 Hz (0.97 mT, 3 hrs/day) for 50 and 100 days plasma showed an exposure time-correlated increase of lipid peroxidation (by 35% and 65%, respectively)¹³⁰. The same phenomenon was observed in rats exposed to 50 Hz (0.018 T, for 20 days, 2 hrs/day), where lipid peroxidation in female/male rat liver and kidney tissue was increased by 88%/287% and 51%/49%, respectively. In contrast, rats exposed to SMF (0.49 T, nonlinear gradient 0-2 T/m, for the same period) showed no significant alterations in the liver and kidney lipid peroxidation levels in comparison with control groups¹⁴².

In terms of increased lipid peroxidation induced by SMFs, this was shown in a study with mice (adult male Swiss BALB/c) exposed to gradient SMF (-2.9 to +2.9 μ T) or to 50 Hz (1.4 mT), both exposure types for the same period of 30 days. Both fields showed a similar trend of action, with lipid peroxidation levels in the liver being significantly increased ~40%¹⁴³. In another study with rat peripheral blood lymphocytes pre-treated with FeCl₂, lipid peroxidation increased by 152% and this effect was further amplified by an extra 23% when the cells were exposed simultaneously to FeCl₂ and SMF (7 mT, for 3 hrs) apparently via the Haber-Weiss/Fenton reaction mechanism⁹⁹.

RF effects: In a study with volunteers (adult males 20-25 years old) exposed for 4 hrs to 900 MHz (by a cellular phone Ericsson GH 688, placed in their pocket in standby mode with the keypad of the phone facing the body -no SAR value was reported) their blood plasma lipid peroxidation was increased by 11%¹⁴⁴. Increased lipid peroxidation was also documented in human blood platelets exposed to cell phone RF 900 MHz for up to 7 min¹⁴⁵.

Lipid peroxidation induced by RF used by mobile phones and WiFi (WLAN) has been documented in many studies using rats. In a study with rats exposed to GSM 900 MHz continuous wave (1.04 mW/cm², 30 min/day for 10 days) lipid peroxidation in kidney increased by 83%. This effect was attributed to RF-induced oxidative stress since it was reversed by the administration of the free radical scavenger melatonin to the rats before RF exposure¹⁴⁶. In rats also exposed to GSM 900 MHz (from a mobile phone placed approx. 10 cm away from the rats, in the standby position and called intermittently for 4 weeks, 10 min 4 times/day), cornea and lens exhibited an increase in lipid peroxidation (860% and 128%, respectively), which was substantially reduced by antioxidant vitamin C supplementation (before RF exposure), suggesting again that mobile phone RF induces oxidative stress¹⁴⁷. Similarly, in rats exposed to 890-915 MHz (modulation frequency 217 Hz, SAR 0.52 W/Kg, averaged power 250 mW, for 1 month, 20 min/day) lipid peroxidation increased by 52% in brain tissue (without any visual histological alteration)¹⁴⁸, while in rats exposed to GSM 900 MHz (analog phone continuous wave, with brain SAR 2 W/kg and average whole body SAR 0.25 W/kg, for 1 week, 1 hr/day) lipid peroxidation increased by 28% in brain tissue, although it developed histopathological changes. Both effects were attributed to RF-induced oxidative stress since administration of the antioxidant *Ginkgo biloba* extract reversed all these effects to the control levels¹⁴⁹. Same effects were documented in a study with guinea pigs exposed to a cellular phone RF 890-915 MHz [pulse rate 217 Hz, maximum peak power 2 W, SAR 0.95 W/kg, for 30 days, 12 hrs (11 hrs and 45 min in stand-by and 15 min in speaking mode)/day], where lipid peroxidation in brain tissue and blood increased by 13%, and 44%, respectively¹⁵⁰. Significant lipid damage was also reported in a series of studies with rats exposed to 900 MHz (by a cell phone-simulating half wave dipole antenna, pulse modulated with 217 Hz repetition cycle, 2 W peak output power and 1.04

mW/cm² power density, with SAR varying between 0.016 for whole body and 4 W/kg for the head, for 10 days to 3 months, 30 min/day). Lipid peroxidation in retina and kidney increased by 43% and 47% after 10-day and 3-month exposure, respectively^{151, 152}, and increased also in myocardial tissue (after 10 day exposure)¹⁵³. This effect was attributed to increased oxidative stress since it was reversed (to the control level) by the administration of the antioxidants melatonin or caffeic acid phenethyl ester. The oxidative stress mechanism is also involved in the increased lipid peroxidation (by 50%) observed in the plasma of rats exposed to 945 MHz (pulse modulated at 217 Hz, SAR 11.3 mW/Kg at power density 3.67 W/m², for 8 days 7 hrs/day)¹⁵⁴. Increased lipid peroxidation was also documented in two studies with rats exposed to 2450 MHz (continuous-wave, with SAR 9.2 W/kg at an incident power density 40 mW/cm², for 15 min). Heart tissue damage 6 days after exposure was assessed as accumulation of the lipid peroxidation products malondialdehyde (MDA, lipid oxidation end product) and lipofuscins (complexes of oxidized lipids and proteins), which increased by 87% and 43%, respectively¹⁵⁵. Moreover, MDA in rat liver 2, 4 and 6 days after exposure increased to 1.3, 1.5, and 1.7 fold, respectively¹⁵⁶. These effects were partially reversed by the administration of the antioxidant green tea catechin, which supports the hypothesis that RF effects are exerted via the oxidative stress mechanism. Similarly, in rats exposed to GSM 900 MHz (SAR 1.2 W/Kg, for 4 weeks, with cellular phone being in the stand-by position and called intermittently 4 times/day for 10 min in on position), erythrocyte lipid peroxidation increased by 24%, and this was associated with oxidative stress because it was mostly reversed by supplementation of rats with the natural antioxidant vitamin C before RF exposure¹⁵⁷. In another study, rats exposed to cellular phone-modulated 900 MHz EMF exhibited increase of liver lipid peroxidation, which was decreased by administration of the antioxidant caffeic acid phenethyl ester (an active component of propolis extract), suggesting that EMF-induced oxidative changes in liver were reversed by strengthening the antioxidant defense system¹⁵⁸.

