

EMF Science Review Symposium



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Breakout Group Reports

for

**Theoretical Mechanisms and *In Vitro*
Research Findings**

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First EMF Science Review Symposium Theoretical Mechanisms and *In Vitro* Research Findings

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The National Institute of Environmental Health Sciences (NIEHS) sponsored a workshop on March 24-27, 1997 at the Regal University Hotel, Durham, NC. The goal of this meeting was to discuss the utility and applicability of data derived from *in-vitro* mechanistic studies and predictions based on physical theory for characterizing potential human hazards associated with exposure to power line frequency electric and magnetic fields (EMF). This EMF Science Review Symposium was the first in a series of scientific meetings that brought together over 100 experts including physicists, biologists, engineers, mathematicians, and epidemiologists as well as journalists, governmental officials, representatives from industry, and the public. General overviews of selected areas were presented as lectures in plenary sessions. Breakout group sessions provided time for in-depth, small group discussions and evaluations of the quality and reproducibility of experimental findings and biophysical observations and of the degree to which they can support a causal linkage between EMF and biological and/or health effects. On the last day, an expert, scientific panel debated the scientific evidence based upon theoretical mechanisms and *in vitro* research findings for whether exposure to power line frequency EMF is a human health risk.

Included in this booklet are the complete reports from each of the breakout group sessions. The breakout group discussions of EMF effects covered ten research areas under three major categories: biophysical mechanisms; cellular growth, differentiation, and control of gene expression; and enzymes, intracellular pathways, and signal transduction. In addition, sessions were held on the roles of *in vitro* assays and physical theory in understanding and quantifying human health risk. In general, attendees agreed that additional research is needed especially with regard to predicting *in vivo* human hazard from *in vitro* research findings. There also appears to be a need for alternative, physical, mathematical models that can better explain some *in vitro* observations. While the mechanistic findings discussed at this symposium can be used as qualitative support for a decision about whether a hazard exists from exposure to power line frequency EMF, it is not clear that these data provide quantitative information for describing the magnitude of any identified hazard.

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Magnetochemistry and Magnetite

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Introduction

Of the current theoretical mechanisms for magnetic field detection in biological systems, magnetochemistry and magnetite represent the two models that best succeed in trying to explain these phenomena from a starting point in physics and physical chemistry. Magnetochemistry effects arise from changes in radical pair recombination efficiencies in solution and in enzyme active sites. The theory has existed for more than 20 years and many examples of magnetic field dependent chemical reactions have been published in the peer-reviewed chemical literature.

Questions Discussed by the Breakout Group

The break-out session started out by finalizing a list of topics prepared by the facilitator and supplemented by the group. The questions and a brief summary of the resulting discussion forms the remainder of this document. Literature citations are given in References. In addition to reflecting the group's discussion on the assigned topics, Dr. Joseph Kirschvink furnished comments, that have been included in the section, Additional Issues Discussed by the Breakout Group.

A1. Overarching Questions (Magnetochemistry)

1. Based on theoretical mechanisms, what is the plausibility of extremely low frequency electric and magnetic fields (EF, MF) having effects on biological systems?

Free radical (FR) recombination provides a plausible mechanism for biological detection of magnetic fields. There are two biochemical systems (e.g., a heme enzyme and a B₁₂ enzyme) that respond to the application of a dc magnetic field at MF strengths larger than those considered in residential and occupational settings (Harkins and Grissom, 1994; Harkins and Grissom, 1995; Taoka et al., 1997; Taraban et al., 1997). A detailed model combining radical recombination with enzyme kinetics is capable of explaining observed biphasic magnetic field effects on the B₁₂ enzyme systems (Eichwald and Walleczek, 1996). Theoretical models exist that suggest the possibility of responses at geomagnetic field levels (Walleczek, 1995; Schulten, 1986).

2. According to the most plausible theories of biophysical mechanisms, what are reasonable thresholds for which magnetic field effects on biological systems could be observed?

A 20% effect has been observed at 10 G dc in a peroxidase enzyme (Taraban et al., 1997). It is unknown whether biochemical systems exist that will be directly sensitive to dc fields much below 10 G. Lower thresholds may be expected when magnetochemical processes interface to dynamical biological systems with time scales commensurate to the applied frequencies.

3. What are the EMF characteristics associated with producing biological effects and how do these traits, which are controlled for in *in vitro* experiments, translate to measurements made in epidemiological studies?

Magnetic field effects on radical pair recombination occur on a picosecond to nanosecond timescale. Therefore, 50-60 Hz ac EMF fields produce peak fields that persist long enough to alter radical pair recombination. In this respect, it may be very important to study transient strong magnetic fields that persist for only a few microseconds to milliseconds, but have the potential to affect radical pair recombination. Also, for weak ac magnetic fields, it is important to know the relative orientation to the geomagnetic field, as the dc geomagnetic field can either augment or diminish the effect of the ac magnetic field. In particular, the ac components parallel and perpendicular to the static field must be known for a detailed analysis of field effects at low ac fields.

A2. Overarching Questions (Magnetite)

1. Based on theoretical mechanisms, what is the plausibility of extremely low frequency electric and magnetic fields having effects on biological systems?

This is very likely in animal sensory systems that have evolved to sense the geomagnetic field for migratory navigation. The breakout group agreed that even if a magnetic field sensory system exists in modern humans, it is unlikely to affect human disease or result in pathology (however, see comment from Kirschvink below).

2. According to the most plausible theories of biophysical mechanisms, what are reasonable thresholds for which magnetic field effects on biological systems could be observed?

Dc magnetic fields might be detectable at 0.1 μ T-levels. Ac field frequency dependence is theoretically expected and has been experimentally observed (Kobayashi and Kirschvink, 1995). It is possible that 50-60 Hz magnetic fields may oscillate too quickly to be biologically effective in some systems.

3. What are the EMF characteristics associated with producing biological effects and how do these traits, which are controlled for in *in vitro* experiments, translate to measurements made in epidemiological studies?

The relative orientations of ac and dc environmental fields may be important. An ac component perpendicular to a (larger) static field might be expected to cause a biological effect by inducing rotational motion of a magnetite crystal or crystal chain oriented by the dc field. A parallel ac component could not induce rotational motion because the force vector is in the same direction as the dc field.

B1. Breakout Group Specific Questions (Magnetochemistry)

1. What are the lowest fields known to exhibit effects via radical pair (RP) recombination?

A 20 % effect at 10 G (Taraban et al., 1997); 1 mT, photo process (Scaiano, 1995); and 200–500 μ T Walleczek (1995).

2. Theoretical expectations on effects (thresholds)?

It is not possible to extrapolate to the lower fields produced by a 50-60 Hz powerline. The theory of magnetochemistry predicts a nonlinear dependence on applied field (Grissom, 1995). Walleczek (1995, p. 416) discusses limitations of extrapolations to human health effects of power frequency magnetic fields based on currently available RP recombination data.

3. How large do these effects need to be in order to be of biological significance?

Rare events may be important if they are outside of the normal physiological chemistry. In this sense, it is important to consider natural fluctuations in competing processes. It is impossible to predict the degree of biological change and the downstream effect from the biophysical mechanism alone. The biochemical regulatory mechanisms which achieve physiological homeostasis are very efficient at damping out deleterious variations in metabolite levels. However, the interaction of a magnetic field with a signal transduction pathway may allow the detection of a weak signal.

4. What is necessary to implement RP recombination as a biologically relevant mechanism for signal transduction?

The group agreed that amplification and integration of the basic mechanism is necessary in order to observe biological effects at low level fields, possibly through interactions with biochemical systems that exhibit non-linear (oscillatory) behavior (e.g., Walleczek 1995). Detailed models have been presented to explain avian migratory magnetosensing through changes in radical pair recombination (Deutschlander, 1995; Schulten, 1986).

5. What are the “special requirements” for RP recombination to exist *in vivo*?

Special requirements are (Grissom, 1995):

- a) Compartmentalization to increase the radical pair lifetime (range is 0.1 ns–0.1 ms).
- b) Must have a mechanism by which MF dependent intersystem crossing (ISC) can occur (most often a metal ion is involved).
- c) Escape must compete with recombination.
- d) Spin-orbit coupling must not dominate.
- e) The reaction is very dependent on the substrate. A candidate biochemical system should be checked against these prerequisites to determine the plausibility for magnetic field detection.

6. What is the characteristic of biological effects from radical pair models; is it either beneficial or detrimental?

It is difficult to conclusively identify *in vivo* effects that arise through changes in RP recombination. Parallel/perpendicular field combinations may provide information on the transduction type for small ac fields ($B_{ac} \ll B_{dc}$), where a perpendicular component is expected to contribute only very weakly compared to a parallel field combination.

7. Are ac fields (50-60 Hz) the same as dc fields?

For a pure RP-combination mechanism, an ac field is expected to produce a net effect proportional to the time-averaged rectified field. This is not true if the RP-recombination step feeds into a dynamical system (micro- or macroscopic) responsive on the 50-60 Hz time scale. In that case, ac fields could show effects well below dc thresholds. Transients might be an important source of very high magnetic fields that could profoundly alter RP recombination for a brief time.

8. Are there higher frequency ac effects?

Untested theoretical models suggest that RP recombination might be affected at frequencies over 100 kHz (Canfield et al., 1996). Transients may have an impact on RP based mechanisms.

9. How do we define “good” data and “bad” data?

The group did not address this question in a meaningful way. However, the question can be rephrased to: **“What level of experimental proof is required to demonstrate a robust effect?”** This depends very much on the predictions of the theoretical model that underlies the experiment. At dc magnetic field strengths above 10 G, where theory agrees with experimental evidence in many chemical systems, only normal scientific practices are required. At very low magnetic field strengths (below 10 G), where there are at most one or two examples in chemical systems, the level of proof required to document an effect in a biological system is

much higher, as magnetic field effects within this range have not received detailed study in more simple chemical systems.

10. What degree of accuracy do we need in experiments to detect an effect?

In a purified biological system (i.e., purified enzyme preparation), the detectability of the effect is determined by the biological assay and typically ranges from 5-15%. In a whole organism (cell culture or whole animal), it is more difficult to control all of the important variables and an effect typically has to be on the order of 50-100% to be unambiguously detectable above the assay background.

11. Will all enzymes with RP intermediates be (equally) sensitive to applied fields?

No. There are some hints that enzymes containing Co, Fe, and other transition metal ions will be better candidates for magnetic field effects through changes in RP recombination.

12. Are "F-pair" (free radicals that diffuse together and can undergo reaction or escape) field effects important?

There was little discussion of this point.

13. Are magnetic field effects possible with $\bullet\text{OH}$, $\bullet\text{RO}$, $\text{NO}\bullet$ and $\text{O}_2\bullet$ radicals?

Magnetic field effects on radical pair recombination with these species have not been demonstrated in a simple chemical system. It will be important to demonstrate magnetic field dependent RP recombination with these chemical species *before* seriously proposing biological effects involving these heteronuclear radicals. One example of magnetic field dependent recombination with alkoxyl radicals ($\bullet\text{RO}$) has been reported (Buchachenko, 1995).

14. Where do we go from here with the experimentation?

It is important to design experiments that critically test theories of the interaction mechanism.

B2. Breakout Group Specific Questions (Magnetite)

1. How large do magnetite effects need to be in order to be biologically relevant?

The system must overcome the randomizing effect of thermal noise in order to realize a physical mechanism based on magnetite. Experiments on honeybees

(Walker and Bitterman, 1989) claim a threshold below 0.1 μT and there is indirect evidence for magnetite being the operative mechanism in this system. Evidence in newts (Deutschlander, 1995) shows sensitivity to 0.1 μT fields, but the observation is not uniquely linked to a magnetite mechanism. Magnetosomes (a subcellular organelle containing magnetite grains) can provide very high magnetic moments, thus increasing the sensitivity of the detection step.

2. Is detected magnetite real, or an artifact of isolation?

The observation of magnetite in some organisms appears to be robust and repeatable. Some evidence for magnetite exists in all five classes of vertebrates (e.g., Kobayashi and Kirschvink, 1995).

3. Do magnetosomes exist [in the human body]?

Magnetite may have been observed in various human tissues (Kirschvink et al., 1992b; Dobson and Grass, 1996; Dunn et al., 1995) and there is crystal structure information that suggests the magnetite might have been organized into magnetosomes.

4. How can a magnetic field be "sensed" by an organism using magnetite?

One biophysical mechanism has been proposed by Kirschvink et al. (1992) in which an ion channel might be opened by means of a filament attached to a magnetite grain. The magnetic field will exert torque on the grain and can thereby mechanically control the gating of the ion channel.

5. What are the properties and possible biological significance of the magnetic field in the microscopic neighborhood of a magnetite crystal?

Single domain magnetite crystals are magnetically saturated and produce a localized magnetic field with peak flux densities between 0.25 and 0.5 T. These flux densities are sufficiently high to alter radical pair chemistry and therefore provide a mechanism for potentially altering cellular biochemistry. Should an ELF external magnetic field cause magnetite grains to rotate, then a strong, greatly varying local magnetic field would be produced. The corresponding local electric field might be sufficiently strong to alter membrane function. These speculations about biological significance have not been tested experimentally.

6. Frequency dependence is expected, does it exist?

The mechanical model of magnetite grain behavior in a viscous medium suggests a drop-off in response as a function of increasing frequency. Experiments with honeybees demonstrate a reduced response with increasing

frequency (Kobayashi and Kirschvink, 1995). Only marginal detection may be possible at 50/60 Hz.

7. Is contamination an issue for *in vitro* experiments?

Contamination of buffers and various solutions with ferromagnetic particles (Kobayashi et al., 1995) may occur with *in vitro* experiments, but the breakout group thought it is probably not relevant to the outcome of these experiments. Furthermore, there is no reason or requirement for an extrinsic role for magnetite in the large body of *in vitro* experiments (e.g., no ion channel infrastructure in place). However, there is currently little data to support either side of this argument. Experiments addressing this question are currently underway at Dr. Joseph Kirschvink's laboratory. One attendee of the group notes that most reported weak field EMF studies use membrane based experimental systems which, in principle, may be affected by magnetite binding.

8. Is there a body of MF effects that can be ascribed to magnetite? Human vs. non-human? Sensory vs. non-sensory systems?

There is a large body of MF effects ascribed to magnetite in connection to animal navigation. There was general group consensus that magnetite has been implicated in several different animal navigation studies and in magnetotactic bacteria. Some work exists on humans (Baker, 1987), but little work in the way of replication exists. The group largely agreed that while magnetite reasonably explains some sensory perception data, there is little support for direct health effects on humans that would be attributable to magnetite (however, see Additional Issues Discussed by the Breakout Group).

9. What constitutes reliable data?

The group did not address this question.

Additional Issues Discussed by the Breakout Group

A. Other Issues Raised In Breakout Group

The list of issues discussed in the break-out group was decided at the outset of the session, and no additional issues were discussed.

B. Additional Comments Collected After the Meeting

A comment on a possible cancer link as suggested by Dr. Joseph Kirschvink.

If one postulates a magnetosome bound via a cytoskeletal filament to a mechanically-activated ion channel in a calcisome, then EMF exposure during cell division could lead to disruption of spindle fibers (because of increased Ca^{++} levels) and a chromosome non-disjunction event. Non-disjunction can directly promote cancer by producing daughter cells haploid in one or more chromosomes, unmasking cancer-generating mutants.

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The lists below are not exhaustive, but simply a representation of literature references relevant to the discussion during the breakout session.

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B. Magnetite

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Summary and Recommendations

The radical pair recombination and magnetite models have led to a good understanding of magnetic field influences of simple biochemical systems and animal navigation, respectively. However, it is unclear that either model is directly applicable to judging the implications for possible human health effects of residential or occupational level magnetic fields.

The theory and observational evidence for the magnetochemical model is thus far well known only for magnetic fields at 10 G and greater, although predictions and theoretical modifications exist that may extend the class of mechanism to significantly lower field levels (1 G and below).

Biological magnetite is very likely operative in the sensory detection of geomagnetic fields by some animals. Observations of magnetosomes/magnetite in some life forms indicate that its biological function evolved early, and it could reasonably be suspected to be present in humans as well. A serious objection to linking magnetite with a human health risk is the lack of a compelling pathway to human disease and the expected low response at power frequencies of 50-60 Hz.

Ion Resonance and Electron Transfer Mechanisms

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Rapporteurs: Joseph D. Bowman, Ph.D. and Charles Polk, Ph.D.

Introduction

Mechanistic models of the interaction between EMF and biological systems can be useful in guiding experiments. Starting in 1985, ion resonance models were developed in order to explain experimental observations of amplitude and frequency "windows" involving the Ca^{2+} ion (Blackman et al., 1982, 1985). There have been a series of such models: ion cyclotron resonance (Liboff, 1985), ion parametric resonance (IPR) (Lednev, 1991, 1994), and a variation of IPR (Blanchard and Blackman, 1995). Each model has built on its predecessor in attempts to increase physical and biological plausibility and to produce better agreement with experiments. The ion resonance theories have serious theoretical flaws and have been heavily criticized. However, these models do make very specific predictions on the magnetic field characteristics that should produce biological effects and have inspired many experiments to test their predictions. Some experiments have found biological effects with the magnetic field exposures predicted by these models, but only a few have been replicated thus far. While theoretical questions are unresolved, the ion resonance models do not increase the plausibility of the experimental findings of EMF biological effects. At the present time, ion resonance models serve primarily as heuristics for organizing experimental findings and for suggesting designs for experiments.

Mechanisms involving electron transfer and non-linear interactions between magnetic fields and spontaneous oscillations of intracellular ions were also considered. Since the theories for these mechanisms are less developed, they received less attention from the breakout group. What these three classes of mechanisms have in common is a certain degree of experimental support.

One attendee of the group commented at the outset that a "summary" of theoretical results is futile if further research is to be cut off; the present results raise more questions than they answer. It was stressed further the need to indicate in this report areas where more research is needed.