Lipid peroxidation can be induced by RFs even in plant tissue. This was shown by a study on Duckweed (*Lemna minor* L.) exposed from 400 MHz to 300 GHz (both RFs at field strengths of 10, 23, 41 and 120 V/m, for 2 and 4 hrs). At 400 MHz, lipid peroxidation increased by 16% and 33% at 23 and 120 V/m, respectively, while the other exposure treatments did not have an effect. However, at RF 900 MHz almost all exposure treatments significantly increased lipid peroxidation between 13% and 23%, suggesting that 900 MHz preferably induces lipid damage in plant tissue¹⁵⁹.

6. EMFs increase oxidative stress by direct change of the levels of ROS/RNS and of oxidant enzymes

Lipid peroxidation, DNA damage and alteration of antioxidant and metabolic enzyme activities are well known effects of ROS/RNS on cell metabolism¹⁶. It has been experimentally shown that ROS/RNS production can be induced by a combination of EMF exposure and stimulation/amplification by internal and external factors (see sub-section 3., p. 86). This sub-section presents experimental evidence that EMFs alone can induce production of ROS/RNS, possibly as result of the increased activity of certain oxidant enzymes.

ELF/pulsed magnetic field (MF) effects: In a study with human umbilical cord blood-derived monocytes and human monocytic Mono Mac 6 cells exposed to 50 Hz (1 mT, for 45 min) there was an increase (1.2 and 1.5 fold, respectively) of ROS/RNS

(measured non-specifically by dihydrorhodamine 123 fluorescence) and equal increase (1.4 fold) of superoxide radical (measured non-specifically by nitroblue tetrazolium chloride). This increase concurred with activation of the superoxide radical-producing enzyme NADH oxidase¹²⁷. Cellular activation processes were also observed in another study with murine macrophages and their precursor cells. When exposed to 50 Hz (1 mT, for 45 min to 24 hrs) ROS/RNS production (measured by dihydrorhodamine 123) increased by 25%. In 50 Hz-exposed promonocytes an increase (by 25%) was also observed for superoxide radical (using the non-specific nitroblue tetrazolium chloride assay), and this was attributed to NADH oxidase activation. Furthermore, in differentiated macrophages, a significant increase (up to 33%) of superoxide radical production was observed after ELF exposure¹⁶⁰. Post-exposure cell activation was observed in a study with HL-60 cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts exposed to 50 Hz (0.50, 0.75 and 1.0 mT, for 3-72 hrs). There was a ~18% increase in ROS levels (non-specifically measured by dihydrofluorescein diacetate fluorescence) as early as 3 hrs after exposure to ELF, and this increase persisted after 24-hr exposure¹¹².

ROS production by ELFs, independent of cell stimulation, was shown in the following studies: Phorbol 12-myristate-13-acetate (PMA)-stimulated mouse bone marrow-derived macrophages exposed to 50 Hz (0.5-1.5 mT, for 45 min) showed the same as the non-stimulated cells increase in phagocytic activity (36.3%) and superoxide radical production (33%, assessed by the nitro blue tetrazolium dye)¹²⁶. In another study, ELFs (50 Hz, 0.05-1 mT, for 45 min to 48 hrs) contributed to a general activation of mouse macrophages (lipopolysaccharide-activated or not), resulting in changes of numerous immunological reactions such as in increased ROS formation (1.4 fold, as measured with dihydrorhodamine 123 fluorescence), in an enhanced (by 1.6 fold) phagocytic activity, and in an increased interleukin-1 β release (up to 12.3 fold)¹⁶¹.

ELFs and pulsed DC MFs induce also RNS production, as it was shown in a study with adult guinea pig exposed to continuous or intermittent 50 Hz (1.5 mT, continuous 4 hrs/day, or intermittent 2 hrs on/2 hrs off/2 hrs on, for 4 days). Intermittent exposure caused increased NO \cdot levels (by 58%), while continuous exposure caused increase in both plasma myeloperoxidase (MPO) activity (by 45%) and NO \cdot levels (by 77%). Moreover, MPO in blood increased by 30% at intermittent exposure, and decreased in liver by 25% at both ELF exposure modes¹³⁶. It should be noted that MPO catalyzes the oxidation of H₂O₂ to the very potent oxidant product hypochlorous acid. Analogous results have been reported by Seyhan and Canseven (2006) in a review on studies with guinea pigs exposed to 50 Hz (1-3 mT, for 5 days, 4 or 8 hr/day), where NO \cdot levels and MPO activity were increased in lung and kidney, respectively, possibly in response to ELF-induced increased oxidative stress¹³⁷. In a study using pulsed DC MF (0.1 mT, for 1 hr), even crude solutions of rat cerebellum nitric oxide synthase (the enzyme that forms the free radical NO \cdot from L-arginine and NADPH; see sub-section 7.1) exhibited 11.2% increase in activity⁴⁶. Increased concentrations of NO \cdot were also observed at much higher ELF exposure levels such as those attained by a magnetic resonance imaging (MRI) apparatus. In a study with 33 male volunteers (aged 18-26 years old) exposed to a 1.5 T static magnetic field for 30 min (against a control group aged 19-26 years old) their NO levels were increased by 18%¹⁶².

RF effects: These have been shown by the following studies associating RF exposure with the RNS component NO \cdot and with ROS producing and oxidant enzymes. Rabbits (adult male albino, New Zealand type) were exposed to GSM 900 MHz (by a commercially available cellular telephone emitting 2 W peak power, average power density 0.02

mW/cm², for 7 days, 30 min/day). Serum NO⁻ levels decreased by 60% in the exposed animals compared to the sham group, suggesting a probable role of RNS in the RF-induced adverse effect¹⁶³. However, in rats also exposed to GSM 900 MHz (for RF exposure details see sub-section 5., p. 90) brain tissue NO⁻ levels and the activities of xanthine oxidase (O₂⁻-producing enzyme) and adenosine deaminase (ADA) increased by 106%, 71% and 39%, respectively. ADA, in particular, is responsible for the deamination of toxic adenosine to the physiologically less active inosine. ADA activity affects also brain function because adenosine can act as a neuromodulator and/or neurotransmitter in CNS and some peripheral systems¹⁶⁴. These effects were attributed to RF-induced oxidative stress since they were reversed (to the control levels) by the antioxidant *Ginkgo biloba* extract¹⁴⁹. In rats also exposed to 900 MHz (for RF exposure details see sub-section 7.5) NO increased by 210% and 155% in the retina and kidney, respectively^{151,152}, as well as in myocardial tissue¹⁵³, and this effect was related to RF-induced increased oxidative stress since it was reversed by administration of either one of the antioxidants melatonin and caffeic acid phenethyl ester. In another study, GSM 1800 MHz exposure [at modulations GSM-non DTX (speaking only), GSM-DTX (hearing only), GSM-Talk (34% speaking and 66% hearing)] of human Mono Mac 6 and K562 cells (at SAR 0.5, 1.0, 1.5 and 2.0 W/kg) induced a significant increase in O₂⁻ and ROS production when compared to sham and/or incubator conditions¹⁶⁵. ROS are produced at even higher RFs. Yeast cultures exposed for 20 min to a 9.71 GHz pulsed electromagnetic field (at SAR 0.5 W/kg) exhibited 20 and 50% increase of free radical production in the intra cellular compartment¹⁶⁶.

Increased ROS production via RF exposure and its relation to ROS -inducing oxidant enzymes has been documented in a study with rats exposed to 2450 MHz (for RF exposure details see sub-section 5., p. 90). Six days after exposure heart tissue exhibited an increase (by 35%) in superoxide radical production (measured *in vitro* in heart homogenates prepared after RF exposure by the SOD-inhibited reduction of ferricytochrome *c*), which slightly decreased (to 30%) after administration of the antioxidant green tea catechin. Moreover, cytochrome *P450* level was increased by 85% (and lowered to 62% in the presence of catechin), with concomitant increase of the NADPH-cytochrome *P450* reductase activity by 29%/22% (-/+ catechin, respectively)¹⁵⁵. It has been already established that ROS can be produced by cytochrome *P450* (being also a biological damage indicator) as well as by 'futile cycling'⁵⁵ e.g. of other cytochromes *P450*¹⁶⁷. In another study with rats exposed to cellular phone RF 900 MHz (for exposure details see sub-section 5., p. 90) XO activity in erythrocytes significantly increased by 50%. However, XO and ADA activities in the kidney/heart tissue decreased by 10%/22% and 22%/20%, respectively. These results were mostly reversed to the control levels by supplementation of the antioxidant vitamin C, which, again, is a strong indication of ROS involvement. Similarly, Sprague-Dawley rats exposed to cellular phone-modulated 900 MHz EMF ± the antioxidant caffeic acid phenethyl ester (CAPE) exhibited increase of XO activity, which was decreased by CAPE administration. It was concluded that CAPE may prevent the 900 MHz EMF-induced oxidative changes in liver by strengthening the antioxidant defense system via ROS reduction¹⁵⁸.

RFs can induce ROS increase even in plants as it was shown in a study where duckweed (*Lemna minor* L.) was exposed from 400 MHz to 300 GHz (for RF exposure details see sub-section 5., p. 90). At 400 MHz H₂O₂ content in duckweed increased ~30% only when exposed to 23 and 120 V/m, while at 900 MHz H₂O₂ content increased between 12% and 34% almost at all exposure treatments, and it was concluded that H₂O₂ and oxidative stress are mostly induced at 900 MHz in plant tissue¹⁵⁹.

7. *EMFs affect the antioxidant defense (enzymic/non-enzymic) and the activity of enzymes associated with biological damage/disease/metabolism*

EMFs can change the activity of the main antioxidant enzymes (SOD, GPx, CAT) and make cells more vulnerable to ROS/RNS attack. They can even affect (decrease/increase) the activity of enzymes that serve as indicators of perturbed metabolism and disease.

EMFs (ELF and RF) can induce protein oxidation: The decrease in enzyme activity, besides being indirectly controlled by gene expression¹²¹, can be due to degradation of oxidized proteins possibly resulting e.g. by EMF-induced free radical oxidative attack on crucial for activity protein domains. This is supported by the finding that in rats (Wistar-Albino female, 8 week-old) exposed to 50 Hz (1 mT, for 45 days, 4 hrs/day) a substantial increase (by 77%) of 3-nitrotyrosine was observed in female liver¹⁶⁸, suggesting a deteriorative effect on cellular proteins due to possible formation of the protein oxidant RNS component peroxynitrite (from $O_2^{\cdot-}$ and NO). For example, nitrotyrosine accumulation has been correlated with many diseases such as the prototypical autoimmune disease systemic lupus erythematosus¹⁶⁹, Alzheimer's disease and aging¹⁷⁰. Protein damage was also reported in rats exposed to 2450 MHz (for exposure details see sub-section 5., p. 90), where their heart tissue exhibited increase of protein carbonyls and lipofuscins (i.e. oxidized protein-lipid complexes) by 10% and 43%, respectively¹⁵⁵. In another study with guinea pigs exposed to power frequency electric (E) field (50 Hz, 12 kV/m, 7 days/8 h/day), no statistically significant changes occurred in protein carbonyl content, advanced oxidation protein products and 3-nitrotyrosine levels with respect to the control group. However, liver hydroxyproline level was significantly diminished in the E field exposure group compared to the control and protein carbonyl content, and hepatic hydroxyproline and 3-nitrotyrosine levels changed significantly in antioxidant N-acetyl-L-cysteine-administrated groups¹⁷¹.

ELF/SMF effects: These have been documented by studies on man and other organisms including plants. In steel workers (working at processing shops in the presence of a heater were exposed to 50 Hz, 1.3 mT) those working less than 3 years exhibited no significant changes in the activity of SOD and GPx in red blood cells. However, the activity of both antioxidant enzymes decreased by 13% in those working from 3 to 10 years, and also by 19% and 12%, respectively, in those working more than 10 years, while CAT activity was increased by 19% and 32%, respectively. Furthermore, plasma GPx showed a non-significant tendency to decrease. These effects were attributed to oxidative stress because they were accompanied by an increase of lipid peroxidation (by 28% and 56% for workers working from 3-10 years and more than 10 years, respectively)⁹³. In another study of the same research group with rats, female/male liver and kidney tissue in animals exposed to 50 Hz (0.018 T, for 20 days, 2 hrs/day) showed an increase in the activity of SOD (by 30%/67% and 62%/47%, respectively), CAT (11%/68% and 59%/85%, respectively) and GPx (17/5% and 30/4%, respectively). However, when the rats were exposed to SMF (0.49 T, non-linear gradient 0–2 T/m) for the same period, they showed no significant alterations in the activities of the antioxidant enzymes in either organ¹⁴². The combination of 60 Hz exposure (1.2 mT, for 3 hrs) and treatment of mouse brain homogenates with the lipid hydroperoxide analogue *tert*-butyl-hydroperoxide increased SOD activity by ~50% in response to increased oxidative stress¹⁰¹.