Questions Discussed by the Breakout Group

1. What are the best developed models of ion resonance and electron transfer?

The breakout group ranked the proposed ion resonance and electron transfer models roughly in order of their theoretical development. For each model, the site of interaction, EMF coupling mechanism, amplification mechanisms, and sources of noise were identified along with an assessment of the model's physical and biologic plausibility.

a. *Lednev's ion parametric resonance model.*

Site of interaction: Ion bound in a protein complex.

EMF coupling mechanism: Static and alternating magnetic fields in parallel directions couple directly the ion vibrating at infrared frequencies. Resonant field combinations produce preferred orientations of the ion's vibration.

Amplification mechanisms: The directed oscillation may lead to the ion's dissociation or isomerization with the protein complex. If the protein is an enzyme or affects an enzyme (e.g., calcium calmodulin), the altered binding of the ion would affect the enzymatic activity leading to biochemical amplification.

Sources of noise: Collisions with the bound ion, random magnetic field fluctuations, and electric field effects on the ion vibration (i.e., Stark effect).

Plausibility: The IPR model requires long ion residence time in the excited state. In most proteins, collisional damping produces residence times much shorter than 1/60 sec. The model also assumes that very small modifications of the vibration frequency and the ion's angular velocity change the ion binding. Lastly, the model assumes that the ion is not hydrated. An ion-protein complex, which meets these conditions, has not been conclusively identified.

Additional comments not directly recorded in the breakout session: Lednev's IPR model has been examined in depth by Adair (1992) and Engström (1996), who were not in this breakout group. However, the facilitator and rapporteurs solicited their comments on the ion resonance models for this report. With regard to plausibility, Dr. Robert Adair writes: "The ion resonance ideas are simply nonsense; no better based than astrology. The papers by Lednev, and by Blanchard and Blackman Z (see below), are simply wrong; the physical assumptions are ludicrous and both make serious mathematical errors." Dr. Stefan Engström comments that the Lednev model is "not plausible" because the physical assumptions are "very strained." He points out the following theoretical problems:

- 1) The resonance mechanism requires millisecond time scale to sense 50/60 Hz fields, which is difficult to justify in a biological system.
- 2) Symmetry requirements to allow the model to see very low magnetic fields would most likely invalidate the theory.
- 3) No strong observable data exist for the proposed models.
- 4) It is biologically unrealistic to find free calcium or other ions.
- 5) One attendee noted in a written comment after the session, that as observed in the far infrared absorption spectrum, energy levels corresponding to chemical bonds between heavy objects are not a line but a very broad band. It includes so many sublevels that Zeeman splitting suggested by Lednev must be a very small portion

of them. The contribution to Lednev's splitting must therefore make no difference. Moreover, the chemical bond is anisotropic.

The breakout group discussion was generally in accord with Dr. Engström's assessment about the "very strained" assumptions in Lednev's model. However, participants disagreed with Dr. Adair's assessment that the Lednev model is "nonsense." The 1994 Lednev paper, which we considered, has no "serious mathematical errors." The group commented that the model's assumptions require a biological environment, which is very unlikely, but not totally impossible (see question 4). Experimental data from several laboratories are consistent with some of the predictions of the resonance models (see Table 1 and Blackman et al., 1996). The existing models can therefore provide the foundation for future model development.

b. Blanchard & Blackman version of IPR

Model specifications: This version is identical to Lednev's model except for an assumption that the forces, which the ac and dc magnetic fields exert on the moving ion, obey different physical laws. Blanchard and Blackman have demonstrated that this unphysical assumption is needed in order to fit a large set of experimental data. Dr. Carl Blackman was a member of this group and provided direct comment to discussion of this model.

Plausibility: The Blanchard and Blackman model has all the theoretical difficulties of the Lednev IPR model in addition to their unphysical assumption about the ac coupling constant. Therefore, the model has limited validity from the theoretical point of view and serves mainly as a heuristic one that organizes a body of experimental evidence.

Another criticism of the Blanchard-Blackman model has been its application to multiple ions. These authors consider all combinations of ions and their subharmonics, so a resonance could be due to several ions (some of which have no biologic significance). In Blackman et al. (1994), the linear combination of IPR functions for three ions is invoked to fit the experimental data. With so many degrees of freedom, some argue that the Blanchard-Blackman model can fit any data set and is therefore not falsifiable. In the breakout group, Dr. Martin Blank commented on the theory and noted that the process of "systematizing data (as was done for atomic spectra before they were understood in terms of the Bohr theory of the hydrogen atom)" is an important process; however, such categorizing does not in itself constitute a theory. This requires the establishment of a "link between the biological property and the biological measurements if they are to be considered in the same context. The IPR 'theory' relates properties of ions, which have no demonstrated role in the biological measurement (neurite outgrowth). Additionally, the q/m ratio, the physical ratio, which determines the IPR prediction, is ambiguous in the sense that it can give similar values for several different ions. Therefore, the IPR theory is not a theory that explains the biological data and there

has been no unambiguous test to fit the data to the theory." In rebuttal, Dr. Blackman pointed to experiments that demonstrate unique responses of a biological system tuned only to hydrogen resonance (Trillo et al., 1996 and recent data presented by Blackman et al., 1996 at the BEMS and DOE meetings).

c. *Liboff's ion cyclotron resonance model*

The ion cyclotron resonance mechanism has been applied mainly to ion transit through membrane channels. It is subject to the same sources of noise and other limits of plausibility as the IPR models. In addition, the time required for the ion to travel through channels is far too short for resonance with a 60 Hz field.

d. *Non-linear interactions between magnetic fields and spontaneous oscillations of intracellular ions.*

Site of interaction: Intracellular ions like Ca^{2+}

Coupling mechanism: Lorentz forces on moving ions, torque on endogenous magnetic moments, forces on endogenous charges, and currents by magnetically induced electric fields or effects on chemical reaction rates depending on electron spin (treated in the Magnetochemistry and Magnetite breakout group).

Amplification: The EMF interaction drives ions or magnetic moments at frequencies preferentially amplified by a biological oscillating system. If the magnetic field affects receptors and mimics a biological pathway, the signal transduction then induces a calcium response (Lindström et al., 1995). This is reflected either as induction of oscillations or change in spontaneous oscillations on exposure to the magnetic field. Amplification is the same as that following a physiological signal, such as cross-linking of a receptor complex.

Stochastic resonance is a method of amplification that can apply to many transduction mechanisms. Non-sinusoidal oscillations of intracellular ion concentrations are a manifestation of biochemical phenomena, which are described by the nonlinear differential equations characteristic of nonlinear systems. Application of low intensity perturbing "signals," e.g., low intensity, sinusoidal electric or magnetic fields, to such systems may then lead to "stochastic resonance." This is a counterintuitive process where noise can aid in the detection and transmission of coherent information. In its simplest manifestation, stochastic resonance needs only three ingredients: a threshold, a subthreshold information carrying signal, and noise. Information is transmitted by means of threshold crossings mediated by noise (Moss and Wiesenfeld, 1995; Adey, 1972).

Noise: Competing EMF fluctuations.

Plausibility: For detailed treatment of non-linearity, the exact coupling mechanism in these models has not been specified thus far. For Lorentz forces by weak magnetic fields to be effective, implausibly large ion velocities would be needed. Endogenous magnetic moments have only been identified in small quantities or specialized animal systems (discussed by the Magnetochemistry breakout group). Induced electric fields due to weak magnetic fields are likely to be below thermal noise. Magnetic effects on chemical reaction rates appear not to involve (at least not directly) the frequency of the applied field.

However considerable work exists showing that noise in non-linear systems can actually enhance detection of a weak signal under very specialized circumstances (see references on non-linear oscillations and stochastic resonance). Of particular interest are the theoretical papers by Eichwald and Kaiser (1993, 1995) and the papers on sensory systems by Levin and Miller (1996), Moss and Pei (1995), and Collins et al. (1996) connecting theory with experiment. Behavior of non-linear, self-oscillating systems under the influence of weak applied fields, enhanced by stochastic resonance, could possibly explain the reported observations. Much further work needs to be done to extend the existing analyses involving stochastic resonance to electrically non-excitable cells, to coupled networks of cells and to enzyme systems. On the basis of present knowledge, stochastic resonance in non-linear systems appears to be a plausible mechanism for biological detection of weak fields.

There is also extensive evidence on spontaneous oscillations of intracellular calcium (Jacob, 1991; Fewtrell, 1993; Berridge, 1993), and such effects from EMF exposures have been demonstrated with Jurkat cells (Lindström et al., 1993, 1995). However, this experiment has thus far not been performed in another laboratory (except at least in part by the original investigator), and the observations reported by Lindström could possibly be interpreted as just transient increases in Ca^{2+} rather than Ca^{2+} oscillations. One problem with explaining EMF effects in Jurkat cells in terms of interactions with endogenous Ca^{2+} oscillations is that the details of the control mechanisms for Ca^{2+} entry into Jurkat cells are still unknown.

e. *Electron transfer* (Nair and Liburdy, 1996).

Long-range intermolecular and intramolecular transfer plays an important role in conferring the specificity and directionality of numerous biological processes (Gray and Winkler, 1996). These include classical enzyme reactions that have been suggested as a medium for field interactions (Grundler, 1992).

Site of interaction: Electrons in enzymes. Also, electron transfer in DNA has recently been experimentally observed (Murphy, 1993; Dandliker, 1997).

Coupling mechanism: Direct interaction of magnetic field with electrons.

Amplification mechanism: Effect of changes in electron transfer rates may be magnified in the action of enzymes.

Plausibility: Although a low energy input requirement is one feature that suggests the mechanism is plausible, there has been only indirect demonstration. Details of the theory are yet to be worked out.

2. What are the model's predictions about the EMF exposures that might produce biological effects?

The clearest predictions of EMF exposure characteristics come from the ion resonance models. The term "resonance" means that the effects come only from magnetic fields whose characteristics satisfy conditions determined by the biological system and its environment. Thus, biological effects should occur when the EMF exposure characteristics are "in resonance" and disappear when the characteristics deviate from the resonance values by amounts greater than the system's bandwidth.

The resonance frequency f_R predicted by both the IPR and cyclotron resonance models depend on the magnitude of the static magnetic field B_0 and the ion's charge-to-mass ratio (q/m):

$$2\pi f_R = (q/m) B_0$$

Additional resonances can also occur at sub-harmonics of the resonance frequency ($f_R/2$, $f_R/3$, etc.) in the IPR models. Resonances can also occur at harmonics ($2f_R$, $3f_R$, etc.) in the cyclotron resonance model and Engström's version of IPR.

The ion resonance models also require that the magnetic field B_1 with frequency f_R is parallel to the static field vector. In the IPR models, the exposure-response relationship is a Bessel function of B_1/B_0 . The fluctuations of the Bessel functions means that IPR responses should be non-linear, actually going to zero at particular values of B_1/B_0 . Experiments at the predicted zeroes of the Bessel function are important tests for IPR models. For most residential magnetic fields, B_1 is around $0.1 \mu\text{T}$ (1 mG) and B_0 $50 \mu\text{T}$ (500 mG). With $B_1/B_0 < 0.01$, the Bessel function is essentially a linear function, so the "windows" in the exposure-response are not expected from the 50/60 Hz fields found in homes and most workplaces.

In order to assess exposures to ion resonance conditions, the following magnetic field characteristics should be measured:

- frequency spectrum, i.e., the ac field magnitude for all frequencies in the extremely low frequency range
- static field magnitude
- spatial alignment of the ac field relative to the static field vector

These detailed magnetic field characteristics can be measured, for example, by instruments like the Multiwave II (Electric Research and Management, State College, PA).

3. To what degree does the experimental evidence agree with the predictions from physical theory?

a. *Ion resonance effects.*

The breakout group considered ten studies from seven independent laboratories that reported bioeffects in accord with ion resonance models (see Table 1). Most of this evidence is for frequency resonances dependent on the dc field. More limited evidence shows bioeffects with the complete IPR pattern including the Bessel function windows as a function of B_1/B_0 .

Two ion resonance studies might have broader implications for EMF research. Prato's snail study (1996) had magnetic field exposures designed to compare the Lednev model with the induced current and magnetite mechanisms, and clearly supported the ion resonance mechanism. Similar designs comparing different mechanisms should be used with experimental systems. Important support for the IPR model is provided by experiments that show the biological responses return to null effects exactly at the ac/dc values predicted by the model (Blackman et al., 1995). The ICR and both versions of the IPR model (Lednev's and Blanchard-Blackman's) require that ac and dc magnetic fields be parallel. Another demonstration with nerve cells, as well as the existence of a non-linear effect from orthogonal ac/dc fields, has recently been reported (Blackman et al., 1996). The latter shows that mechanisms other than IPR may be acting and may dominate under appropriate conditions. The epidemiologic study by Bowman et al. (1995) is the only data linking ac/dc resonances to increased cancer risk. This study and the *in vitro* work show the importance of measuring static magnetic field exposures in epidemiologic studies.

As for replication, the neurite outgrowth with PC-12 cells (Blackman, Blanchard et al., 1994) was replicated by another investigator working in the same laboratory (Trillo et al., 1996). The radish seed experiment (Smith et al., 1995) has recently been replicated (Davies, 1996). Several attempts to replicate resonance effects have failed. For example, Lednev's experiments with calcium-calmodulin (Shuvalova et al., 1991) could not be replicated (Markov and Pilla, 1993). The RAPID program has sponsored a study to replicate the PC-12 experiment with full cooperation from Blackman's group.

b. *Non-linear interactions between magnetic fields and spontaneous oscillation of intracellular ions.*

Intracellular oscillations of calcium and other ion concentrations as a result of events following signal transduction have been observed in about 25 varieties of excitable and non-excitable cell types (Jacob, 1991; Berridge et al., 1988). It is now well established that frequency and amplitude of various perturbations can affect oscillatory chemical reactions, as well as stable steady-state reactions that, when perturbed, decay with an oscillatory component (Lazar and Ross, 1990). Thus, it has been suggested that ELF magnetic fields may influence normal signal transduction, and the resulting effects may be observed through the study of

calcium oscillations Lindström and colleagues have induced such oscillations by 50 Hz magnetic fields in Jurkat cells, a human leukemic T-cell line (Lindström, 1993, 1995). Dr. Mats-Olof Mattsson reported that this Swedish laboratory is continuing to observe these effects (although replication of the earlier findings did not come easily). At a plenary session, Dr. Kjell Mild presented preliminary results from these intracellular calcium studies that seem to show a 50 Hz resonant interaction depending on the dc magnetic field.

As an amplification mechanism, stochastic resonance has been observed in many different types of both inorganic and living nonlinear systems, including sensory neurons (Douglass et al., 1993; Collins et al., 1995). Application to explaining observations of biological effects of low intensity magnetic and electric fields has been suggested (e.g., Grundler et al., 1992; Kruglikov and Dertinger, 1994; Kaiser and Eichwald, 1992; Eichwald and Kaiser, 1993). Direct experimental demonstration of stochastic resonance has thus far only been tried and achieved on animal and human sensory systems using low intensity mechanical stimulation (Douglass et al., 1993; Levin and Miller, 1996; Collins et al., 1996), but there have been no tests of this mechanism with externally-applied EMF.

c. Biological electron transfer

Although electron transfer was not discussed at length at the session, Dr. Blank presented some supporting data on effects of 60 Hz fields on cytochrome C oxidation by the enzyme cytochrome oxidase (previously presented at the DOE Contractors Review in San Antonio 1996). This electron transfer reaction was accelerated in the field. These results were similar to his Na, K-ATPase studies where 60 Hz EMF affects electron transfer mechanisms at electric field intensities as low as 0.5 mV/m at 100 Hz (Blank, 1992) and 60 Hz magnetic fields of 5 μ T (50 mG) (Blank and Soo, 1996). Dr. Blank stressed two points about these studies:

- The effect of the magnetic field is related to the biological state. It varied inversely with the enzyme activity. That is, if the enzyme activity is high, the field effect is minimal; if the activity is low, the field effect is maximal.
- The dose response data for this one electron transfer experiment allow the extrapolation to the threshold of 5 to 6 mG.

4. How can theoretical problems be overcome?

The theoretical conditions necessary for the IPR model are highly unlikely to occur in biological systems, but not impossible. Since the experimental evidence is very limited, more experiments are needed which give unequivocal proof for the existence of ion resonances that are determined by the applied static magnetic field. Participants in the breakout group suggested several developments that could possibly solve the inadequacies in Lednev's IPR model:

- Chiabrera and coworkers (Chiabrera et al., 1987, 1991, 1993, 1994) have proposed that ion binding sites exist where ion-neutral collisions

are negligible. Absence of collisions would enhance the credibility of ELF resonance models since collision damping is probably the most important objection against their validity. Chiabrera et al. proposed, with reference to some incomplete experimental evidence, that electric field gradients at ion binding sites can be sufficiently large to extract dipolar water molecules by dielectrophoresis (Pohl, 1978).

- Hydrophobic binding sites where the ion is unhydrated but still bound weakly enough to be affected by the parametric resonance.
- Non-spherical protein binding sites with sufficient symmetry to energy levels separated only by the Zeeman splitting, e.g., octahedral or trigonal bi-pyramids.
- Development of a more detailed model of the chemical dynamics in order to identify the observable biochemical property altered by the magnetic field.
- Experimental evidence for nuclear magnetic resonance at static magnetic fields on the order of magnitude of the geomagnetic field (this would involve mutually perpendicular rather than parallel oriented static and alternating fields).
- Development of more detailed models for endogenous oscillations and electron transfer that correspond to the biological systems where the experiments show EMF effects (see question one above).

5. What experiments are needed to critically test the theory?

It was suggested that in general, hypothesis testing experiments need to be set up for all the theories. For example, in both ion resonance and electron transfer, systematic examination of the biochemistry or signal receptor information can yield likely locations and processes where the requirements of the theory may be met. Then, hypothesis-driven tests can be designed and done. Using well-understood systems such as hemes and ion channels should aid this process. Slow biological processes such as protein folding and calcium and potassium channels opening should receive special attention to "match" the frequency realm of ELF fields.

- a. The breakout group had many specific suggestions for experiments to test the theoretical mechanisms.

Ion resonances Those tests of the IPR model where resonance conditions may be satisfied simultaneously for several ions can still be criticized as ambiguous. More convincing tests, which yet need to be independently replicated, are those for H^+ resonance (Trillo, 1996) and others thus far only reported by Blackman et al. as abstracts at the 1996 DOE and BEMS meetings. To provide convincing tests of the theories, it is necessary to postulate the ion in well understood reactions (e.g., calcium in calmodulin) and to test the resonance conditions for this ion. This has been done in the past (Liboff,

Lednev) but not always reproducibly. Specific biological sites are relevant for different ion species, and this information should be used to select systems for testing the theory. The long residence time of excited state of Ca^{2+} in calmodulin may make it a good candidate for testing for ion resonance as has been suggested by Lednev. It is also desirable to design experiments with simpler biological systems to find the protein complexes where resonance occurs.

Non-linear oscillations Apply magnetic fields to an oscillating *in vitro* chemical system, e.g., a biochemical Ca^{2+} analog to the Belousov-Zhabotinskii reaction (Miller et al., 1985).

Electron Transfer (ET) One proposal is to measure effects of magnetic field on reaction rates of electron transfer in cytochrome C. Cytochrome C is a well characterized system, and the possible effects of ac and dc fields on this system could be studied for hypothesis generation for ET as a mechanism.

- b. The breakout group also had comments that apply to all these experimental tests of theory.

Replication There was discussion of the need to replicate experiments such as the neurite outgrowth (in PC-12) experiments, and the calcium oscillation experiments. Dr. Steven Miller pointed out that the PC-12 assay has been perfected and other labs need practice to get it right. It is important to continue working with systems in which effects have been observed and to resolve discrepancies.

Biological state of the cell or system Several experiments have shown (Blank in K,Na-ATPase; Lindström in Jurkat cells) that the effects are dependent on the initial state of the system. It was remarked by one attendee that "cells evolve" with accompanying changes in rate changes of expression and this may affect the site of interaction and hence experiments may not replicate (e.g., T-cell receptor complex in Jurkat cells). It was further suggested that it is important to persist in these cases and find causes of the non-replication. This may actually yield insight into the mechanism.

Suggestion for experiment It was suggested that the c-AMP signal transduction system would be ideal for testing EMF effects. A proposed hypothesis would be that EMF affects membranes so as to interfere with hormone/drug-induced adenylyl cyclase interaction. This interferes with down-stream cyclic AMP-dependent reactions such as PK-A activation or protein phosphorylation. This can be tested using new techniques such as fluorescent imaging of adenylate cyclase, c-AMP, phosphorylation of connexin, or gap junction proteins, etc.

6. What theoretical limits (if any) have been derived from these models for the lowest magnitude to which biological systems should respond?

The ion resonance theories in their present form set no lower limit on the magnitude of the ELF magnetic field. For an ELF field to be effective according to these models, ions would have to remain in an excited state at least for periods on the order of 50 ms. This is impossible at the collision frequencies corresponding to the viscosity of water (Galt et al., 1993; Muehsam and Pilla, 1996). Therefore, the ion resonance theories, which have been compared with experiment, assume no collisions and therefore allow resonance effects at extremely small ELF magnetic field magnitudes, provided the field's spectrum has one of the resonance frequencies determined by the static field magnitude (Lednev, 1994; Blanchard and Blackman, 1994). Of course, a lower limit on the ELF magnitudes could be deduced empirically from IPR experiments. In the experiments done so far (see Table 1), only one experiment has been done below 1.3 μ T (13 mG), and that was not designed to determine a lower limit of detection (Trillo et al., 1996). Trillo et al. reported a detectable change at 9.5 mG rms.

Additional Issues Discussed by the Breakout Group

These issues have been included within the text of the previous section.

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(The numbered references are summarized in Table 1).

1. *Lednev's Ion parametric resonance (IPR)*

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Summary and Recommendations

The first overarching question is whether the proposed mechanisms increase the plausibility of biological effects from EMF exposures. Unfortunately, the ion resonance models have theoretical deficiencies, which has created controversy. While theoretical questions are unresolved, the ion resonance models do not increase the plausibility of the experimental findings of EMF biological effects. At the present time, IPR models serve primarily as heuristics for organizing experimental findings and for suggesting designs for experiments. One attendee put forth an historical analogy with the Balmer formula and the other mathematical expressions for systematizing the hydrogen spectra that was eventually explained by quantum theory. Likewise, the body of experimental data, which fit IPR models, may lead to better EMF biophysical theories.

The non-linear biology and stochastic resonance mechanisms are better established theoretically, although there have been few direct experimental tests of these mechanisms with externally applied EMF. Stochastic resonance may explain biological effectiveness of very weak stimuli from electric fields and magnetic fields acting through induced electric fields and Lorentz forces as well as through free radical reactions (see the breakout groups reports for Magnetochemistry and Magnetite and for Internal Fields Produced EMF, Dosimetry, and Endogenous Fields).

The second question is what exposure thresholds can be derived from the theory. IPR models in their present form do not set a lower limit on the magnitude of the ELF magnetic fields that produce biological effects. However, these models predict that bioeffects occur only with specific combinations of the static and alternating magnetic fields. As only a small proportion of environmental magnetic fields meet these resonance conditions, the IPR model predicts that only a limited proportion of ac/dc magnetic field exposures could affect people. This prediction

suggests that effects in people exposed to IPR resonances may be greater than the observed associations with measurements of the ac magnetic field alone.

The last overarching question is to identify the EMF characteristics that produce biological effects. Here, the IPR models are highly specific, dictating the combinations of frequency, static magnetic field, spatial orientation, and dose-response of the oscillating field. These predictions mean that the measurements of the time-averaged ELF magnetic field magnitude conducted in most epidemiologic studies may be inadequate to characterize the biologically-relevant exposure. Since the biological state of cells evolves with accompanying changes in the relative importance of different biochemical processes at different time points, it is important that reports on *in vitro* experiments indicate the developmental stage of cells, as well as environmental parameters. When a replication experiment gives different results than an original experiment, it is essential to find the reason for the discrepancy. This is likely to help to understand biological, chemical and physical mechanisms that lead to field effects.

In conclusion, the weaknesses of the ion resonance and electron transfer theories contrast sharply with the experimental results. Ion resonance models make clear predictions that have major implications for the design of laboratory and epidemiologic studies and for risk assessment. However, theoretical objections suggest that these models in their present form are not a very plausible representation of actual physical processes as presently conceived. It is therefore necessary (1) that experiments, which report ion resonances at ELF, be replicated in several laboratories, and (2) that additional *in vitro* experiments be designed that can identify the specific biological systems and experimental conditions under which ion resonances at ELF are most likely to occur. Such experiments should preferably be combined with numerical models of physiologically-realistic situations to improve the theoretical models. On the other hand, theories invoking stochastic resonances and non-linear oscillations are better developed, but have thus far been tested only on a few sensory systems with perturbations other than externally-applied EMF. At the present time it appears that these proposed theoretical explanations can make substantial contributions to the design of experimental research, particularly when parameters of the biological system known from other research can be related to the model.

Table 1
Experimental Papers Reporting "Resonances" Depending on the Static Magnetic Field (B_{DC})

No. in Reference s	Freq. of exposure (Hz)	B_{AC} (μT)	B_{DC} (μT)	f_R (Hz)	Ion?	Cells / organism	Exposure duration	Effect	Notes
1	60	299 peak	78 ± 1	60	$^{40}Ca^{2+}$	Snails	15 min.	Attenuation of opioid-induced analgesia by approximately 20%	B_{AC} and B_{DC} variable
2	16	42.1	23.4	16	$^{45}Ca^{2+}$	Mitogen-activated lymphocyte	60 min	Inhibits Ca influx triggered by ConA	No effect: DC only AC only
3	32	150 rms 75 rms	42	-	$^{40}Ca^{2+}$	Human lymphocytes	72 hrs.	Micronuclei formation frequency increased 97 to 100% 6 subjects (marker of chromosomal damage)	Genotoxic effect
	50 32	150 rms. 75, 150 rms.	0	-	-			No effect	Off resonance (no DC)
4	45	7.7-46.8	36.6	45	Mg, V, Mn,	PC-12 Neurite Outgrowth	23 hrs.	Reduction of % cells with neurite outgrowth	Subharmonics, B_{AC}/B_{DC} dependence
	25	7.8-18.1	20.3	25	Li, H			No effect	Off resonance (effect depends on NGF concentration)
	45	0.79-2.1	2.0	-	-				
5	45	0.29-4.11 rms	2.96	45	H^+	PC-12	23 hrs.	Reduction of % cells with neurite outgrowth according to IPR, except near (B_{AC}/B_{DC}) = 0.85	Effect at main resonance (n=1)
	30	0.79-2.05 rms	1.97	30					
	45	0.79-2.05	1.97	-	-			No effect	Off resonance
6	60	10 peak	78.4	60	$^{40}Ca^{2+}$	Planaria	300 hrs.	Delay of cephalic regeneration by 48 hrs.	Controls: $B_{DC} = 18.2 \mu T$, or $B_{AC} = 0$
	60	10 peak	51.13	60	K^+			No effect	
7	60	20 peak	78.4, 39.2, 26.1, 153.3, 76.6, 51.1, 47.5, 9.5	60	$^{40}Ca^{2+}$ K^+ Mg^{2+}	Radish seeds	21 days	Ca tuning slowed germination, stimulates growth, K tuning speeded germination, inhibits growth. Mg tuning did not affect germination, stimulates growth.	Also AC unexposed controls

Table 1 continued
Experimental Papers Reporting "Resonances" Depending on the Static Magnetic Field (B_{DC})

Ref. in section IV	Freq. of exposure (Hz)	B_{AC} (μT)	B_{DC} (μT)	f_R (Hz)	Ion?	Cells / organism	Exposure duration	Effect	Notes
8	60	>0.3	38 ± 4.5 50.6 ± 4.5	29 39.5	$^{40}Ca^{2+}$	Children	1-11 yr.	Increase in childhood leukemia risk.	Resonant DC fields $B=f_R / n$ for $n = 2$ & $3/2$ are derived from Ca-efflux studies
9	13.5-18	20 peak	20	16.3	$^{45}Ca^{2+}$	TE-85 Sa OS-2	10 min. 40 min.	Increase of ^{45}Ca uptake into bone cells	2 Hz, 50% band w.
10	15.3	20 peak	20 ± 2	15.3	$^{40}Ca^{2+}$	TE-85	10 min. optimum	Increase of IGF-II release (human osteosarcoma)	DNA synt. incr. during 24 hrs. post expos. Max. $E=1.6 \times 10^{-5}$ V/m

Appendix 1

Ion Resonance & Electron Transfer Mechanisms

Submitted by Carl Blackman, Ph.D.

Executive Summary

The report of the breakout group on Ion Resonance and Electron Transfer Mechanisms does not fully reflect all of the views expressed during the session, nor those expressed during private conversations during the workshop as a whole. It is essential to present all of these views in order to allow the NIEHS to prepare the most objective and accurate view of the research field in this area for its report to Congress.

The most egregious deficiency in the report is the failure to recognize the true role of theoretical models vis-à-vis experimental results. The report inappropriately places theory as the sole arbiter of what experimental results are possible and what are impossible. It is widely accepted in the general scientific community that there is a natural and essential interplay between experiment and theory, neither subjugated to the other, but rather one refining the other, by which theory and experiment are eventually refined. The fundamental flaw of assigning experiment as subservient to theory in the report distorts the important progress that has been made to date, particularly in ion resonance studies.

The report also applies an unequal standard of proof for the experimental data cited as tests of various theoretical models. In some cases, a single experimental report is cited as support for a particular theoretical concept even though that model lacks predictive capabilities. In another case, one laboratory has published several reports of testing predictions from another model and has even demonstrated application to a second biological test system and endpoint, yet this support of that model is discredited by unproven theoretical arguments. In yet another case, several laboratories have experimental reports of very similar effects. But these more persuasive experimental findings, some of which are at odds with a given physical model, are essentially ignored, when instead they should be highlighted so as to provide important guidance to theoreticians for devising new, more descriptive and predictive physical models.

Finally, the report misrepresents the breadth of scientific opinion regarding what needs to be addressed by future research. By citing selected personal opinions and giving them equal or greater weight than extensively peer-reviewed journal articles, the report diminishes the value of important experimental data. The fact that some theorists at the meeting described specific experimental results as "impossible" should be put into the proper perspective rather than simply quoted without judgment. Theories require simplifications so that models can be developed. It is instructive to quote Charles Palus (1997) on Maxwell: "Maxwell was a 'super-visualizer.' He constructed an imaginary world to facilitate his learning: a hydraulic world of plumbing and flowing; a world of tubes, vortices, and eddies, within a fluid called *ether*. His genius became clear when, having worked out his historic mathematical formulation of electromagnetism, he then discarded the imaginary model he used to formulate it. The model was scaffolding." Thus,

even imperfect models can have great utility. In the cases of claims of "impossible," it is equally, if not more likely, that the current theoreticians have not yet incorporated a sufficiently complex biological model into their theories so as to explain the experimental results. The report is strangely silent on this simple truth, and thus, it provides a grossly distorted view of progress that has occurred in this research area over the last 30 years.

These comments, and those below, reflect my experience at the meeting as a whole and at the breakout session in particular. I hope the inclusion of these comments as an appendix to the report will help to provide a more equitable view of the state of science in this area, and thus, to allow the NIEHS to prepare an objective and accurate view of the research field in its report to Congress.

Introduction

The introduction does not capture the sense of the breakout session combined with the general session as I heard them. When considering the utility of various models, we would do well to consider what Dr. Thomas Tenforde so eloquently noted in his remarks to the general session: namely that "theory cannot invalidate biological observations, but experimental findings can invalidate particular theories." The flip side of this message is, of course, that models are only as good as the accuracy of their guidance in telling us where to look for something and then in predicting how much of it we will find. This is consistent with comments by Dr. Theodore Litovitz (Catholic University) in other EMF meetings that the only real test of a model is whether it gives us accurate directions about where to look for an effect and then whether it predicts how much of an effect we get. These ideas are more consistent with my recollection of the overarching theme of this particular session and of this conference in general.

The statement that "... ion resonance models do not increase the plausibility of the experimental findings ..." is not true (it is the model that is validated by data, not vice versa). This is only part of the concepts that were discussed at the breakout session. There are many ways that the plausibility of experimental findings can be enhanced and these should be explicitly stated since they were discussed in the session. For example, enhancement of findings can occur from replication and extensions of experimental results obtained by the same research team, from independent replication of results by other research teams, from extensions/extrapolations of results to other biological systems and endpoints, and from identification and isolation of specific biochemical entities that are affected by exposures and the fitting of those entities into known reaction schemes that lead to the observed effects. Data, published and presented both at this session and elsewhere, demonstrate experimental findings that support and extend initial results, and do so in a way consistent with predictions of IPR and other models, whatever criticisms are currently placed on the specifics of the models. Furthermore, there are reports of data obtained with different biological systems and endpoints that are precisely fit by the IPR model using scaling parameters developed for the original biological endpoints. Biologists are now

testing experiments that will more directly tie model predictions to specific observable biochemical endpoints. Although these advances are obvious, they were omitted from the text of this introduction (or elsewhere in the breakout report).

Discussion Questions

1. What are the best developed models of ion resonance and electron transfer?

a. Lednev's ion parametric resonance model

Plausibility:

The first paragraph under "Plausibility" adequately describes the situation associated with ion resonance models as agreed to by the session participants. It should be noted, however, that Lednev's model formulation applies to calcium ions and perhaps to magnesium ions, but not to other ions.

The second paragraph describes hearsay comments. Neither Dr. Adair nor Dr. Engström were present at the session. These un-verifiable quotations from them were not discussed by members of the breakout group nor were they rebutted by presentation of opposing views. Their inclusion here only serves to bias the report.

The problems listed with ion resonance models, attributed to comments by Engström, are in fact problems theoreticians have with constructing conceptual models that meet these requirements. This limitation on the part of theoreticians has nothing to do with the plausibility of experimental data because experimental data have an entirely different set of requirements to meet in order to enhance their plausibility. By failing to acknowledge this fact, the text presents a distorted view.

The report comments that the biological environment required to meet the requirements of the resonance model are "very unlikely but not totally impossible." This is a very weak admission on the part of physical scientists that the experimental data from several labs have caused them to "hedge their bets." The report would be far more valuable if it explicitly admitted that experimental data from several laboratories are consistent with some of the predictions of the resonance models, and thus, it appears that the "very strained" assumptions of the models nevertheless merit further examination because they appear to have predictive utility for biological responses. It is important to put this situation into historical perspective: Physical scientists suggest exact and accurate knowledge is possible in their realm of expertise, but there are many instances in which even physical science leverages itself via hypotheses and models that somewhat imperfectly predict data. The Ritz electrodynamic theory attempted, for example, to explain experimental data without requiring a constant speed of light, independent of reference system (Stratton, 1941). The Ritz combination principle is an empirically derived mechanism that found the second Lyman series line frequency is the sum of the first Lyman series frequency and the first Balmer series frequency (Atkins, 1990). Characterization of such observations as good or bad

overlooks the critical contribution provided by such a model: it, like any model, tells us where to look for results in an otherwise complex space of many variables. To the extent that its predictions are consistent with data, the model provides the foundation for later model development which, presumably, gets closer to the "truth" as its predictions more accurately predict a growing number of observed results in an ever wider group of systems. One salient example of the value of implausible models, is the use by Maxwell of the ether concept as he developed his now-famous mathematical formulation for electromagnetism. As he refined his models he found he could discard the ether concept with no loss of applicability. But it was crucial in helping to formulate the original theory.

b. Blanchard and Blackman version of IPR

Model specifications:

The IPR model developed by Blanchard and lackman differs from the Lednev model in several ways not stated in this report. The site of interaction for this model was expanded from the Lednev formulation to include ionic cofactors in biological/ biochemical processes, thus, all ions used by biological systems are potentially available for such interactions. In addition to the statement that the IPR model assumes that the ac field acts other than as just a quasi-static field, it should be noted that the model allows for multiple ion resonances at the same time (but ONLY for those ions whose frequency indexes are integer values, a notion that in practice severely restricts the range of possibly involved ions; ultimately the biological system determines which ions are actually involved), including the summation of influences from ions at different frequency indices. The IPR model also includes the concept of a hydrogen resonance trigger that provides a lower limit on the Bac that will create a response. Although the concept that the ac field has actions other than as a quasi-static field has met with substantial lack of acceptance by the physical science community, the IPR model must be considered a exceptional heuristic model because the model has an unequaled success rate in predicting the most extensive set of non-linear data to date. The predicted response form for this data is not intuitively obvious without resort to the IPR model.