ELF-induced alteration of the enzymic antioxidant defense has been documented in other studies as well. In a study with 3T3-L1 preadipocytes (from murine 3T3 fibrob-

lasts) exposed to 180-195 Hz (120 μ T, for 2 days 36 min/day), MnSOD and Cu/ZnSOD decreased by 70% and 20%, respectively, after 24-hr exposure, and CAT increased by 45%, while no change in activity was observed in GSSG-reductase. Exposure for 48 hrs reduced significantly all antioxidant enzymes except of GSSG-reductase, without affecting the proliferation rate of 3T3-L1 cells⁹. The unchanged activity of the glutathione (GSH)-regenerating enzyme GSSG-reductase suggests that glutathione (GSH) is not involved in the antioxidant defense of these cells. In another study by the same lab, the activity of MnSOD and Cu/ZnSOD but not GPx in murine squamous cell carcinoma line (AT478) was increased upon 50 Hz exposure, and this effect was in response to ELF-induced increase of oxidative stress since it was reversed after a combined treatment with antioxidant melatonin before ELF exposure¹³⁸. Moreover, ELF-MF exposure (sinusoidal 50 Hz, 0.1 mT for 10 days) of female Sprague–Dawley rats significantly affected antioxidant capability both in young and aged animals, although in opposite ways. Exposed young individuals enhanced their neurotrophic signalling and anti-oxidative enzymatic defence (SOD, GPx, CAT) against a possible ELF-MF-mediated increase in oxygen radical species, while aged rats underwent a significant decrease in the major antioxidant enzymatic activities (CAT, GR, GPx), suggesting that exposure to ELF-MFs may act as a risk factor for the occurrence of oxidative stress-based nervous system pathologies associated with ageing¹⁷².

ELFs and SMFs can cause even extensive disturbance in metabolism as it was shown by the following study using mice (Swiss BALB/c, adult male) exposed either to SMF (gradient -2.9 to +2.9 μ T) or to 50 Hz (1.4 mT) for 30 days. Both fields showed similar trend of action; gradual body weight loss and significant decrease in serum glucose concentration, in alkaline phosphatase activity and in total protein levels (possibly resulting in decrease of the levels of important for antioxidant defense metabolic enzymes); significant increase in lactate dehydrogenase activity in serum and liver, paralleled by significant activity elevation in hepatic γ -glutamyl transferase (e.g. related to the infiltration of fat in the liver and to hypertension¹⁷³); significant increase in GSH-S-transferase (the enzyme that neutralizes oxidative stress-inducing toxic xenobiotics¹⁶) and decrease in the antioxidant thiol GSH in the liver. Furthermore, a significant decrease in the counts of monocytes, platelets, peripheral lymphocytes as well as splenic total T- and B-lymphocytes levels was observed, and the granulocyte percentage was significantly increased. These results strongly suggest a causative relation between SMF/ELF exposure and increased oxidative stress via redox balance alteration leading to extensive physiological disturbances¹⁴³. Significant perturbation of the main antioxidant thiol GSH was also shown in guinea pigs (a) in a series of studies by Seyhan and Canseven (2006) after exposure to 50 Hz (1-3 mT, for 5 days, 4 or 8 hr/day), where they reported an increase of GSH in lung and kidney¹³⁷, and (b) in another study after exposure to continuous/intermittent 50 Hz (1.5 mT, continuous 4 hrs/day, or intermittent 2 hrs on/2 hrs off/2 hrs on, for 4 days), where both modes of ELF exposure resulted in a slight decrease of GSH in blood and intermittent exposure caused GSH decrease in brain by 35%¹³⁶. These adaptive responses were possibly due to ELF-induced increased oxidative stress. Decrease of GSH upon ELF exposure was also shown in two studies with rats fed/not fed with ZnSO₄ and exposed to 50 Hz (at 5 μ T, for 6 months, 5 min/day). GSH concentration decreased in erythrocytes and brain by 40%¹³⁹, as well as in testicle and kidney¹⁴⁰. Since GSH levels were elevated to the control by the administration of Zn, these effects can be explained by the RF-induced oxidative stress mechanism given the antioxidant function of Zn¹⁴¹ and its participation in the active center of the important antioxidant enzyme CuZnSOD¹⁶.

RF effects: Antioxidant defense can be altered by RFs in man and in various experimental systems, including plants. In the previously mentioned study (sub-section 5., p. 90) with the 12 adult male volunteers exposed to 900 MHz by a cellular phone, erythrocyte antioxidant enzymes SOD and GPx decreased (by 7% after 4 hrs and by 9% after 1 hr exposure, respectively), while the levels of CAT were unchanged¹⁴⁴. Decreased SOD activity was also observed in human blood platelets exposed to cell phone RF 900 MHz for up to 7 min¹⁴⁵.

Antioxidant defense perturbation has been observed in many studies using RFs emitted by mobile phones. In rats fed/not fed with vitamin C and exposed to GSM 900 MHz (from a mobile phone, see exposure conditions in sub-section 5., p. 90) cornea CAT activity was increased by 220% while SOD was decreased by 50%. However, lens CAT and SOD activity increased by 33 and 16%, respectively, while cornea/lens GPx activity was not significantly changed. Vitamin C supplementation reduced rat eye impairments to the control levels, suggesting that the alteration of the enzymic antioxidant defense was in response to RF-induced oxidative stress¹⁴⁷. Changes in antioxidant defense were also seen in a study with rats exposed to cellular phone 900 MHz (exposure details in sub-section 5., p. 90), where erythrocyte GPx activity increased by 12% and kidney tissue CAT activity increased by 29%. These effects were mostly reversed by administration of vitamin C¹⁵⁷ and for this reason they can be attributed to antioxidant defense adaptation in response to RF-induced increase in ROS production (possibly the CAT and GPx substrate H₂O₂¹⁶). Same conclusions were drawn by another study with rats exposed to GSM 900 MHz (exposure details in sub-section 5., p. 90), where brain tissue SOD activity increased by 12% and returned to normal upon administration of the antioxidant *Ginkgo biloba* extract, while that of GPx remained unchanged¹⁴⁹. The oxidant effect of mobile phone RFs on antioxidant defense was also shown in a study with rabbits exposed to 900 MHz (by a commercial cellular telephone, see exposure details in sub-section 6., p. 93), where serum SOD activity increased by 10%¹⁶³, and in another study with rats exposed to 945 MHz (see exposure details in sub-section 5., p. 90), where erythrocyte SOD activity increased by 41% and total blood GSH decreased by 59%¹⁵⁴. In another study, rats exposed to cellular phone-modulated 900 MHz EMF exhibited increase of CAT activity, which was decreased by administration of the antioxidant caffeic acid phenethyl ester¹⁵⁸.

The alteration of the non-enzymic antioxidant defense by mobile phone RFs alter has been shown also in a study on guinea pigs exposed to RF 890-915 MHz (exposure details in sub-section 5., p. 90). The levels of the blood antioxidant vitamins A, D₃ and E, and the activity of CAT were all increased by 44%, 127%, 45%, 42%, and 13%, respectively, and they concurred by 18% decrease of GSH. Moreover, GSH and CAT in brain tissue were both decreased by 18% and 29%, respectively, while the concentration of vitamins A, E and D₃ remained unchanged¹⁵⁰. Similar non-enzymic defense changes were reported in another study with rats exposed to mobile phone GSM 900 MHz (whole body SAR of 0.25 W/Kg intermittently for 4 days, 15 min/day, or acutely for 1 hr), where there was a decrease in the plasma vitamins C (by 47% or 59.8%, respectively), E (by 33% or 65.7%, respectively) and A (by 44.4% or 46.8%, respectively). This was accompanied by a decrease in the main plasma GSH (by 19.8% and 35.3%, respectively), as well as in the antioxidant enzymes CAT (42% or 52%) and SOD (19.5% or 22%)¹⁷⁴. These results, besides their direct relation to the oxidative stress mechanism, indicate that the effects of acute mobile phone RF exposure on rat's antioxidant status are significantly higher and thus more hazardous than those of the intermittent exposure.

Similar conclusions were derived by a series of studies on rats exposed to 900 MHz (exposure details in sub-section 5., p. 90), which concurred with activity changes in enzymes-indicators of biological damage/disease. The activities of SOD, CAT and GPx in retina were reduced by 30%, 20% and 22.5%, respectively, after 60-day exposure¹⁵¹. The same enzymes showed exposure period dependent activity decreases in kidney (15%/25%, 0%/26% and 25%/18,5%, for 10-day/3-month exposure, respectively)^{152,175}, which were exhibited also in myocardial tissue after 10 day exposure¹⁵³. Increased activity (by 250%) was observed in the urinary *N*-acetyl- β -D-glucosaminidase (marker of oxidative stress-induced renal tubular damage) after 10-day exposure¹⁷⁵, which further increased to 350% after long-term (3 month) exposure¹⁵². All these effects were oxidative stress-dependent since they were reversed to the control level by the administration of the antioxidants melatonin and caffeic acid phenethyl ester. Similar effects were observed in a study with rats exposed to GSM 900 MHz (continuous wave, at 1.04 mW/cm², for 10 days, 30 min/day), where their kidney showed a 360% activity increase in urine *N*-acetyl- β -D-glucosaminidase and decrease of SOD, CAT and GPx (by 25%, 25% and 19%, respectively). Again, these effects demonstrated RF induction of oxidative stress since melatonin supplementation reversed them and ameliorated oxidative tissue injury in rat kidney via its free radical scavenging and antioxidant properties¹⁴⁶.

In another study using even higher RFs such as those used by WiFi (WLAN), rats (Wistar) exposed to 2450 MHz (exposure setup in sub-section 3, p. 86) showed a significant increase in ornithine decarboxylase (by 247%) activity and a decrease (by 57%) in the calcium-dependent protein kinase activity, both enzymes being associated with ROS/RNS controlled¹²¹, tumor-associated cell proliferation and differentiation¹²⁰. RF exposure at 2450 MHz affects antioxidant defense by inducing oxidative stress, as it has been documented in two studies with rats (for exposure details see sub-section 5., p. 90). Six days after exposure, heart tissue SOD activity decreased by 34%/25% at \pm antioxidant catechin supplementation, respectively, and so did GPx activity (28%/0%, respectively)¹⁵⁵. Moreover, SOD activity in liver decreased on the 4th day after exposure, and increased to the control level by catechin supplementation on the 8th day. Furthermore, liver GPx activity decreased on the 8th day and increased to the control level on the 16th day, an effect also attained by catechin supplementation on the 6th day. In addition, SOD and GPX activities decrease concurred with decrease in expression of the corresponding genes, which were cancelled by catechin supplementation¹⁵⁶.

Mobile phone emission has been shown to interfere with electron transfer processes that take place during the enzymic reactions of lactoperoxidase, ascorbate oxidase and laccase. The biochemical reactions catalyzed by these enzymes proceed by generating free radical intermediates, which are paramagnetic species sensitive to electromagnetic fields. Particularly, RF's emitted by a dual band mobile phone (915-1822 MHz, in receiving mode at electric field emitted intensity of 3 V m⁻¹) altered both conformational and configurational features of the steady-state transition complexes formed by these enzymes⁴⁹.

Antioxidant enzymic defense can be perturbed even in RF-exposed plants as it was shown in a study with duckweed (*Lemna minor* L.) exposed from 400 MHz to 300 GHz (for exposure details see sub-section 5., p. 90). At 400 MHz, CAT activity was increased after most exposure treatments while both activities of pyrogallol peroxidase (PPX) and ascorbate peroxidase (APX) did not change. Exceptions were the reduced PPX and APX activities after longer exposure at 23 V/m, and the increased PPX activity after exposure at 10 and 120 V/m. By contrast, at 900 MHz almost all exposure treatments decreased

mostly PPX activity and did not affect CAT activity. Exceptions were exposures to a modulated field and to the field of 120 V/m, which increased both PPX and CAT activities. At this RF, APX activity was significantly decreased after exposure at 10 V/m and 23 V/m, but it increased after a shorter exposure at 23 V/m. It was concluded that perturbation in the activities of the plant antioxidant enzymes occurs mostly at 900 MHz¹⁵⁹.