Plausibility:

The opinions expressed in this paragraph are either incorrect or outdated. Both the information presented at the breakout session and a large number of published, peer reviewed papers (which have been inexplicably ignored in this report) that unequivocally demonstrate the success of the IPR model in describing the response of a biological system tuned only to hydrogen ion resonance. Of particular note is the use of only two fitting parameters for 72 data points. Moreover, recent data presented at both the DOE and the BEMS meetings (Blackman et al., 1996a, 1996b; and noted at the breakout session) showed that the fitted equation for neurite outgrowth from PC-12 rat pheochromocytoma cells

could be used to predict, without any adjustable parameters, the changes in gap junction communication in Clone 9 rat liver cells exposed under the same field conditions. Since the fit of the equation to the data was not improved by allowing the parameters to float for best fit, the argument advanced in this paragraph about too many degrees of freedom in the model is moribund and should be removed. The report incorrectly asserts that the ions we listed in the original publication have little or no biological role; this assertion is untrue. We cited references in our initial publication (Blanchard and Blackman, 1994) that demonstrate all those ions have activity in some biological system.

It is important to clear up some misunderstandings reported by the session leaders. The quotation attributed to Dr. Martin Blank asserts that no role for the ions identified by the model, presumably magnesium, manganese, and hydrogen, have been demonstrated in neurite outgrowth is factually incorrect. References cited in Blackman et al. (1994) and noted by Dr. Blackman in the breakout session, clearly refute this claim. In response to further statements attributed to Dr. Blank, it is important to explicitly recognize the value of models in early stages in the development of a principle, even heuristic models, because they can be of extreme utility to critical members of a scientific community; a model does not have to receive the blessing of all members before it "constitutes a theory."

The subsequent attribution to Dr. Blank that the IPR model is predictions cite several ions potentially at resonance and thus it is an ambiguous model, propagates a misunderstanding of the IPR model that is also applies conceptually to the Lednev model. In addition to citing publications of Dr. Blackman and colleagues without much explanation, the report could also quote Dr. Blackman's response to Dr. Blank at the breakout session: resonance conditions refer to potentially resonant ions based on their charge-to-mass ratio and activity in biological/biochemical systems. The Lednev model ignores this fact while the IPR model explicitly indicates that experimental tests must be done to determine what ions can be eliminated. It is true that certain exposure conditions, for example, when tuning for calcium ions, both the Lednev and IPR models also predict simultaneous tuning for nickel, cobalt and iron. Yet, other exposure conditions, such as tuning for hydrogen alone (and not other ions) provides results that fit the requirements Dr. Blank has described for a theory. This response was inexplicably left out of the report.

c. Liboff's ion cyclotron resonance (ICR) model

Plausibility:

Despite subsequent concerns about physical details of the model, the ICR model was a crucial development that helped focus the research field to develop more sophisticated models and to perform experimental research to test the concept of ion resonance. Those contributions should be explicitly acknowledged. The main limitation of the ICR model is that no description is given of the influence of the ac flux density on the system response.

- d. Non-linear interactions between magnetic fields and spontaneous oscillations of intracellular ions.

Site of interaction:

It is incorrect to assume the only site of action is on intracellular calcium directly. Alterations in membrane processes and extracellular ionic concentration changes are also noted to perturb intracellular systems, including spontaneous oscillations of intracellular molecules. Thus, the membrane and extracellular ions are additional sites of action.

EMF coupling mechanism:

In addition to the potential mechanisms identified in the report, please add nuclear spins (see Blackman et al., 1988a).

Amplification mechanism:

In addition to the mechanisms cited in the report, interested readers should also refer to concepts advanced by Adey (1992) and Blackman (1992) that invoke field-induced changes in membrane dynamics.

Plausibility:

There is data in reports by Bawin and Adey (1976), Blackman (1992), Dutta et al., (1984, 1989), Schwartz et al. (1990), and Lee et al. (1987) that demonstrate influences on changes in the release of calcium ions in various biological systems caused by specific frequencies and intensities of electric and magnetic fields. In two reports the influence of the earth's magnetic field was demonstrated, and in others, the importance of temperature and pH changes that sensitized the test specimens to the field exposure conditions. These environmental conditions are exactly those that affect phase changes in cellular components. Since systems at transitions points (critical points) are known to have long range order and extreme sensitivity to perturbation, it was hypothesized that such systems were the basis for the effects that were seen, which exhibited aspects of non-linear responses described by Kaiser and Frohlich (see Blackman et al., 1989). Thus, it is fitting to include these studies of extracellular calcium changes to support and augment the emerging data on intracellular calcium changes.

It is important to emphasize that not all scientists agree with the possible underlying bases for non-linear effects. To be consistent in the consideration of each model, Adair's statement on record (1996) explicitly states that stochastic resonance effects cannot provide a mechanism for biological effects of very weak electromagnetic fields where those effects are bounded by thermal noise.

It should be explicitly noted that no theoretical models have been developed that predict the form of experimental results to the extent that the ion resonance

models have. From this perspective this research area is far more primitive with primarily experimental observations motivating the attention to non-linear models.

- e. Electron transfer (Nair and Liburdy, 1996).

Plausibility:

It is appropriate to indicate that this concept has not been directly tested. By the standards of proof that the report has placed on ion resonance models and the supporting data, this concept needs more development at the theoretical level, particularly to estimate field strengths that would be needed to affect process and the form of the parameter space that should be tested and then some confirming experimental tests before the concept can be seriously considered as a vehicle for NIEHS considerations. It is only a hypothesis at this time.

- 2. What are the model's predictions about the EMF exposures which might produce biological effects?

Contrary to the text in the report, experimental results testing the null effects predicted by the IPR model have been published by Blackman et al, 1995b (and presented at the breakout session) that show exactly what the text requests, namely, that biological responses return to null effects exactly at the ac/dc values predicted by the IPR model. It is unfortunate that the text was not revised to acknowledge this fact and to state what this added accomplishment means for the resonance theories; it puts resonance theories on a much firmer basis than any other models at this time.

Work at Pacific Northwest Laboratory, which has been presented at DOE meeting by Hansen (1996), shows there are some special settings where resonance conditions might be obtained in the work place. Further, it would be more useful in future research to identify ranges of residential fields that might meet resonance conditions. Work by Blackman and Most (1993) identified possible dc flux densities that could correspond to resonance conditions observed in calcium efflux experiments. Liboff and McLeod (1995) have also addressed this issue.

- 3. To what degree does the experimental evidence agree with the predictions from physical theory?

- a. Ion resonance effects.

The evidence for Bessel function windows is the most detailed (most number of experimental points consistent with the predictions of a theoretical model, in this case using the exposure metric of B_{ac}/B_{dc}) of any yet developed and shows not only multiple null biological effects occurring at B_{ac}/B_{dc} values predicted by theory, but also shows the substantial impact of orientation of B_{ac}

relative to Bdc. Further, it shows that the Bdc magnitude serves as a metric for the Bac flux density and that the proper exposure quantity is Bac normalized by Bdc.

There are several other studies that have broad implications for EMF research. Studies by Blackman et al. (1993a, 1993b) using different subclones of PC-12 cells were specifically designed to test the hypothesis that alterations in neurite outgrowth were caused by the induced electric field. The results in both cases identified the magnetic field alone as the causative agent. The subsequent report by Prato (1996) confirmed this result in a snail assay. These studies clearly discount the induced electric field as the causative agent at least for the specific systems tested.

There is an increasing series of reports supporting the general concept of ion resonance effects. It is a fact that no better theory has been developed, that is, one that predicts the series of experimental results that have been reported as well as the IPR model does. It is time to say that the theoreticians have so far failed to develop "acceptable" theoretical models that can predict experimental effects and begin to ask them to work on models that help to clarify what is being seen in many labs. For example, numerous labs have reported biological results at exposures less than 1 G. Surely all of these are not due to artifacts. We know that biological systems utilize conformational foldings of proteins that are controlled by ions or charged molecules in binding sites. While at this time we have only imperfect biological data and incomplete heuristic models to guide the theoreticians, there is already much work using the experimental results that can be done to expand the limited concepts of environments in biological systems that are critical to understanding the fundamental processes. Rather than citing opinions that were neither carefully and fully formulated nor accompanied by the rebuttal statements to provide a balanced perspective, it would be more appropriate to correct the lack of explicit awareness of the full literature by including the five-page summary of the experimental work developed to test the IPR model. For purposes of completeness, that five page summary is affixed to the end of this appendix.

It should be noted that replication work does not appear to be on-going or planned for Prato's snail study.

b. Non-linear interactions between magnetic fields and spontaneous oscillations of intracellular ions.

It should be noted that there is no physical theory that predicts responses as a function of exposure parameters so that experimental testing can be done.

Amplification mechanism have been discussed above, under "d." Principally, it involves systems poised at chemical phase-transition points or at sub-optimal stimulation or inhibition conditions, where systems may be particularly sensitive to perturbations induced by fields.

Readers should be reminded that Dr. Adair has voiced strong opposition to stochastic resonance having any applicability to explain low level EMF biological effects, as noted earlier in my remarks. Since Adair's opinion was solicited for other aspects of this report, it is only fitting that his opinions of this theoretical model not be censored.

Although there is a lack of independent replication of internal calcium changes, there has been independent replication and extension of non-linear results using calcium efflux as the endpoint.

c. Biological electron transfer [Electron transfer (Nair and Liburdy, 1996)]

This is a nascent concept. There is no predictive model and no explicit experimental tests on electron transfer. The results of Blank do not address electron transfer directly, only enzyme activity, and these results have not been independently replicated.

4. How can theoretical problems be overcome?

It is obvious that there must be a continuing interaction between theory and experimentation to refine the critical tests and provide overwhelming experimental evidence that current theory just cannot explain. But to do this, theorists will need to consider ALL the literature not just what is convenient to support a particular bias. Also, higher credence must be given to peer-reviewed literature and considerably less to off-hand remarks that conveniently bolster otherwise not-well-supported arguments. Theorists need to be more creative in their construction of biological models, and experimentalists need to characterize all known critical exposure conditions, as well as, the particular limitations in the test application and to continue attempts to reduce the biological/biochemical system to the minimal unit that is still responsive to exposure. Perhaps most importantly, experimentalists as a group need to perform experiments that will solidify any particular biological response to fields among their peer group because this will focus the theoretician's energies on model development and away from unsupported criticisms of experimentalist's techniques which merely serve to dilute their efforts.

The report ignores at least two different experimental reports that explicitly address the issue raised here. The existence of field effects at orthogonal ac/dc fields has been reported for two different experiments, calcium release from chick brains (where interaction with nuclear spins was hypothesized (see Blackman et al., 1988a) and the requirement of orthogonality was demonstrated (Blackman et al., 1990) and for neurite outgrowth (1996c).

5. What experiments are needed to critically test the theory?

The report is incorrect when it states that previous experimental work was not hypothesis driven. Such a claim does an injustice to the science and the researchers who performed such experiments.

In fact, there are many developments in the history of this research area that were hypothesis driven. They should not be discounted or ignored. These historical studies give the scientific community a sense of an accumulating, developing science occurring over decades that lead to the current understandings of how fields might interact with biological systems. The development of the

calcium efflux test was hypothesis driven (Adey, 1981). In early explorations of the calcium efflux phenomenon, numerous test were hypothesis driven, for example, tests of the importance of

- a) temperature and pH (Blackman et al., 1981; Blackman et al., 1991) to examine conditions that would indicate some involvement of a phase transition process, which could provide the sensitivity demonstrated for such low field strengths;
- b) frequency sensitivities to guide studies of resonance type reactions (Dr. Blackman et al., 1988a); and
- c) the potential involvement of the static magnetic field of the earth (Blackman et al., 1985, 1990) as a cofactor that might influence the frequency selectivity that was observed (for reviews see Adey, 1992; Blackman, 1992).

These results provided the impetus for research that lead to the development of the ICR model.

Subsequent early neurite outgrowth experiments directly tested whether the magnetic field or the induced electric field was responsible for the changes (Blackman et al., 1993a; 1993b), and whether there was a frequency specificity for the changes observed (Blackman et al., 1995a). Observation of a frequency specificity in the neurite outgrowth results, lead directly to testing for an involvement of the static magnetic field (Blackman et al., 1994). In the later PC-12 cell and Clone-9 cell tests, Blackman et al. (1996a,b) for example, the critical field exposure conditions that were tested were selected based directly on hypotheses derived from the IPR model. These were subsequently found to produce biological results that were in fact consistent with the predictions of the IPR model for those exposure values. It is disingenuous for the report to suggest otherwise.

The report identifies areas of study that exclusively address development of physical models. Also needed are studies to test biochemical hypotheses for mechanisms of action. It is essential to continue to explore the ranges of exposure parameter values and of biochemical and biological conditions that sensitize the systems to field exposures because it is quite likely that all the environmental conditions that shape the biological system and cause it to be responsive to fields have not yet been determined.

It is indeed appropriate that "hypothesis testing experiments needed to be set up for all the theories," but what is missing in all the text in the subsequent sections is the concept that there may be a minimum biological/biochemical complexity that is needed to provide the receptive environmental conditions so that effects can be manifest. That concept should have been explicitly stated in the introductory paragraph, and included in the subsequent paragraphs where evidence was presented demonstrating biological effects in complex biological/biochemical systems. What also needs to be stated explicitly is the need to test all aspects of the theoretical model predictions in order to determine those aspects that may not be critical or may not create predictions consistent with the observed responses. These results will provide essential elements for future

model builders who will be attempting to develop more realistic models, both from theoretical and biological/biochemical perspectives.

a. Ion resonances

What constitutes a convincing test of any model? The answer depends on the perspective of the members of the discipline responding to this questions, and on the degree of convincing that is required. There is much work that needs to be done, but it is instructive to examine and contrast the developments that have occurred in the ion resonance category with those in the other categories treated in the report. Ion resonance reports have been published by numerous investigators. They focused primarily on demonstrating an effect.

All tests of the ion resonance for calcium, using models developed by Liboff, Lednev or Blanchard/ Blackman, simultaneously satisfy resonance conditions for several ions based on the ion charge to mass ratio and a bandwidth of 10% (which was experimentally determined for hydrogen by Blanchard et al., 1994). It is in the nature of the theoretical model. The biological or chemical system may impose further restrictions on the ions that can participate in resonance due to local conditions for specific ion types or in defined chemical conditions rather than in cells. This ambiguity is not a fundamental flaw in the model, as some would propose.

The original IPR reports showed a consistency between model predictions and 66 independent data points, with only three variables in the model prediction which provided a means of calibrating the prediction to the form of the reported data. In fact, Blackman et al. (1994) tried to "fit the data" using low order Bessel functions not specified by the IPR model with the same number of variables to calibrate the fit, and found it impossible to model the data this way. Further, the original test of the IPR model which used neurite outgrowth from NGF-stimulated PC-12 cells (a rat adrenal pheochromocytoma cell line) as a monitor of cell response to fields (Blackman et al., 1994) introduced a concept of hydrogen ion triggering (at a high frequency index) that appears to take precedence over the influence of tuning for the primary ions (those at a frequency index of one) and this has been seen in the biological response.

When this hydrogen trigger concept was first proposed, some members of the research group believed it was highly unlikely, but agreed that it nevertheless served as an interesting post-hoc evaluation. It was from this hypothesis that another set of experiments were performed under exposure conditions which tuned for hydrogen ions as a primary ion. The charge-to-mass ratio for hydrogen is so high that it is uniquely possible to tune exclusively for ONLY that ion. Thus, the published, peer-reviewed paper by Trillo et al. (1996), quoted elsewhere in the report, directly demonstrates that the first sentence of the report summary is in error.

Other tests have been done with the IPR model. For example, critical results of this body of work show that (1) off-resonance conditions have demonstrated the dc flux density is essential (Blackman et al., 1994), (2) increased flux density of the ac field produces biological responses near null values at

exposure conditions that the model predicts to be non-perturbing to the biological system (Blackman et al., 1995b), (3) relative magnitudes of the IPR-model-predicted response and the observed biological response in PC-12 cells are consistent over several cycles of the non-linear response form (the relative magnitudes are fixed by the response function predicted for the first cycle and cannot be changed by any fitting parameters beyond that), (4) changes in the ac frequency gave results consistent with the predictions of the IPR model, and (5) a totally different biological system, rat liver epithelial cells (Clone 9 from ATCC), and a totally different biological endpoint, dye transfer between adjacent cells as a monitor of gap junction communication, produces experimental data that can be well-fit by the IPR model equations with parameter fixed to those determined by the best fit of the IPR model to neurite outgrowth from NGF-stimulated PC-12 cells (Blackman et al., 1996a,b) when both systems were exposed under the same conditions. This is an extraordinarily difficult test for any model to meet, yet it is a test of generalizability that any model should be required to meet. The IPR model has met that test, and that milestone needs to be explicitly recognized. And still further, the specific orientation of the ac and dc fields were tested in the neurite outgrowth assay and found to be critically important for previously reported results (Blackman et al., 1996c). In contrast to these experimental tests of the ion resonance model, the other categories generally have a greatly diminished experimental base. Only the non-linear oscillation category, when enlarged by the calcium efflux experiments, show independent replication of results.

It is a mistake, as urged in the report, to focus on calcium ions for future tests of the ion resonance models, exactly for the reason cited in the report, and above, about the ambiguity of which resonant ion is actually involved. It would be far more appropriate to select hydrogen as the test ion to examine in various systems.

b. Biological state of the cell or system:

The caveat stated here "... effects are dependent on the initial state of the system ..." needs to be emphasized for all EMF research. For example, this statement is entirely consistent with the published report of Blackman et al. (1988b). This report demonstrated the importance of establishing or characterizing the growth conditions before testing the influence of given EMF field conditions because prior exposure history can dramatically influence the results of the test. Specifically, exposure of chicken eggs to one of two extremely low frequency electric field frequencies, 50 or 60 Hz, was the determinant of whether exposure of chick brains of the hatched chickens showed enhanced calcium ion release or not in a subsequent test.