Oxidative stress induces disease in man

Living systems and man maintain a balanced reducing state within their cells preserved by antioxidant and reducing power forming enzymes through a constant input of metabolic energy. This balance is upset under increased levels of oxygen free radicals (high oxidative stress), depletes cells from ATP and prevents their controlled (apoptotic) death, thus causing cell necrosis and disease^{176,177}. Most of the oxygen-derived species are produced at low levels by normal aerobic metabolic processes, and the damage they cause to cells is continuously repaired. Normally, regulated levels of ROS/RNS can be metabolically beneficial, since e.g. they contribute to the immunological defense by attacking and killing various pathogens. In addition, they are involved in transduction signaling pathways, and in order for these redox-signaling rôles to be exercised a balance must exist between reactive oxygen production and consumption¹⁶. Therefore, disturbance of ROS/RNS normal levels, as in the case of EMF exposure, could cause cascades of biochemical reactions that may induce amplification of the primary response and result in disease in man (fig. 7).

The numerous studies already presented above show beyond any doubt that EMF exposure causes perturbation of normal redox state and results in a multiplicity of adverse biological effects through the production of various organic/inorganic ROS/RNS (oxygen and nitrogen free radicals, peroxides, hydroperoxides etc) that damage all structural and functional cell components, especially DNA¹²⁴. Besides damaging important biomolecules, which can be mostly repaired, EMFs can cause perturbation of cell/organism antioxidant defense and normal metabolism, with most prominent long term effect the non-repairable DNA damage¹⁷⁸ known to be directly associated with carcinogenesis.

Reviewing the literature on EMF (ELF and RF) effects up to 2004, Simkó and Mattsson proposed that EMFs might be a stimulus to induce an 'activated state' of the cell (such as phagocytosis, signal transduction pathways involving calcium metabolism etc), which then enhances (amplifies) the release of free radicals, leading in turn to genotoxic and other disease-causing biochemical processes¹⁷⁹. They envisage that EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels via four distinct processes: (1) Direct activation e.g. of macrophages (or other cell types) by short-term exposure to EMF leading to phagocytosis or other cell specific responses and consequently to free radical production; (2) EMF-induced cell activation includes direct stimulation of free radical production; (3) an increase in the lifetime of free radicals by EMF leads to persistently elevated free radical concentrations -in general, reactions in which radicals are involved become more frequent, increasing the possibility of DNA damage; (4) long-term EMF exposure leads to a chronically increased level of free radicals, subsequently causing an inhibition of the effects of the pineal gland antioxidant hormone melatonin. Taken together, these EMF-induced reactions could lead to a higher incidence of DNA damage and therefore to an increased risk of tumour development.

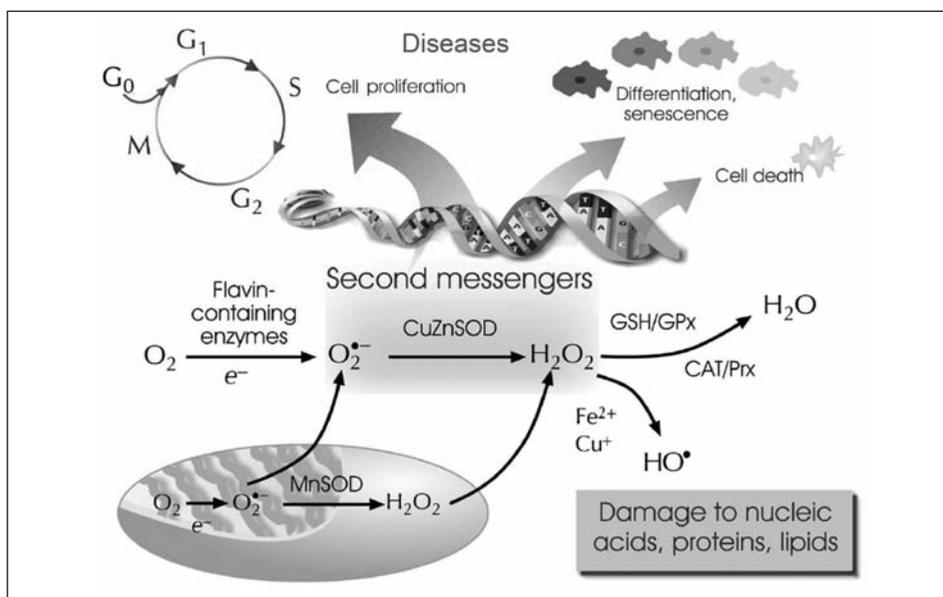


Fig. 7. Cell free radicals are responsible for disease development in man on a multistage level. EMF-induced ROS generated mainly in mitochondria or by various biochemical reactions (catalyzed by flavin-containing enzymes) can cause diseases either by inducing (as second messengers) abnormal cell proliferation and differentiation (e.g. various cancer types) and cell death (e.g. neurodegenerative diseases), or by destroying crucial for cell/organism physiological function biomolecules (e.g. DNA, proteins and lipids via hydroxyl radical attack)

In man, oxidative stress is implicated in the pathophysiology of a wide range of diseases such as multistage carcinogenesis (e.g. brain, breast cancer and cancer-prone diseases), in autoimmune, cardiovascular and neurodegenerative diseases (Parkinson's, Alzheimer's, Lou Gehring's and Huntington's disease, cerebral ischemia), in mitochondrial and respiratory diseases, human reproduction, Down's syndrome, ulcerative colitis, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, even in aging and HIV infection^{102,180-184}. Numerous epidemiological studies have linked EMF exposure with cancer and oxidative stress^{185,186}. In particular, ELF (classified as "possible human carcinogen" by the International Agency for Research on Cancer) have been linked with childhood leukemia and with increased risk for all cancer and brain tumors in relation with oxidative stress¹⁸⁷⁻¹⁹⁰. EMFs (ELFs and RFs) have also been related to oxidative stress-induced neurodegenerative diseases (as well as with suicide and depressive symptoms)¹⁹¹, and they have been linked to various long/short term diseases especially in people hypersensitive to the electromagnetic pollution¹⁹².