The caveat about initial conditions has direct implications for the suggestions made in this section of the report describing what experiments are critically needed to test theories. One logical conclusion is that it may not be possible, or even reasonable, for future research funding to exclusively focus on simple, isolated biochemical systems to obtain effects under conditions of direct

assistance to theoretical model builders, regardless of how desirable a goal that may be.

6. What theoretical limits (if any) have been derived from these models for the lowest magnitude to which biological systems should respond?

Additional reports showing low intensity effects include experiments showing magnetic-field induced neurite outgrowth from PC-12D cells (Blackman et al., 1993b) demonstrated a threshold below 22 mG rms., while calcium ion efflux from chick forebrain, which was subsequently shown to be sensitive to the dc magnetic field flux density and orientation (Blackman et al, 1985; 1990) was reported to occur below 3 mG rms. (Blackman et al., 1982). Dutta et al., (1994) reported field induced changes in enolase activity in *E. coli* bacterial cells exposed to magnetic field flux densities under 10 mG rms. (97 nT rms., or 0.97 mG rms., for 16 Hz ac fields and 65 nT rms., or 0.65 mG rms., for 60 Hz ac fields). These reports provide severe requirements on new models to be developed by theoreticians.

Summary and Recommendations

The statement made in the first sentence of the Summary and Recommendations gives mechanistic developments an enormously distorted importance which is highly misleading. Any biological result showing the influence of exposure to EMF will achieve plausibility by independent replication and extension to other biological systems, as well as by dissection of the biological system into subunits in order to identify the site of action. These results are determined by the biological systems themselves and not by whether there is a theoretical model that predicts the results that are observed. The interested reader should examine the Summary and Recommendations section in the report on "EMF Effects on Signal Transduction Cascade" for an example an alternative perspective, expressed from a biologist's viewpoint, on what is needed to demonstrate a biological effect that could help in risk assessment.

Theoretical models give guidance about where to search and how to extend studies. In an area as complex as the EMF area, with exposure variable(s) that must be characterized by more than one parameter (e.g., magnitude, orientation, duration, and, in the case of ac fields, frequency), model development is critical to clarifying the useful testing parameters. As experimental results are repeated and extended by one research group and other independent research groups are able to replicate some of the findings, the lack of a theoretical model only means that the theoreticians have failed to construct an appropriate model, nothing more. Theory is essential to validating a paradigm shift, but such a shift does not depend on theory; it is frequently led by experiment. Theory is valuable only to the extent that it can provide organizing principles and predictions of interconnected parameters that lead to more experimental testing.

The IPR and the PR models do more than just serve to organize the experimental data and to suggest designs for experiments as suggested by the

report. They can be used to predict the response form of various experimental endpoints that are sensitive to the exposure conditions. With the recent experimental data from Clone 9 cells being predicted by the IPR-fitted equations for PC-12 cell responses (see discussion above), the IPR model becomes a heuristic predictor of responses by systems that have similar sensitivities. This is a major step, perhaps in the development of a paradigm shift, that could be similar to the historical utilization of the Ritz Combination Principle (and the Balmer formula) that had no complete foundation in the physical theory of the times, but eventually lead to the development of quantum theory, or to the use of ether that led Maxwell to develop the mathematical foundations of electromagnetic theory. There are several models of interaction mechanisms presented in the literature, but a number have so little experimental support that they cannot even be evaluated for relevance to experimental problems at hand. There are several other theories that are coupled with varying amounts of experimental evidence; these theories have flaws from the current perspective that require us to use the qualifier, heuristic, when describing them. Nevertheless, even if no better EMF biophysical theories are developed in the near term, these heuristic theories can serve as very useful guides for biological scientists to determine critical EMF exposure parameters that need to be controlled in any experiment, especially those designed to develop biological/biochemical models of interaction mechanisms. It is a major deficiency in the report that the value of heuristic models mentioned.

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Appendix 2

Ion Resonance & Electron Transfer Mechanisms

Submitted by Ross Adey, M.D.

Current and future bioelectromagnetic research on ion transfer mechanisms in biological systems, and the possible functional significance of their susceptibility to imposed electromagnetic fields, must take account of relevant cell membrane ultrastructure. Cell membranes function as the cell's window on the world around it and the citadel within. Theoretically, although magnetic components of imposed fields may interact directly with intracellular molecules and organelles, much experimental evidence points to their interactions with cell membrane structural and functional systems in the first transductive steps.

Theoretical models and experimental studies that have ignored virtually all aspects of relevant cell membrane ultrastructure have accomplished little in their experimental validation because of serious mismatches between the models and the real world of biological substrate. There are gross disparities between sensitivities reported for induction of conformation changes in ribosomal RNA in pure solution by pulsed electric fields of 20 kV/cm (Neumann and Katchalsky, 1972) and significant effects on DNA synthesis in cultured cells with pulsed magnetic fields in the range 0.1-0.01 microtesla (Takahashi et al., 1986).

Cell membranes as a structural substrate influencing transmembrane ion transfer

Future research will require identification of the role in transmembrane ion transfer of the huge static (but not immutable) electric potential of 200 kV/cm across the plasma membrane. In addition, protein strands inserted into the lipid bilayer of this plasma membrane have highly negatively-charged strands protruding onto the membrane surface (the glycocalyx). Their presence in the plasma membrane is associated with coherent motions in surrounding phospholipid molecular tails (McConnell, 1975).

Two emergent aspects of this organization relate directly to current and future research on transmembrane ion transfer. This surface sheet exhibits a high potential of 30-40 mV and its modulation by the extracellular ionic environment directly influences cell excitability by drugs and neurotransmitters (Van der Kloot and Cohen, 1979). Also, magnetic field stimulation of the intracellular enzyme ornithine decarboxylase (ODC) leads to synthesis and export to cell surfaces of polyamine molecules bearing intense electric charges of opposite sign (polycationic) to the polyanionic glycocalyx (Hawel et al., 1994). By these molecular interactions there may thus be an autoregulation of net cell surface charge through actions of imposed magnetic fields.

Exported polyamines also interact with the glutamate (NMDA) receptor (McBain and Meyer, 1994). Activation of the NMDA receptor in brain tissue by

extremely low frequency magnetic fields (Bawin et al., 1994, 1996) is associated with an inward calcium current that initiates nitric oxide synthesis through activation of nitric oxide synthase. As a free radical with either reactive-oxygen-species (ROS) or reactive-nitrogen-species (RNS), nitric oxide regulates cell membrane ion transport in calcium-dependent potassium channel proteins of neural and vascular cells, in stimulation by ionizing radiation of potassium channels of non-excitabile cells, and in G-protein-mediated inhibition of chloride channels in gastric parietal cells (Lander, 1997). Nitric oxide is recognized as mediator in a variety of oxidative stress diseases, including Parkinson's and Alzheimer's diseases, coronary heart disease, cancer and aging (Youdim and Riederer, 1997).

"Clearly, we are at the early stages of understanding free radical signal transduction and using this knowledge for intervention in pathophysiology. Future work may place free radical signaling beside classical intra- and intercellular messengers and uncover a woven system of communication that has evolved to yield exquisite specificity" (Lander, 1997).

Inward signaling along cell membrane proteins with special reference to non-excitabile cells

Much important bioelectromagnetic research remains for the future in the search for understanding of transmembrane ion transfer in excitable and non-excitabile cells (Adey, 1993).

In their simplest forms membrane receptor proteins appear to cross the plasma membrane only once. In a second group the strand crosses the membrane seven times or more. The most striking feature of these proteins is in the structure of the extremely short chain of 23 amino acids that lies within the cell membrane at each consecutive membrane crossing. These short segments are composed of hydrophobic amino acids (and therefore non-conducting), in contrast to the hundreds of hydrophilic amino acids forming the rest of the strand inside and outside the cell membrane. Because of their hydrophobicity and their location in a highly hydrophobic environment, Ulrich et al. (1985) concluded that a hydrophobic segment as short as 23 amino acids is probably too short to be involved in conformation changes; and that its hydrophobic character makes unlikely its participation in either ionic or proton movement by coulombic forces.

Direct measurement of receptor-mediated inward Ca^{2+} currents in epidermal cells (Moolenaar et al., 1986) and mast cells (Hoth and Penner, 1992) indicates that it is not voltage-activated and would be consistent with ion translocation by molecular vibrational modes. Binding of the epidermal growth factor (EGF) to its specific receptor is followed by a 4-fold increase in intracellular calcium within 30 sec. All this calcium increment is derived from extracellular sources without a change in membrane potential. In mast cell it shows a characteristic inward rectification. For mast cells Hoth and Penner (1992) have proposed that this may be the mechanism by which electrically non-excitabile cells maintain raised intracellular calcium stores after receptor stimulation.

Possible role of highly cooperative electron transfer in proteins in cell membranes as a basis for electromagnetic field interactions

Studies by Chance (Devault and Chance, 1966) first disclosed the physical nature of the first steps in activation of the cytochrome c-bacteriochlorophyll complex by millisecond electron transfer. Since the transfer was temperature-independent from 120K to 4K, they deduced that the reaction proceeded through a quantum mechanical tunneling mechanism, perhaps taking place over distances as large as 30 angstroms and thus spanning distances equivalent to the entire thickness of the cell's plasma membrane. Their studies coincided with Mitchell's development of a chemiosmotic model, which proposed that the key electron-transfer steps of respiration operate across the full 35 angstroms of the cell membrane.

Extension of these early concepts by Moser et al. (1992) has suggested options for much further research on detection and coupling of electromagnetic fields at cell membranes. They conclude that a variation of 20 angstroms in the distance between donors and receptors could change the electron-transfer rate by 12 orders of magnitude. In the time domain there would also be a strong dependence on distance. Thus, in considering electron-transfer across the full thickness of the cell membrane, with edge-to-edge distances between 25 and 35 angstroms, the optimal transfer rate is shifted from time scales of seconds at the shorter distance, to days for the greater dimension.

In future research using this model to study biological membranes, it would be sufficient to select distance, free energy and reorganizational energy, in order to define rate and directional specificity of biological electron-transfer, thus meeting physiological requirements in describing a wide range of cytochrome systems. Here, protein would behave like an organic glass, presenting a uniform electronic barrier to electron tunneling and a uniform nuclear characteristic frequency.

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Internal Electric Fields Produced by EMF, Dosimetry, and Endogenous Fields

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Introduction

Issues addressed by this breakout group spanned a broad range of engineering and scientific questions. Electric currents induced inside the body by exposure to environmental electric and magnetic fields have been studied in some detail and were one of the first interaction mechanisms given serious attention. A relevant study of this topic must include consideration of dosimetry (the relationship between the exposure fields and induced internal fields), mechanisms through which the induced fields interact with anatomical structures and physiological processes, and the background of naturally occurring endogenous electric fields. The endogenous fields must be further categorized into noise fields resulting from random physical processes and physiological fields that are either directly functional (e.g., transmembrane fields) or simply by-products of physiological processes (e.g., electric fields due to cardiac activity occurring in surrounding body tissues).

Early dosimetry studies approximated the body as simple geometric shapes (e.g., ellipsoids) and in many cases underestimated peak currents that can arise due to the inhomogeneous body structure. More recent studies by Dr. Om Gandhi and Dr. Maria Stuchly have revealed that the spatial resolution of the body model used in dosimetry studies is important in producing accurate estimates of local peak currents and fields. A plenary lecture by Dr. Gandhi elucidated some of these principles and provided recent modeling results. While such dosimetry studies can be accomplished by straightforward application of physical and engineering principles, they represent a crucial link in the overall electric field mechanism problem. Their importance can be understood by noting that the central issue in any detection problem is the competition between a signal of interest and all other signals which may be lumped under the heading of noise. Dosimetry studies provide a quantitative approximation of the magnitude of the signal of interest - in this case, the electric fields induced by environmental fields. In very recent studies Dr. Gandhi has also begun to model the magnitude of endogenous fields resulting from normal cardiac activity (Gandhi et al., 1996).

Once the magnitude of the induced fields is determined, the next step is to evaluate possible biophysical interactions. Two general approaches have been employed. The first is to compare the induced fields to the best available estimates of macroscopic endogenous fields (e.g., from cardiac activity). An advantage of this approach is that it does not require one to identify all possible target structures which might be relevant at the macroscopic or microscopic level. The second approach is to choose a target structure such as the cell membrane and then try to predict the magnitude of competing noise sources at that specific location. In the case of the cell membrane, considerable information (based on

both experiment and theory) is available for estimating Johnson noise, channel noise, and other fluctuations in membrane potential. Both of these approaches are based on a comparison of the induced fields to the competing fields, and bottom-line estimates of minimum detectable signal strengths will change when better information about either induced field strengths or local noise competition is obtained.

In order for an electric field interaction to take place, a target physiological process must depend on movement or distribution of electric charges. Such processes are common in biological systems. An obvious example is voltage-gated ion channels which occur in cell membranes. Considerable attention has been given to this mechanism because the cell membrane tends to concentrate electric fields occurring in the surrounding tissues and the physics of these channels is relatively well understood.

Dr. Kenneth McLeod gave a short presentation on sources of endogenous fields at the beginning of the breakout session. His main points are summarized below:

- 1) Electrically active organs: Brain and heart generate electric fields ranging from 1 to 10 mV/m and neural tissue generates fields from 0.1 to 10 mV/m. In measuring the EEG on the cranium, the typical voltage between electrodes ranges from 1 to 50 mV.
- 2) Electro-kinetic or streaming potentials result from the deformation or strain of connective tissue containing fluid which has an ionic composition. It is expressed as the electric field per unit strain (ϵ), where strain is the relative deformation of the tissue. One microstrain ($\mu\epsilon$) is a deformation of one micron in the length of a one meter rod. In bone it ranges from 0.05 to 0.4 mV/m per $\mu\epsilon$, which is on the order of 0.1 mV/m per $\mu\epsilon$. For cartilage it is about 10 times larger or 1 mV/m per $\mu\epsilon$.
- 3) Piezo-electric effects result from the deformation of crystalline bone, which ranges from 0.1 to 1 mV/m per $\mu\epsilon$ in dry bone. It has not been measured for wet bone *in vivo*.

It was noted by several participants that some of the best data for electric field effects comes from studies of bone, but caution is needed in applying these results because some phenomenon such as electro kinetic and piezo-electric effects, are most relevant to bone and other specific tissue types.

Dr. Gandhi presented excerpts from his talk presented earlier in the day, which dealt with his calculations of the current density and electric fields in several tissues due to three sources of fields:

- 1) a person standing under a power line, characterized by an electric field, $E=10$ kV/m, and magnetic field $B=33 \mu\text{T}$ ($B=330$ mG);
- 2) a 1 mT (10 G) magnetic flux density, which is the ACGIH occupational standard as well as the CENELEC occupational standard [the latter is actually 1.33 mT (13.3 G) according to his morning lecture];

- 3) the endogenous fields from the heart, where that data comes from measurements of the surface potentials at 64 points on a dog's heart. His group had earlier (Xi et al., 1994) published results of modeling with a larger voxel size than the present work.

He presented two series of calculations - one for the head region with a voxel size of 2x2x3 mm and the other for the entire body at a resolution of 6x6x6 mm. His results were presented in graphs of the average and peak value (of current density or electric field) of all the voxels at a particular vertical height above the feet (or below the top of the head). In response to a question, he said he could provide a histogram of the distribution of the quantity (current density or electric field) at each height. The latter would provide a measure of both central tendency and range of the quantity, which was thought to be of some interest. Dr. Gandhi forwarded these statistics for the entire assembly of voxels from all vertical heights combined. The heart has several frequency components, and although he did calculations at a variety of frequencies, he presented the results only in the range from 40 to 70 Hz. (It is noted that this frequency range is the most relevant range for the NIEHS goals, since it is the band that encompasses the 50-60 Hz power frequencies in Europe and the US. In contrast, the justification of WHO and IRPA standards on the basis that skeletal muscle contributed large biopotentials may be less relevant, since the bulk of the power in the spectrum of fast skeletal muscle activity (such as intercostals and to some extent the diaphragm) is skewed to considerably higher frequencies.)

Dr. Gandhi showed selected slides from his earlier lecture to the group, and he reiterated his conclusions that both power line and occupational exposures produce much larger induced current densities and electric fields than endogenous currents produced by the heart (Figures 1-4.) for exposures at the upper limits allowed by IRPA, WHO and ACGIH, especially the latter. In the discussion the group noted from the slides that with a 1 mT (10 G) exogenous magnetic field probably 100% of computational cells (or voxels) had electric fields of 50 mV/m at the low end of the distribution and 99% of cells would probably have electric fields of less than 500 mV/m, with a mid-range of about 250 mV/m. The results of Dr. Maria Stuchly were brought up; they showed that a 1 μ T (10 mG) magnetic field produced a 0.2 mV/m induced electric field and that agreed with Dr. Gandhi's results.

Dr. Jan Stolwijk recalled from his work at ICRP that muscle was the tissue that gave the highest current density - 3 to 10 mA/m² - and that this was the basis of their standards, namely exogenous fields should not produce internal currents greater than those from endogenous sources. A discussion of which cells or organs should be the basis of standards was inconclusive; some believing that induced currents had no effects on some organs (cells), so these cells should have no influence on standards; others believing that the electrically-active organs (cells) had background currents of their own and may be less affected by induced currents from exogenous fields than other cells. Although there was some sentiment for not using these data for setting standards, the matter was not pursued to the point where the group reached consensus.

After the meeting, Dr. Robert McGaughy summarized the slides presented by Dr. Gandhi (Table 1). Since each exposure situation involves a population of either voxels or organs, Dr. McGaughy decided to compare the distributions (e.g., power lines versus endogenous sources) by calculating the ratio of their medians. This could be done with the data on 15 organs of the body. This leads to somewhat lower ratios of exogenous to endogenous currents and electric fields (40 to 100) than quoted by Dr. Gandhi in his talk (50 to 200), but his conclusion that current densities and electric fields from exogenous sources are larger than those from endogenous sources is still valid. A question was raised as to whether there are thin (less than the voxel size) membranes around organs or groups of cells that might have an electrical resistance high enough to appreciably reduce the currents inside them. Nobody knew of data to check that hypothesis, but it was acknowledged to be a real possibility. This is a generic problem for the current modeling efforts; it is not a specific limitation of work by Gandhi but is shared by Stuchly and other modelers as well. Dr. McLeod thought that the bone marrow was shielded by surrounding bone and that currents there would probably be less than the models showed. He also noted that bladder epithelium had a high resistivity.

Theoretical limits on cellular responses to currents and fields were the subject of vigorous discussion. The committee's opinion of new developments since the Adair paper (1991) for physical limits on detection by cells, was solicited.

The first topic was the paper given by Dr. James Weaver that morning, which concluded that the effects of weak magnetic fields (undefined, but presumably less than 2 mG) are in principle indistinguishable from temperature changes of 1 micro-degree C. He concludes that some researchers think they are measuring the effect of the applied fields but are actually seeing the effect of tiny temperature fluctuations caused by the fields. He also noted that the small effects reported by some researchers could be expected to result from temperature variations of far less than one degree centigrade and advised experimenters to measure carefully the temperature-dependence of their reported effects. The breakout group did not agree with Dr. Weaver's conclusions for several reasons:

- 1) Dr. Ted Litovitz adds a noise field to a coherent signal that induces a biological effect and the effect goes away with the extra field (Litovitz et al., 1994);
- 2) If temperature change is the factor, there would be no reproducible effects, and there are reproducible effects both within and between laboratories;
- 3) His calculations assume equilibrium conditions, and biological systems are far away from equilibrium;
- 4) Temperature control in the laboratory can not practically be tighter than plus or minus 0.1 degree, so the theory would be hard to prove directly;
- 5) Dr. Gayle Woloschak noted that many gene effects in the lab are stable within 2 or 3 degrees, so EMF effects in these systems can not be explained by temperature changes.

It was also pointed out that Dr. Robert Adair and Dr. Weaver present their calculations as if they were model-independent and simply reflected fundamental constraints of nature. In fact, their calculations are made by making a number of assumptions about the biological systems, and the group and others at the meeting pointed out that some of those assumptions were either incorrect or at least not universal or even common in biological systems. Two examples were given: The first, provided by Dr. Christopher Portier during Dr. Weaver's talk, pointed out that the assumption of high basal flux in metabolic systems is not general and may even be the exception; Dr. Antonio Sastre elaborated on a related flaw when Dr. Weaver makes calculations where ion flux across the membrane is determined by voltage-gated ion channels; for the case of Ca^{++} , the resting flux is extremely low; lower by many orders of magnitude than Dr. Weaver's assumptions. Drs. Weaver and Adair have never provided evidence that their "most field sensitive model" in fact converges to realistic values in the limiting cases of low basal flux, such as the cases that Drs. Portier and Sastre brought up. For these and other reasons, the group felt that the constructive contributions that the physicists could make to this debate still awaited calculations with biologically realistic numbers and assumptions.

The next topic was cellular ensembles, and measurements and theory showing that detection thresholds for these ensembles are more sensitive to small fields than are single cells; this effect was not discussed in Dr. Adair's 1991 paper, where the detection limit was derived as 100 V/m (as presented by Dr. Weaver's morning talk). Dr. Ad Kalmijn presented his experiments of electric field detection by sharks and other elasmobranch fishes. The sense organ consists of two orifices 10 cm apart. These orifices are connected to tubes filled with sea water which bring the fluid into contact with two adjacent layers of sensitive specialized epithelial cells. Behavioral experiments show that the animals can respond to an external electric field of 1 to 5 nV/cm, which puts 10 to 50 nV between each orifice. Therefore, each layer of epithelium has 5 to 25 nV across it at the limit of detection. About 2,000 cells of this layer are connected to a nerve fiber; several of these nerve fibers converge to a ganglion cell where the inputs are summed and modulate the frequency of the axon of the ganglion cell. The precise comparison of this threshold with the prediction from Dr. Adair's paper was not discussed. Dr. Paul Gailey also mentioned some of his thesis work on the conceptual and practical problems of calculation of what represents noise when dealing with electrically-connected cell ensembles. It was generally agreed that this problem needed more study before Signal/Noise (S/N) calculations were universally accepted in an ensemble context.

There are several other examples of large arrays of cells electrically connected via gap junctions, such as bone, liver, glial cells in the brain, heart, skin and other epithelia (Pilla et al., 1992, 1994). The thresholds of these ensembles are determined in part by their connectivity, not just the properties of individual cells.

Dr. Sastre next discussed his measurements of transient magnetic fields in California homes and his calculation of the expected induced currents in the body from these transients (S), compared with the electric field noise limit of detection

by single cells (N) derived in Dr. Adair's paper (Dr. Sastre noted the noise computed is Nyquist noise. Dr. Adair first endorsed it as the cellular noise floor and has since rejected it). He found that there were several thousand of these transients each day in the homes. Sastre, Kavet and Weaver studied a sample of 160 transients (10 from each of 16 homes) and found that they all had a signal to noise ratio (S/N) of greater than one (100% had a S/N of greater than 1 and 149/160 had a S/N of 3 or higher). A typical transient lasted 200 μ sec on the average and was 2 to 4 mG in peak-to-peak amplitude. A number of them induced voltages across the cell membrane that were comparable to the miniature end-plate potentials across the neuromuscular junction. Therefore, they are large enough to exceed theoretical noise limits and to potentially cause biological effects. What is not clear is whether biological processes can be induced by pulses as short as 200 μ sec. In Litovitz's work with low level magnetic fields, the pulse needs to be greater than 100 msec to be detected, but Dr. Litovitz was not sure that this was relevant to the large amplitude fields that Dr. Sastre was discussing. (It was suggested, but no consensus was reached, that the Litovitz coherence and the effects, if any, of transients may activate different mechanisms and pathways in cells and tissues.)

Electric field thresholds of *in vitro* effects were next discussed by Dr. McLeod. In the frequency range of a few 10's of Hertz, the thresholds are 0.1 to 1.0 mV/m. Considering the whole literature, the occurrence of effects below this is sparse, and above 1.0 mV/m the occurrence of effects is abundant. Dr. Gailey noted in his plenary talk that from a theoretical point of view, there is widespread agreement (even among the strongest critics of EMF biological effects) about the possibility of electric field biological effects at the 1.0 V/m level. Based on the latest dosimetry studies, this internal electric field level can be induced by an external magnetic field in the range of 10 μ T to about 100 μ T (100 mG to about 1000 mG, 1 Gauss). Thus, theoretical studies provide little guidance concerning effects in this range or above other than the observation that direct electric field effects are plausible. At lower field strengths, currently proposed models can not explain direct electric field interactions.

Questions Discussed by the Breakout Group

1. Best estimates of internal fields produced by EMF due to environmental exposures

Internal electric fields of 1 mV/m can be produced by 60 Hz magnetic fields in the range of 100 mG to 1000 mG. For more detailed information see the attached table and consult original references as noted in bibliography (Tenforde, 1986; Foster and Schwann, 1986).

a. Important sources of noise

Noise considerations have been raised from a theoretical point of view (Weaver and Astumian, 1992; Weaver, 1992; Pilla, 1994; Barnes, 1986). However, as with any theoretical model, exact prediction of complex interactions cannot be made with certainty. If experimental results indicate an effect at field strengths below which theory suggests effects are possible, then a much greater burden of proof is placed on the experiment. In general, multiple replications involving sham-sham runs, blinded protocols, and other quality assurance measures are necessary. Experimental lists should determine the temperature coefficient of the effect they are studying. If the ELF effect they are reporting could also result from a temperature change of a fraction of a degree, then extreme control of the experimental setup will be necessary.

b. Biological sites of interaction and amplification mechanisms which have been modeled

Most modeling attempts have focused on electrical effects at the cell membrane. However, some studies have considered the effect of transmembrane electrical fields on chemical fluxes (Astumian and Robertson, 1993; Liu et al., 1990). It is noted that amplification mechanisms alone cannot overcome the signal-to-noise problem. Such a mechanism would also amplify local noise as much as the signal of interest. Amplification may be necessary once a signal is physically transduced, but the greatest problem in explaining weak ELF effects is identifying a biophysical sensor that does not experience noise greater than the signal.

c. Important competing factors and theoretical constraints

Theoretical studies have focused on competition from endogenous physiological and non-physiological noise. Physiological competition from cardiac, neural, electrokinetic, and piezo electric activity have been considered to varying degrees. Non-physiological noise from thermal action (Johnson noise), ion channel fluctuations, and the discrete nature of microscopic fluxes have been the subject of a various of papers. Most of these studies take a classical signal-to-noise approach in establishing a theoretical lower limit for effects.

2. Predicted EMF exposure levels that might cause a biological effect

There is increasing agreement and experimental evidence that internal 60 Hz electric fields of 1 mV/m could cause biological effects (McLeod and Rubin, 1992; Robin, et al., 1993). Some evidence and models suggest that fields weaker by one or two orders of magnitude might also exceed local noise in some circumstances. A member expressed surprise at the magnitude for the electrically-induced electric fields, which he considered to be too large.

a. EMF characteristics associated with biological effects

Most *in vitro* and *in vivo* EMF studies have employed sinusoidal 60 Hz electric or magnetic fields, although some work has been done at other frequencies. Work by Litovitz (Litovitz et al., 1992) has indicated that the temporal characteristics (intermittency in this case) are important. A number of studies by Blackman, Liboff, and others have suggested that certain combinations of static and alternating fields produce varying effects (Blackman et al., 1990, 1994; Blanchard and Blackman, 1994; Liboff et al., 1987; Smith et al., 1987). Because of the lack of widely replicated *in vitro* results, no specific field characteristics can be singled out as particularly biologically active.

b. Impact of EMF characteristics on experimental studies and epidemiological studies

While a number of epidemiological studies have suggested an association of disease with various direct or indirect measures of power frequency magnetic fields, it is not clear from these studies, which, if any, of the field characteristics are biologically active. Even if an association with sinusoidal 60 fields is observed, it is possible that the biologically active factor is harmonics, transients, electric field strength, or some other associated field characteristic such as geomagnetic fields (Liboff, 1995; Wertheimer and Leeper, 1992). It is for this reason that the epidemiological findings provide an insufficient basis for choosing a mitigation strategy and that they provide little guidance for designing laboratory experiments. This observation underlines the need for a sound physical theory relating field exposure to biological effects.

Concerning internal electric fields, there are fewer theoretical objections when higher frequencies are considered. Higher frequency fields produce higher magnitude internal fields. Transients, which can occur near power lines and home appliances, are a source of such higher frequency components. Few experimental studies have addressed this issue.

3. Theoretical limits for biological effects

There is reasonable experimental and theoretical support for biological effects at internal electric field strengths of 1 mV/m and above. This internal field strength corresponds to 60 Hz magnetic field exposures in the range of 100 mG to 1000 mG (discussed in Dr. Gandhi's lecture). Evidence for effects at lower internal electric field strengths (and lower external magnetic field strengths) is weaker from both an experimental and theoretical point of view. Biological effects at about one-tenth the above exposure levels are more difficult to explain, but are conceivable under special conditions such as large ensembles of connected cells or near interfaces where electrical properties change over short distances (interfaces of dissimilar tissues). Presently available theoretical models are unable to explain effects at magnetic field exposures of less than 1 μ T (10 mG, about 0.01 mV/m)

(discussed in Dr. Gailey's lecture). Our current understanding of this mechanism suggests that it is unlikely to explain biological effects of exposures at this level.

4. Comparison of fields induced by environmental EMF exposures to endogenous fields

Estimates of induced fields due to cardiac activity have been modeled by Dr. Gandhi and there is some information available concerning other sources of endogenous fields. As described above, these levels vary at different points within the body and it is difficult to make one-to-one comparisons. It is also unclear whether coherent 60 Hz signals can affect tissues in a way different from near 60 Hz components of other organized physiological activity such as cardiac function. Any limits suggested by comparing induced signals to endogenous activity are subject to change as new information about microscopic and macroscopic physiologic function is obtained.

5. Relation between theoretical limits for cells and thresholds for humans

Many of the models developed for biological effects at the cellular level are assumed to apply to humans as well. In most cases the relationship between external field exposure and the modeled internal fields is based on whole-body dosimetry. One obvious caveat is that higher local internal fields have been predicted as model resolution improves. It remains possible that even greater local fields will be predicted as models are further improved to include interfaces with relatively discontinuous electrical properties. Another important caveat for available cellular models is that they ignore the coherent functionality of the body. For example, MEG recordings of brain electrical activity suggest that ten thousand to a million or more neurons may fire coherently during brain function. Such large ensembles are likely to exhibit far lower interaction thresholds than single cells or small groups of cells. Extrapolation of simple mathematical models to the full complexity of integrated body function is extremely naive in that it ignores the higher levels of organization and function that characterize human behavior. One example is the influence of ELF magnetic fields on the neural activity of the hippocampus in rats (Bawin et al., 1996). A weak influence on the rhythmic activity of the suprachiasmatic nucleus (which represents a population of coherently firing cells), for example, could affect pineal function and in turn affect the numerous subsystems entrained to circadian rhythms. Questions of this nature cannot yet be addressed by available theoretical models.

6. Implications of *in vitro* laboratory findings on animal/human studies

In the area of bone regeneration, relatively good agreement between *in vitro* and *in vivo* findings have been reported. For other *in vitro* effects, it is unclear whether the reported effects are due to induced electric fields or some other mechanism. Further work to identify effects clearly resulting from induced electric fields should help address this question.

Additional Issues Discussed by the Breakout Group

Points of agreement:

- Modeling results by Weaver, Adair, and others may not yet apply universally.
- Thresholds for E-field responses in the range that Dr. McLeod reviewed (~ 1 mV/m); later at the meeting Dr. Thomas Tenforde gave a lower threshold about 10 times higher than McLeod's but volunteered that he could accept McLeod's as the lower DEMONSTRATED limit.
- External B-field dosimetry needs to take into account the potential for both direct B-field interactions and the induced E-fields.
- The relevant range of ENVIRONMENTAL exposures to be considered by NIEHS in their review was from the low end of residential [$\sim .02$ μ T (~ 0.2 mG)] to the upper end of permitted occupational exposures (1 mT, 10 Gauss).
- There is little or no theoretical basis for excluding the possibility of bioeffects at internal electric field strengths of 1 mV/m which corresponds to 10 μ T to 100 μ T (100 mG to 1 G) external exposure for humans.

General agreement but not unanimity on:

- Potential importance of coherence in creating bio-effects (Litovitz)
- Potential importance of magnetic field transient exposures as a biophysically-plausible exposure
- Need to add more biological realism (anatomy, cell numbers, connectivity, ion channel numbers and kinetics, metabolic rate constants) in modeling efforts.

Points in which group was unable to reach general agreement:

- How problematic were Dr. Gandhi's results with respect to the logic used by WHO and IRPA in setting standards
- How to determine the usable ranges in Dr. Gandhi's calculations
- How to ascertain possibility of effects in electrically-connected cells given problems highlighted by Dr. Gailey in determination of the relevant "noise" metric.

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Summary and Recommendations

Bioeffects due to induced electric fields are reasonably well established experimentally at internal field strengths of 1 mV/m and higher [corresponding to external magnetic fields of 10 μ T to 100 μ T (100 mG to 1 G or higher). Effects at lower field strengths are less certain. Theoretical analyses also agree with the possibility of effects at or above this level, but noise considerations make it difficult to explain electric field effects at field strengths more than an order of magnitude below this level. There are strong theoretical arguments against the possibility of induced electric field effects due to magnetic fields below 1 μ T (10 mG). However, the group noted that current theoretical analyses are based on assumptions that may not be universally applicable in the human body. These assumptions include basal flux rates, numbers of cells acting coherently, the relationship between external fields and induced fields, and approximations based on equilibrium statistics. Older standards are based on early, less refined dosimetry studies and the bases for these standards need to be revisited with the newer dosimetry data and models available now.

Table 1
Induced Electric Fields and Current Densities from Power Lines,
Magnetic Fields and Endogenous Sources

	Power Line	1 mT Field	Endogenous Source (Heart)	Line/Endog	1 mT//Endog	Tissue Examined
E(mV/m)	3.2 (10.2) 20.6	2.1 (17.1) 34.6	0.03 (0.23) 4.5	44	74	organs
		20 --> 450				voxels
J (mA/m ²)	630 (1451) 5.574	575 (3401) 8316	3 (35) 490	41	97	organs
		2 -->40				voxels
	Hair Dryer	Hair Clipper	Endogenous Source (Heart)	Line/Endog	1 mT/Endog	Tissue Examined
E (mV/m)	0.01 (0.02) 0.05	0.1 (0.28) 0.65	0.03 (0.04) 0.08	0.5	7	
J (mA/m ²)	2.4 (7.6) 29.8	17.7 (110.6) 467.3	3 (8) 26	1.0	14	

Notes:

For individual voxels, the range is the eyeball estimate from 5% to 95% of the voxels.

For separate organs, the range is lowest organ (median of all organs) highest organ.

Ratios are based on median of the 15 organs for upper table (whole body) and 5 organs (head only) for the lower table.