Opinions and implications

Low-level EMFs can interact non-thermally with biological systems primarily by spin-polarized chemical steps that can be enhanced by non-linear biological amplification mechanisms that can be triggered with internal and external factors. Free radicals

occur widely in normal biochemical reactions. Free radicals originate mostly from homolytic geminate singlet reactions. It is only the reactions involving the combinations of free radicals themselves that are EMF-dependent. Two different processes are essential to the reactions of free radicals in solution; spin evolution and diffusion. Biological effects at low EMF strength are more likely to arise in geminate radical pairs due to spin shifting from the S to T state, which would result in an increase of the non-recombined radicals largely due to the possibility of restricted molecular motion in them being more probable within cells. It has been known that an increase in the oxygen centered free-radical concentrations in the body is potentially harmful mainly because free radicals tend to be highly reactive and mostly indiscriminating in their reactions. Tissue free radical interactions with EMFs disturb tissue thresholds which control ensemble or domain functions of populations of cells, cooperatively whispering together in intercellular communication and organized hierarchically at atomic and molecular levels¹⁹³.

There are many experimental lines of evidence towards the existence of an oxidative stress mechanism implicated in the development of non-thermal biological effects by EMF (ELF and RF) and SMF exposure. This evidence strongly suggests the involvement of the free radical pair mechanism on the oxidative stress-inducing effect of EMF and SMF as amplified by various extracellular and intracellular stimulants (fig. 8). This has been shown by indirect evidence that oxygen free radicals are generated in experimental organisms and cells during and/or after exposure to EMFs. Oxygen/nitrogen free radicals uncover their presence by the various biological alterations they cause; serious damage on lipids (lipid peroxidation) and DNA (fragmentation and nicks), decrease in the activity of important enzymes involved in the antioxidant protection of the cell, and alterations in the activity of a variety of other important metabolic enzymes, all of which reflect on the harmful perturbation of the general cell/organism metabolism.

The overemphasized and monotonous argument of scientists supporting the idea of no casual connection between EMF exposure and disease in man is that there is no biochemical mechanism by which such relationship can be established. Based on this argument, then, they discount as experimentally and theoretically inadequate even epidemiological studies showing such association. The EMF-induced oxidative stress mechanism uncovered in the present treaty is based on the unification of sound physical, chemical and biochemical processes with fully supportive experimental evidence. Although it may not be the sole mechanism, the rôle of oxidative stress in explaining the adverse EMF effects on man's health may be central since free radicals are part of the physiology (both normal and abnormal) of organisms, and man. Thus, this mechanism can be extended to all future research including epidemiological studies. For example, in designing epidemiological studies based on this mechanism, parameters affecting the antioxidant defense status of the participants should be accounted for. This mechanism predicts that people with low or disease-compromised antioxidant defense due to various factors (e.g. age, poor diet, iron overload, exposure to oxidative stress-inducing working/living conditions and to various environmental pollutants, etc) are more vulnerable to the harmful effects of EMF exposure.

Until now, the evidence of oxidative stress formation under the influence of EMF's is only indirect because it has been based on the non-specific detection of ROS (free radical plus non-free radical oxidants, see Table 1), on measuring oxidative stress-induced biological effects (e.g. lipid peroxidation, DNA and protein damage, perturbation of enzymic/non-enzymic antioxidant defense), and on the reversal of all these effects by natural and artificial antioxidants (such as melatonin, ROS spin traps etc). In