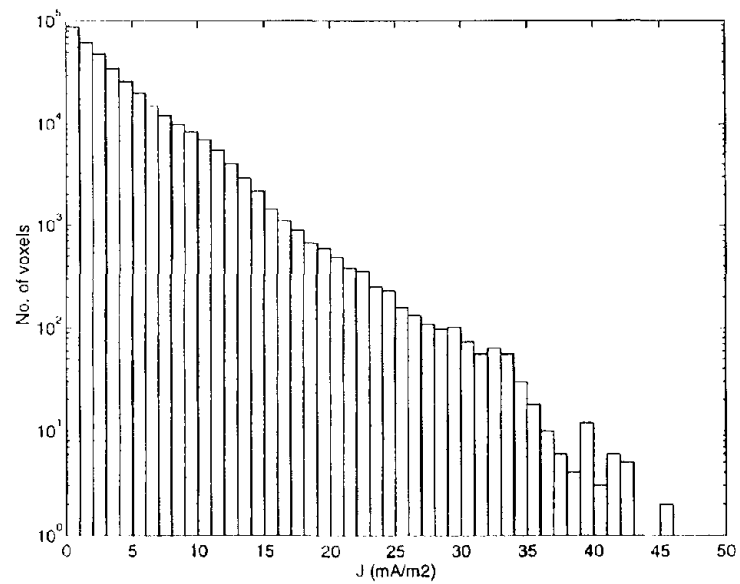


Fig. 1. Histogram of induced current densities in a 6 mm resolution anatomically-based model of the human body for exposure to a uniform magnetic field of 1 mT polarized from front to back of the body. 50 bins each of width 1 mA/m².

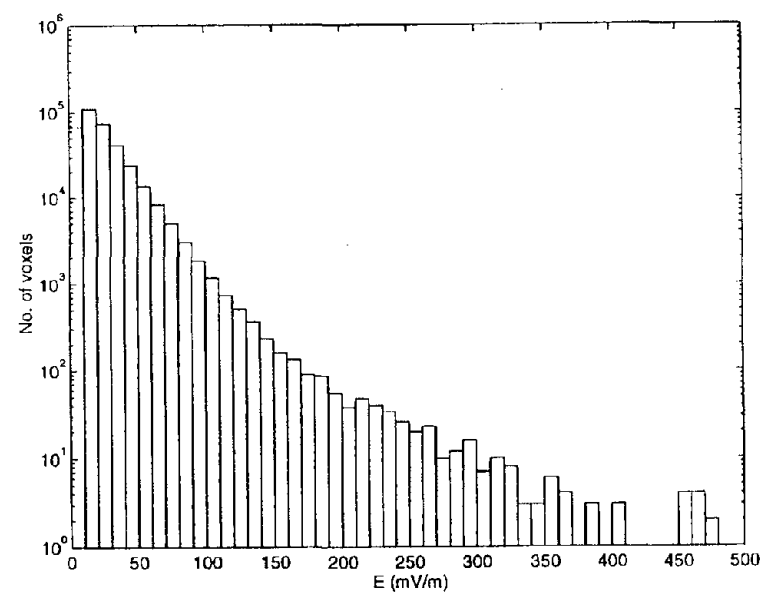


Fig. 2. Histogram of induced electric fields in the anatomically-based model for exposure to a uniform magnetic field of 1 mT from front to back of the body. 50 bins each of width 10 mV/m.

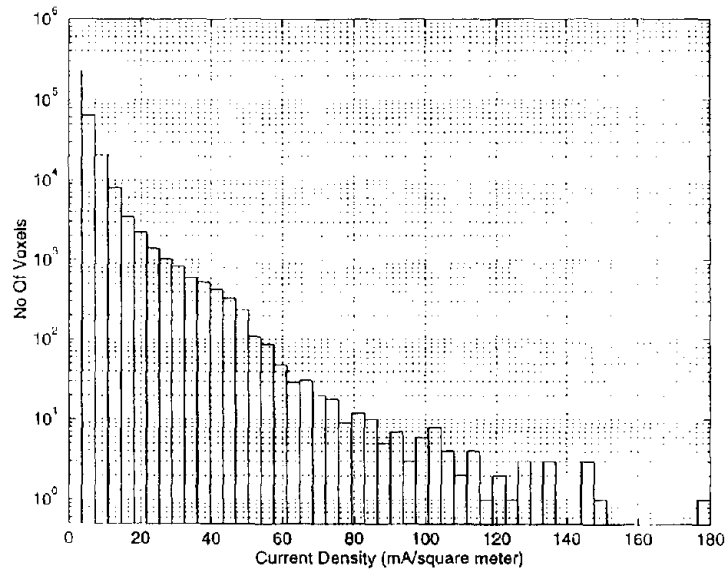


Fig. 3. Histogram of induced current densities in the anatomically-based model for exposure to EMFs under a high voltage power transmission line; $E = 10$ kV/m (vertically polarized), $B = 33$ μ T (from arm to arm of the model). 50 bins each of width 3.6 mA/m².

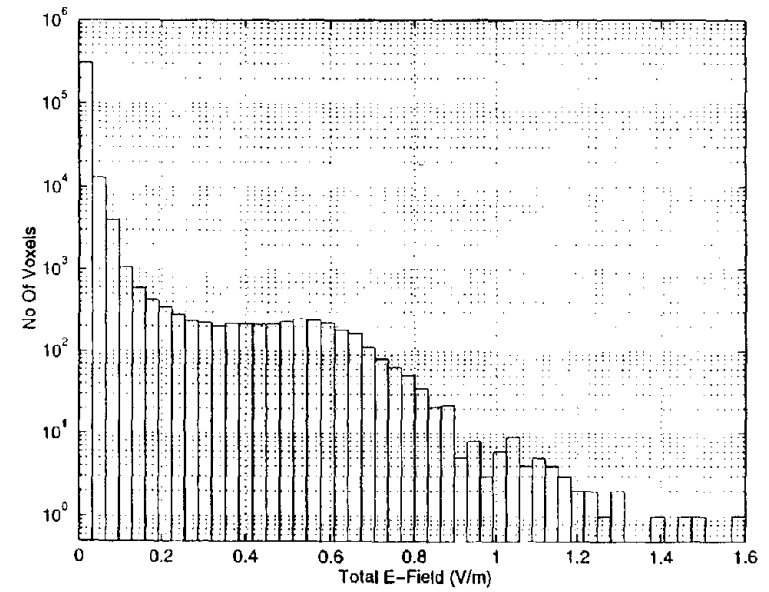


Fig. 4. Histogram of induced electric fields in the anatomically-based model for exposure to EMFs under a high voltage power transmission line; $E = 10$ kV/m (vertically polarized), $B = 33$ μ T (from arm to arm of the model). 50 bins each of width 0.032 V/m (32 mV/m).

Physical Theory and Human Health Risks

Facilitator: Michael H. Repacholi, Ph.D.

Rapporteur: Thomas S. Tenforde, Ph.D.

Introduction

The group understood its task to be a discussion of the feasibility that extremely low-frequency (ELF) electric and magnetic fields (EMF) present a risk to health from exposures at low levels in terms of present theoretical models. In particular, is there a theoretical basis that would suggest health risks from magnetic field exposure, and at what levels? Are there theoretical arguments that seem to preclude any risk from exposures at levels below 10 mG (1 μ T) and is there a threshold?

The group was also asked to discuss the theoretical basis for potential physiological effects of EMF exposure with respect to likely targets (cells, molecules, tissues), and whether such effects have any impact on health. Is there evidence from any theoretical models that there are human health risks from low-level ELF EMF exposure? Does EMF exposure have the ability, based on theoretical models, to influence biological systems directly, or could EMF exposure act as a cofactor interacting with another physical or chemical agent?

In order to focus the discussion within the allotted time, it was directed toward physical models dealing strictly with 60 Hz, sinusoidal magnetic fields. The breakout group briefly discussed models such as the ion parametric resonance model that can predict possible effects as low as 10 mG. However, this model was specifically reviewed by a different breakout group, and therefore, was not subjected to further in-depth evaluation.

Questions Discussed by the Breakout Group

1. Discuss the feasibility of EMF as a health risk at its experimentally observed levels:

- What theoretical arguments allow for risks from magnetic fields and at what levels?
- Are there theoretical arguments that preclude risk from magnetic fields below 10 mG (1 μ T)?

Within the selected set of models considered for this discussion, the group generally believed that established mechanisms such as induction of currents and direct magnetic field interactions can explain biological effects observed at “high field” levels above the thermal or endogenous biological noise levels. From existing laboratory information, the apparent threshold field levels for producing robust biological effects at 60 Hz are B fields (magnetic fields) on the order of 1 G (0.1 mT) and E fields (electric fields) in tissues on the order of 10 - 100 mV/m.

Most, but not all, of the group believed that there are no established biological effects at field levels below 10 mG (1 μ T) and that there are no established theoretical models that predict effects below 10 mG (1 μ T). One finding has been reported as occurring near the upper limit. Three independent laboratories have reported that 12 mG (1.2 μ T), 60 Hz magnetic fields can block the growth inhibitory effects of the hormone melatonin on human breast cancer cells *in vitro*. The original studies conducted in Dr. Robert Liburdy's laboratory (Lawrence Berkeley National Laboratory) (1) have been independently replicated in the laboratories of Dr. Carl Blackman (US EPA), (2), and Dr. Richard Luben (University of California, Riverside) (3) using identical biological materials and exposure conditions; these replication studies are not yet published in the peer-reviewed literature. This is an area for careful analysis of the results and for further research on the underlying mechanisms of ELF field interactions with melatonin receptors.

2. Discuss the theoretical basis for potential physiological effects of EMF:

- What systems are likely targets?
- Are EMF effects adverse to health?

The group agreed that cell membranes and intracellular signaling events may be sensitive biological targets for action by EMF. Further, they believed that there is theoretical support that these may be sensitive to ELF EMF because it is well known that there are signal receptor molecules and amplification processes that could explain disruption of normal cell functions. Evidence to support this possibility comes from studies involving the movement of calcium ions across cell membranes and changes in signal transduction events involving protein kinases.

3. What are the theoretical mechanisms which can explain potential biological effects from EMF exposure?

See discussion for question #1. Also, the group noted the difficulty in correlating fields measured in epidemiology studies (generally the time weighted average of sinusoidal fields, predominantly from power lines) with those prescribed by theoretical models or observed to be biologically active by *in vitro* experimental results. It was agreed that the biological effects observed at low field levels could not be adequately explained by present concepts of "dose" metrics (i.e., models describing a direct relation between the 'amount' of EMF, usually quantified as the field magnitude, and the observed biological result, where more field generally gives more effect).

4. Does theoretical evidence support EMF as a human health risk?

See discussion for question #2.

5. Theoretically does EMF have the potential for affecting biological systems directly or is it likely a co-factor interaction with another physical or chemical agent?

In theory, EMF does have the potential to affect biological systems, but the database is currently so inadequate that it is not possible to form sound conclusions regarding this possibility. Limited studies have suggested that ELF magnetic fields may exert a co-promoting effect in the development of tumors from pre-initiated cells. The reproducibility of these studies, which have primarily involved co-promoting effects of ELF magnetic fields and a chemical tumor promoter (TPA), has been difficult to establish. At the present time there is no firm evidence for a combined effect of ELF fields with other physical or chemical agents in the development of cancer or other adverse health effects. Discussion of the possible influence of other physical agents related to magnetic field exposure, such as the dc field as a co-factor, fell outside the pre-determined scope of this discussion.

One member of the group noted the possibility of frequency specificity in ELF EMF interactions, noting that if a particular process is primed for a particular signal, then receipt of that signal could have biological consequences in excess of the apparent energy deposition provided by the signal itself.

Additional Issues Discussed by the Breakout Group

There was extensive discussion about the "molecular change" model, which focuses on net ionic or molecular changes due to a field exposure, that has been described by Astumian et al. (4, 5) and was presented earlier in the conference by Dr. James Weaver. The model identifies a threshold for electrical noise levels in molecules and cells, above which EMF signal strengths would have to rise before discernible electric field induced biological effects would be possible. This model focuses on selected, postulated characteristics of reactions in biological systems. Specifically it takes into account non-linear systems and signal amplification events where voltage-gated cell membrane proteins are involved. Fundamental biophysical considerations identified by calculations with this model suggest that the threshold induced electric field required to produce detectable biological effects is on the order of 100 mV/m for large, isolated cells. However, the model does not fully account for the occurrence of biological phenomena such as phase transitions or other non-linear effects that could drive the signal above the noise level when EMF levels are below those predicted by electrical noise theory.

In general, the group felt that non-random temperature fluctuations in an *in vitro* sample exposed to EMF were unlikely to explain low-level field effects. Even with temperature control, fluctuations on the order of 0.5 - 1.0 °C occur in living systems, and so one would likely need a temperature rise exceeding this level to observe an effect. The issue of poor temperature regulation was not considered by most attendees to be a probable explanation for effects observed at low field levels or to be a likely explanation for the lack of reproducibility of effects noted in many studies. Moreover, it was concluded that an EMF effect, which can also be generated by a change in temperature within the range of biological homeostatic control, is not likely to have any adverse consequences for human health. At least

one individual felt that the influence of temperature variations in producing molecular changes similar to those of threshold field exposures should not be discounted without experimental testing. Specifically, a recommendation was made that the temperature sensitivity of a reported effect should be determined as well as the quantitative measurements of the temperature variations within the experimental system being used. Together this information should provide insight regarding the magnitude of molecular changes due to thermal effects as compared to the molecular changes due to a field exposure.

The biological database was considered to be incomplete, with numerous examples of contradictory findings. As a result, firm conclusions cannot be drawn regarding the role of several factors that have been discussed as a possible basis for biological effects at low field levels. These factors include combined effects of ac and dc fields, transients and harmonics, intermittent exposures, and ELF exposures in combination with other physical or chemical agents.

References

There are a number of comprehensive reviews of the biological literature on effects of EMF that have been written in the past 2 - 3 years. Perhaps the most valuable single resource is the Handbook of Biological Effects of Electromagnetic Fields, 2nd edition, C. Polk and E. Postow (eds.), CRC Press, Boca Raton, FL, 1996.

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Summary and Recommendations

It was recognized within the group that the role of theory is 1) to describe how effects might occur and 2) to estimate limits or thresholds below which effects are not expected based on known biophysical mechanisms. The group agreed that theory cannot invalidate critically analyzed and accepted biological observations, but theory can invalidate uncritical interpretations of biological observations. Further, critically analyzed and accepted biological observations can demonstrate that particular theories are inadequate.

However, after considering the available information, the group identified several important issues related to the biological effects and health impacts of ELF fields that remain to be addressed, as noted below:

1. There is a need for a more complete analysis of the role of non-sinusoidal fields in causing biological effects. For example, in a recent survey by the Electric Power Research Institute, it was noted that transients are produced within the power distribution system from the switching on and off of appliances. The strength of these transients is, in some cases, sufficiently high to induce signals in biological materials that exceed endogenous electrical noise levels. The possibility that these signals could be responsible for biological effects either alone, or in combination with other agents, merits further study.
2. Mechanistic studies are needed to confirm and to explain the reported biological effects of low-level exposure to ELF fields. In particular, there is a need for predictive models that would allow extrapolation of effects over a broad range of field intensities.
3. Much additional work is needed to relate theoretical models with *in vitro* observations in a way that demonstrates the appropriate application of the models in making reliable predictions of *in vivo* responses and human health risks.

EMF Effects on Cellular Replication

Facilitator: David G. Kaufman, M.D., Ph.D.

Rapporteurs: Robert P. Liburdy, Ph.D. and William S. Baldwin, Ph.D.

Introduction

The breakout group was tasked to review relevant literature and assess scientific findings relating to specific EMF effects on cellular replication. A determination of whether the literature supports a consensus conclusion for the existence/nonexistence of EMF effects on cellular replication is important to understanding the potential for EMF to impact human health. Cellular replication is a fundamental and complex process that involves the interaction of a wide variety of biological processes. The study of replication itself as an endpoint in experimental studies is, therefore, one of the critical factors scientists have evaluated in assessing potential effects of EMF on human health. Identification of specific cell types or the identification of specific biological conditions or the status of the cell (e.g., synergistic interactions with hormones/drugs, cell cycle progression state, or redox state) in which replication is influenced by EMF is important to elucidate. Since cellular replication is a complex process under the control of a variety of different intermediary cellular pathways, it is important that complementary studies are also undertaken to identify, for example, specific EMF effects on early and mid-level events in the signal transduction cascade, including the investigation of direct effects on key enzymes involved in these pathways. In this report reference is made to breakout group reports prepared by other groups addressing these complementary issues.

A number of experimental *in vitro* studies were identified for discussion. A review of the literature resulted in approximately a dozen peer-reviewed reports in which cellular replication itself was explicitly investigated. These reports are listed below and both positive and negative studies are listed. The breakout group discussed a number of these investigations in some detail. Reported findings were assessed from the point of view of quality of the research, and the group discussed critical issues including: experimental design, statistical analysis, control treatment groups, blinding, temperature control, and the appropriateness of biological techniques employed to quantitatively assess replication.

Questions Discussed by the Breakout Group

1. Can EMF alter cellular replication by itself or by acting synergistically with other agents?

a. EMF altering cellular replication by itself

The group felt that at fields above approximately 10 gauss (G, 50/60 Hz) there exists evidence from a number of studies in the peer-reviewed literature to

suggest that EMF can alter cellular replication; these reports derive from a number of laboratories involving different cell lines and different investigators. There are replication studies currently underway in several laboratories, however, these studies are not yet in the peer-reviewed literature. In the aggregate, when the peer-reviewed studies in this high-field exposure range of >10 G are reviewed, these studies indicate that effects on cellular replication are observed across a number of different laboratories and cell types. There also exist several negative studies that have been reported at field strengths approximately 2 G or less; these studies suggest that a direct effect on cellular replication is not observed at these field strengths. Refer to the listing of literature citations below.

There were some important caveats identified by the group that need to be emphasized such as a) none of these high field studies have yet been replicated, and b) each of these studies could have employed improved methodologies in one or more areas of exposure system design, temperature control, or quantitative biological assessment of cell replication. For example, it was agreed that it is best to employ multiple methods to assess cell replication so that a reported effect is not necessarily technique-dependent. In high-field studies it was felt that particular attention should be paid to temperature control during field exposures, since it is possible for fields ≥ 10 G to lead to significantly induced current in some exposure configurations and to media heating. None of these relatively high-field studies are part of the RAPID/NIEHS research program so they have not had engineering site visits to assess field exposure systems.