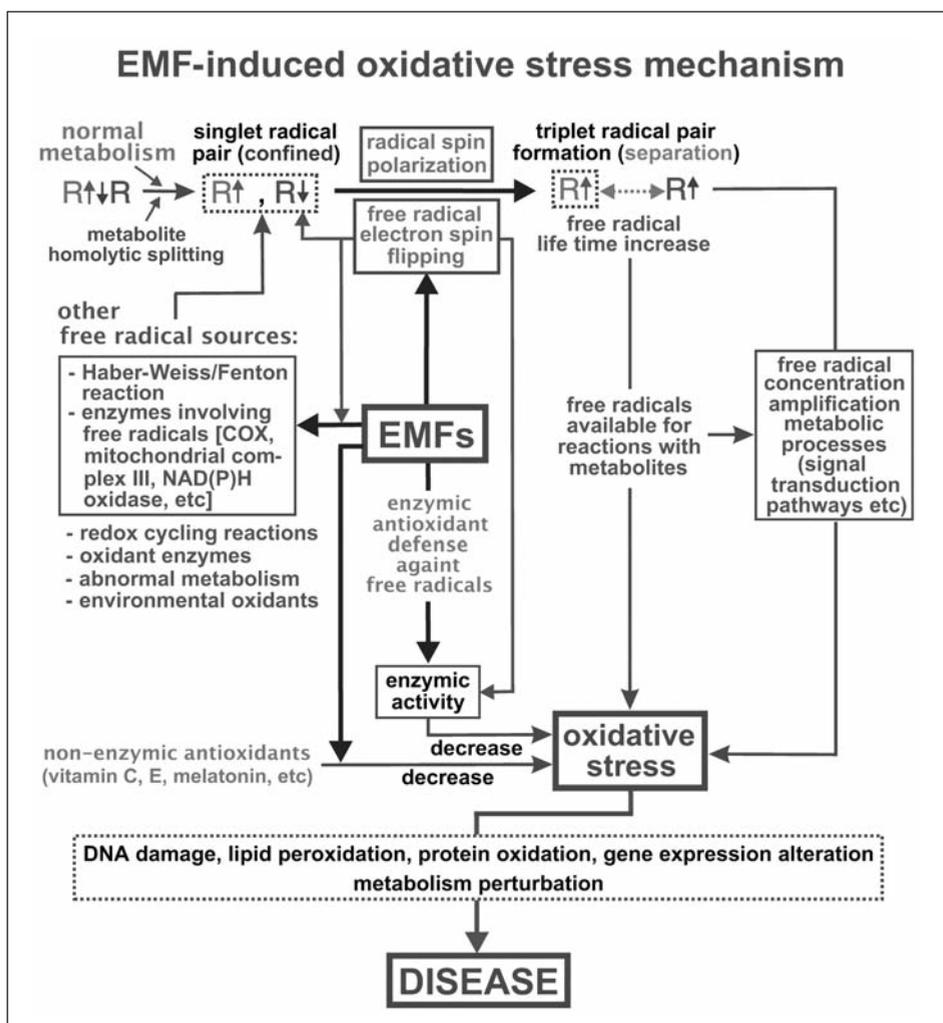


Fig. 8. Diagram of the EMF-induced oxidative stress mechanism. Free radicals are generated by normal metabolism, which involves biochemical homolytic splitting of numerous metabolite molecules, and the formation of singlet free radical pairs. EMFs mostly affect confined free radical pairs; one radical may be immobilized e.g. by attachment to an enzyme surface, with the partner radical able to diffuse around it (or both free radicals may be so attached); or the radical pairs can be localized within a membrane at the time of their creation or immobilized by proteins and DNA. Under these confined conditions and due to magnetic fields from the spin of protons adjacent to free radicals, EMF exposure makes them experience distinct local magnetic fields that can cause electron spin flipping, radical separation and concentration increase (by extending their life time). Electron spin polarization can be caused also on free radicals coming from other sources (such as the Haber-Weiss/Fenton reaction etc), as well as on those localized in the reactive centers of enzymes that catalyze free radical reactions. For antioxidant enzymes in particular, this may result in activity decrease and, subsequently, in the lowering of cell enzymic antioxidant defense. EMFs can also lower non-enzymic antioxidant defense (e.g. decrease in normal melatonin concentration etc) by non-linear metabolic processes, which, in addition, can amplify further the primary EMF effect of free radical concentration increase. This, therefore, will result in amplification of oxidative stress to levels beyond the antioxidant capacity of the cell, and, consequently, in disease development

particular, EMF-induced ROS have been assessed non-specifically by various methods (e.g. using spin traps such as *N-tert-butyl- α -phenylnitron* and *α -(4-pyridyl-1-oxide)-*N-tert-butyl*nitron* and *N-tert-Butyl- α -phenylnitron*^{10,128,129,166}, chemiluminescence¹⁰¹, nitroblue tetrazolium chloride,^{126,127,160,165} and fluorescence traps such as dihydrorhodamine 123^{119,127,160,161,165,194} and dichlorofluorescein diacetate^{32,33,94,104,112}. For example, the dihydrorhodamine 123 fluorescence assay used for detecting ROS does not only discriminate among the various ROS constituents but also between ROS and RNS since it detects indiscriminately superoxide radical, hydrogen peroxide, hypochlorous acid and peroxyxynitrite anions¹²⁷. Even in the exception studies where superoxide radical was specifically detected by the SOD-inhibited reduction it causes to ferricytochrome *c*, this assay is inherently restricted for the *in vitro* detection of superoxide radical secreted by cell cultures (e.g. human neutrophils¹⁰⁴) or in rat heart homogenates prepared after RF exposure and sacrifice¹⁵⁵. Furthermore, lipid damage (peroxidation) and protein oxidation (formation of carbonyls, oxidation of -SH groups etc) and certain DNA damage (such as 8-hydroxy-2'-deoxyguanosine formation) can be repaired by the cell. Thus, their non-detection does not imply absence of oxidative stress necessarily. Moreover, perturbed levels of the antioxidant enzymes (SOD, CAT and GPx) and the natural antioxidants (melatonin, GSH, vitamin C etc) can be attributed to oxidative stress as well as to its absence since antioxidant defense is mostly adaptive. Therefore, the oxidative stress mechanism requires more conclusive *in vivo* quantitative verification by seeking (a) direct evidence for the formation of oxygen free radicals, and (b) indirect evidence for the creation of non-repaired biological damage during and/or after EMF exposure.

It has been already pointed out that the central element of oxidative stress is superoxide radical since it is the primary source of other ROS. Thus, the quantification *in vivo* of this most important free radical during EMF exposure will provide conclusive proof for the involvement of the oxidative stress mechanism and its complementary free radical pair mechanism as well. The methodology for the quantification of superoxide radical has been recently developed^{195,196}, thus, providing an invaluable tool for future studies. On the other hand, the RNS component NO, besides the non-availability of *in vivo* specific assays for its quantification, is not a reliable free radical identifier of oxidative stress because of its many physiological functions. Non-repairable DNA damage constitutes a very valid indirect evidence for the involvement of oxidative stress, as long as it is evaluated quantitatively as DNA fragmentation. Traditionally, genotoxicity in EMF studies has been evaluated by qualitative assays, and it has been disputed as non-reproducible for that matter as well. This problem can be overcome today by the availability of quantitative ultrasensitive assays for assessing non-repairable DNA damage. Such assays measure general DNA fragmentation (0-23 Kb), even small-size (0-1 Kb) necrotic/apoptotic DNA¹⁹⁷⁻²⁰⁰. These assays actually replace the cumbersome and problematic Comet assay and the agarose electrophoresis DNA-smearing assay, both being qualitative assays.

Both superoxide radical and DNA fragmentation assays can be also used in epidemiological EMF-related studies, e.g. to monitor the antioxidant status of the selected participants. The principle behind this approach is that, if antioxidants are taken up by human subjects as part of their every day diet (or in the form of dietary supplements) they should reach the bloodstream and enter the blood cells, enhancing the ability of these cells (as well as of the plasma lipids) to resist oxidative attack when challenged *in vitro* with a source of reactive oxygen²⁰¹. The DNA damage assays, in particular, can be used to monitor the antioxidant resistance of isolated lymphocytes to DNA damage e.g.

induced by H₂O₂. In addition, thiol redox state (TRS) is another parameter for the evaluation of the antioxidant status of man (e.g. by testing blood). Recently available quantitative assays of TRS measure the main TRS components such as the oxidized/reduced protein and non-protein thiol fractions, as well as the specific antioxidant thiols glutathione (GSH) and cysteine (CSH) and their oxidized counterparts (GSSG and CSSC, respectively)^{202,203}. Moreover, the assays that quantify superoxide radical and non-repairable DNA damage¹⁹⁵⁻¹⁹⁹ may be used to derive specific quantitative markers for EMF-induced biological damage, which can be used for the determination of more reliable EMF exposure limits for the general population.

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