Although the group felt that there exists evidence that EMF influences cellular replication itself for magnetic field exposures above ~ 10 G, the group agreed that more research is needed to extend and to replicate these findings and to elucidate a biologically-based interaction site.

b. EMF altering cellular replication synergistically with other agents

The group discussed the work by Liburdy and others in which EMF is reported to alter cell replication in a MCF-7 human breast cancer cell line when co-treated with melatonin or with tamoxifen. Synergy is used in this context by the group to mean that co-treatment of cells with a) magnetic fields and b) drug/hormone were required to alter cell growth; magnetic fields alone did not influence cell growth.

The report by Liburdy (1993) employed "environmental-level" 60 Hz magnetic fields in the low milliGauss [12 mG (rms.)] range and in these studies magnetic fields were observed to block the growth inhibitory action of physiological levels of melatonin on MCF-7 human breast cancer cells. Blackman (1996) and Luben (1996) have recently independently replicated these findings; they have presented their data at scientific meetings, but these findings are not yet published.

These observations with melatonin and the tamoxifen findings discussed below, appear to be a "synergistic" interaction of EMF with the hormone or drug since EMF alone did not alter cell replication. Since no alteration of cell growth itself was observed in the absence of melatonin, these findings are consistent with

the ≤ 2 Gauss high-field studies mentioned above in which no effect on cell replication was reported.

A point of interest to the group was that the melatonin findings have been independently replicated in two laboratories (refer to listings below). Independent replication of these findings in two different laboratories provides a degree of credibility and support which are currently lacking in other studies that the group evaluated.

A recent extension of this work involving tamoxifen was discussed by the group in a paper accepted for publication in Bioelectromagnetics (Harland & Liburdy, 1977). In these studies it was observed that 12 mG magnetic fields act to block or inhibit tamoxifen's oncostatic action on human breast cancer cells. This work is of interest since it extends the "synergistic" action of 12 mG fields with the hormone melatonin, noted above, to the drug tamoxifen. Secondly, tamoxifen is reported to act as an antiestrogen and bind to the estrogen receptor (ER) in ER+ (ER positive) human breast cancer cells. Dr. Robert Liburdy indicated that in parallel studies, reported at scientific meetings (not yet published, see reference list below), his laboratory has also observed that 12 mG fields block the action of ICI 182,780, a pure antiestrogen. These observations and the tamoxifen data suggest that ER receptor-ligand binding may be involved in the field interaction. The group felt that further mechanistic studies are required to evaluate this hypothesis. Also, it was mentioned that performing direct receptor binding experiments in future research would be of value.

The discussion was also directed at technical issues specifically including the areas of field dosimetry, biological variability, temperature control, and statistical analysis. The milliGauss field studies were felt to be associated with a reasonably high level of technical methodology in the areas of field dosimetry, field uniformity, temperature monitoring, CO₂ monitoring, and blinding since this laboratory is part of the RAPID Program of the DOE research program, and has received regular annual field site visits to evaluate and to assess the technical approaches employed. The replication work conducted by Blackman and Luben (noted above) are also part of the NIEHS or DOE programs and are annually site visited.

The melatonin and tamoxifen/ICI 182,780 studies mentioned above were of interest to the group for several reasons.

- 1) This data represents an exception to the above high-field findings since environmental-level (milliGauss) magnetic fields resulted in cellular response.
- 2) The field effect involves the presence of a hormone (melatonin) or drug (tamoxifen), so the interaction appears to be "synergistic" requiring both the hormone/drug and the magnetic field.
- 3) The melatonin findings have been independently replicated by two other laboratories, and these studies were conducted by experienced scientists with a track record in the field of bioelectromagnetics.

- 4) The melatonin findings are interpreted by a number of scientists as playing a role in the melatonin hypothesis, as discussed by Dr. Richard Stevens.
- 5) The tamoxifen and ICI 182,780 studies suggest the possible involvement of the estrogen receptor, and this may have relevance to estrogen-dependent biological processes.

Thus, given the above points of interest, the group, in general, felt that these *in vitro* findings have potential relevance to *in vivo* human health.

There were two recent abstract reports dealing with human brain cancer cells whose results suggest that a synergistic action between magnetic fields and drugs was observed. An abstract report from the laboratory of Dr. L.G. Costa (Fund. Applied Tox. 36:126, 1997, Abstract #643) was mentioned in group discussion. In these studies the growth of human astrocytoma cells in culture was enhanced by exposure to a 1.2 G, 60 Hz magnetic field for 24 hours. In addition, the fields enhanced the mitogenic effects of carbachol and TPA suggesting that magnetic fields can potentiate proliferation of human astrocytoma cells by mitogens. Since a protein kinase C (PKC) inhibitor, Bis, partially blocked the field effect, PKC may play a role in these field responses. A second study by Liburdy and colleagues [Annual Review of Research on Biological Effects of Electricity and Magnetic Fields from the Generation, Delivery, and Use of Electricity (1996), Abstract #A-4 and Second World Congress for Electricity and Magnetism & in Biology and Medicine (1997), Abstract #L-7] reported that exposure to 12 mG, 60 Hz magnetic fields for up to eight days partially blocked the growth inhibitory action of tamoxifen in human glioma cells. These "environmental-level" magnetic fields themselves did not influence cell growth. It is of interest to note that tamoxifen is reported to be a strong PKC inhibitor in estrogen receptor negative (ER-) cells such as human glioma cells.

2. Are there dose-response data supporting these effects? What are the temporal characteristics of the response? How do these exposure patterns relate to long-term effects in cells?

a. Dose-response data

The existing high-field (≥ 10 G) EMF effects studies on cellular replication do not, in general, present detailed dose-response data. In the absence of this information, the group looked across the available literature studies listed below, took into account the field doses employed, and observed that EMF effects on cell replication (in the absence of hormones or drugs) are associated with field levels of approximately ≥ 10 G, while field levels of ≤ 2 G are associated with no alterations in replication. This observation is not a definitive conclusion, but rather represents a statement based on a segregation of these findings into positive and negative studies on the basis of the reported field strengths. Caveats include the fact that a variety of different cell lines were employed, exposure times varied

significantly, different approaches to measuring cell replication were employed, the range of magnetic field intensities was great, and large gaps existed in this range of field levels that were not evaluated.

However, there are existing dose-response data for the milliGauss studies from the Liburdy lab for the melatonin experiments they have reported. This dose-response data has been presented at scientific meetings (see listing below), but it has not yet been published. The group discussed this dose-response data since it suggests that there exists an apparent dose "threshold" between 6 - 12 mG. The discussion emphasized the need for additional data between 6 - 12 mG since this would have the potential to significantly aid future modeling efforts and possibly assist in the design of future epidemiology studies.

b. Temporal characteristics

A review of the high-field (>10 G) literature suggests that a range of exposures as short as several hours to as long as several days has been reported to be effective in altering cellular replication. No definitive conclusions about the effectiveness of specific exposure duration were possible.

The environmental-level (milliGauss) studies by Liburdy were also discussed since data existed indicating that 12 mG magnetic fields required an exposure duration of at least one cell cycle to be effective in blocking melatonin's action on MCF-7 cells. This data was previously presented at scientific meetings, and is not yet published (listed below). The group discussed these findings and the data suggest that a 12 mG field exposure of at least three days is required to achieve a significant blocking of melatonin's action on MCF-7 cell proliferation. A comment was made that it is interesting, and perhaps biologically significant, that the time requirement for the field effect on melatonin is approximately equivalent to one cell cycle.

c. How do these exposure patterns relate to long-term effects in cells?

The group felt it was premature to conclude from the existing database that the available *in vitro* data could be directly extrapolated to long-term effects in cells. The studies available for assessment here did not, in a systematic manner, investigate the reversibility of EMF effects on cellular replication. The group felt that such information would significantly help to address the issue of long-term effects in cells.

3. To what degree does this indicate strong support for an *in vivo* effect on cellular proliferation?

The group did not feel that the *in vitro* data could be easily extrapolated to the *in vivo* situation. Discussion included suggestions for further research that suggested complementary *in vitro* and *in vivo* model systems, which are reported below.

4. Does EMF alter signaling pathways related to cellular growth (cytokines, hormones, etc.)? Is this likely to happen *in vivo*? What health effects might be associated with these types of *in vitro* effects?

The reports discussed by the group focused on cellular replication as an endpoint and these studies did not, as a rule, investigate whether there were concomitant alterations in hormones or cytokines known to regulate cell proliferation. Such studies were thought to be of value as goals for future investigations. Discussion from the group indicated that growth hormones such as TGF β and EGF, cytokines, and cell cycle progression factors such as cyclins, would be potentially fruitful endpoints to measure along with cell proliferation in future *in vitro* studies.

5. Comment specifically on the possible mechanisms involved in the *in vitro* melatonin/EMF studies in MCF-7 cells of Liburdy and others.

Melatonin is a complex hormone and its biological mechanisms of interaction and effects on human health were recently reviewed in the New England Journal of Medicine (A. Brzezinski, 1997; see below); this review was discussed in the plenary talk on EMF Effects on Cellular Replication.

The basic finding in the studies by Liburdy is that a 12 mG (60 Hz) magnetic field exposure applied for at least three days to human MCF-7 breast cancer cells in culture blocks or reduces the growth inhibitory action of melatonin on these cells *in vitro*. This basic finding was recently independently replicated by Dr. Carl Blackman (EPA) and by Dr. Richard Luben (UC Riverside).

There are several possible biologically-based interaction mechanisms that may be operative, and at this time no firm conclusions can be drawn about which one is more likely. Further research is required to differentiate among these testable hypotheses; it is possible that magnetic fields operate on melatonin function via multiple pathways (direct and indirect interactions).

Perhaps the simplest and most direct mechanism is that the magnetic fields prevent melatonin from entering the cell; this would explain the field associated blocking effect on melatonin's action. This is possible, but perhaps not likely; a marked alteration in cell membrane fluidity or permeability would most likely have wide reaching and global effects on cell function. Melatonin is known to be freely diffusible across the cell's plasma membrane, and it rapidly reaches an equilibrium concentration within the cell, so permeability issues do not appear to have the potential to play a critical role. However, this is a testable hypothesis and radioisotope studies could address this issue; such studies are planned.

An alternative, biologically-based interaction may involve magnetic fields influencing melatonin's binding with its receptor inside the cell. This is perhaps more plausible since it has been demonstrated (Harland & Liburdy, 1997) that the magnetic field itself, and not the induced electric field, is the operative field metric. Since direct electric field interactions are restricted to the cell membrane, and the electric field is not the field metric, an electric field (E-field) interaction with the cell surface is ruled out. Magnetic fields penetrate the cell membrane and the interior of the cell, and since the putative melatonin receptor is postulated to be located in

the nuclear envelope, the possibility of a receptor-mediated field interaction is a plausible candidate. For example, Dr. Liburdy mentioned that studies with retinoic acid would be of interest since retinoic acid binds to the nuclear retinoid Z receptor (RZR), as well as melatonin, and this receptor is reported to be present in MCF-7 cells. Dr. William Baldwin concurred and mentioned that the RZR receptor may represent a putative endogenous nuclear receptor for melatonin. The group felt that such studies may help to elucidate whether receptor binding is involved in this field interaction.

There is experimental support for receptor binding as a possible interaction site, and this is based on the follow-on tamoxifen and ICI 182,780 studies mentioned above (see reference list). These two compounds are antiestrogens and bind the ER; ICI 182,780 is a "pure" antiestrogen. In studies discussed by the group and described in Harland & Liburdy (1997) (Bioelectromagnetics, in press) it has been observed that 12 mG magnetic fields inhibit or block tamoxifen's growth inhibition of MCF-7 cells. Additional studies not yet published (reported at scientific meetings, mentioned above, and listed below) also indicate that 12 mG magnetic fields block or inhibit ICI 182,780's action in MCF-7 cells. These findings support the hypothesis that receptor binding, in this case the ER, is involved in the tamoxifen and ICI 182,780 field effect.

It should be emphasized that there are still alternative, biologically-based interaction mechanisms that may be operative. For example, indirect magnetic field effects on signaling pathways "distal" to receptor binding by melatonin, tamoxifen, or ICI 182,780 cannot be ruled out. For example, following ligand-receptor binding there are subsequent signaling pathways, which are triggered, that may themselves be sensitive to EMF. These pathways involve presently unknown co-factors, enzymes, genes, and additional biochemical events that regulate cell growth, but are triggered by melatonin, tamoxifen, or ICI 182,780 receptor binding.

A comment was made that mechanistic experiments involving receptor binding might shed light on the role that receptor binding plays in these field effects. It is possible to perform Scatchard binding studies with MCF-7 cells, to isolate the melatonin receptor, or the ER, and to perform test tube studies to assess ligand-receptor binding in the presence of magnetic fields (see below).

6. What additional studies should be done to strengthen these findings?

The group discussed the need for further *in vitro* studies that take into account some of the following points.

- a. Employ multiple methodologies to measure cell replication in *in vitro* studies.

This is desirable since past studies have either used one technique for assessing cellular replication or have used a non-standard or non-quantitative technique. One improvement would be to use an automated plate reader assay to follow cell growth (e.g., the MTT assay) in combination with hemocytometer

counting or measurement of total DNA content. Another approach is to use two-parameter flow cytometry to measure simultaneously the numeric distribution of cells with a given DNA content as well as S-phase DNA replication where fluorescent antibodies are used to quantitate the incorporation of BrdU in the same cell population.

- b. Employ markers for growth factors or growth regulating hormones in *in vitro* studies.

The group felt it would be valuable to assess changes in important cytokines, cyclins, growth factors (TGFb, EGF), and hormones that influence cell growth, in addition to carefully quantitating changes in cell growth itself.

- c. Further studies in which EMF acts "synergistically" with other hormones or drugs should be investigated.

If EMF acts primarily as a "co-factor" in concert ("synergistically") with other biochemical agents, such as in the melatonin/tamoxifen/ICI 182,780 studies, then it would be important to expand the scope of studies to include investigating how EMF may influence the action of other important growth regulating compounds. Exploratory studies investigating how EMF influences cyclin action, interleukins, TGFb, and EGF action are several examples that the group mentioned.

- d. Since receptor binding is a plausible/possible site of interaction, and some data supports this hypothesis (mentioned above) it would be of interest to perform receptor binding studies.

Scatchard analyses with live cells is a standard approach that could be used to investigate receptor binding during magnetic field treatment. Alternatively, test tube studies (mentioned above) could be performed in which ligand-receptor binding during field treatment is followed directly in the absence of any cell machinery.

- e. Extend cell replication findings to a variety of different cell types.

It is important to determine if a) a given cell type is characteristically responsive to EMF and b) how many different types of cells are responsive to EMF. For example, it was discussed that other human breast cancer cell lines may be sensitive to melatonin and these model systems should be investigated. For example, the human breast cancer cell line, T47D, and the BG-1 cell line, derived from a human ovarian tumor, are reported to be sensitive to melatonin. Human breast cancer cell lines have not, in general, been surveyed for melatonin sensitivity, and this should be investigated. Such cell lines could be used in *in vitro* studies to establish the "generalizability" of a synergistic EMF effect on cell growth involving the hormone melatonin or tamoxifen/ICI 182,780.

It should also be mentioned that there are a number of MCF-7 derived somatic mutants described in the literature that could be used in mechanistic studies (see below in "f").

- f. Utilize "knock-out" somatic mutants derived from the parental stock MCF-7 cell line to test biologically-based mechanisms of interaction.

Dr. Liburdy mentioned that it would be desirable to use a somatic mutant subclone to test hypotheses in which a specific biochemical pathway, receptor, or gene is suspected to be critical in mediating a field effect. There are available MCF-7 mutants that display deficiencies in a number of enzymes such as PKC isotypes and in the ER. If the magnetic field effect is observed in the wild type, parental MCF-7 cell line, and it is not observed in these "knock-out" somatic mutants, this information would be important in identifying specific, focused sites of interaction.

- g. It is important to characterize the influence of temperature on cell lines used in cell growth studies.

The group felt that some cell lines may be more sensitive to temperature than other cell lines, and it is of value to characterize temperature sensitivity. Discussion indicated that mammalian cells are generally not "hypersensitive" to changes in temperature; for example, cell culture incubators can typically vary by as much as 1-2 ° C, and growth curves are not detectably affected. However, it was agreed that changes of 3-5 ° C or greater can have detectable changes in cell growth of typical mammalian cell lines.

- h. Information on exposure duration is needed.

The temporal characteristics of field effects on cell replication should be investigated, including whether the effect is reversible. There is value in investigating the effects of "long-term, chronic" EMF exposures on cell replication; no *in vitro* data are currently available to address this issue.

- i. Some group members expressed the opinion that apoptosis was a valuable endpoint to investigate since cell death and cell growth are tightly linked.

7. What health effects might be associated with the types of *in vitro* effects on melatonin found after cellular exposure to EMF? Discuss the mechanisms by which a loss of melatonin would affect cellular replication? Discuss the relevance of melatonin attenuation to MCF-7 cellular proliferation?

Melatonin has been studied by a large number of investigators and the literature indicates that melatonin is responsible for a wide number of biological

responses in humans. (Refer to the 1997 review by Bzezinski mentioned above and listed below.)

Of relevance to carcinogenesis is the "melatonin hypothesis" of Dr. Richard Stevens (Cancer Research, July 15, 1996). Complementary to this hypothesis are the findings that melatonin has been reported to blunt or inhibit the growth of cancer cells *in vitro* and *in vivo*. Given this oncostatic action of melatonin, any co-factor such as EMF that acts to reduce melatonin's oncostatic action, would increase the probability or risk of cancer. An important caveat must be emphasized; it is not yet determined if the *in vitro* effect of EMF on melatonin action also occurs *in vivo*.

Melatonin has a number of additional biological effects in animals that relate to regulation of sleep, circadian rhythm, immune system responsiveness, and reproductive function. It is not yet known whether EMF has an effect on these actions of melatonin.

8. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of ELF-EMF on cell cycle control *in vivo*?

The group thought it would be of value to examine MCF-7 cell growth in a nude mouse model (pinealectomized) treated with or without melatonin and exposed to EMF. This is a direct *in vivo* correlate to the *in vitro* MCF-7 cell study performed by Liburdy and replicated by Blackman and by Luben.

Additional Issues Discussed by the Breakout Group

1. The group discussed the question of threshold detectability of change in a bioassay. This was of interest since the reported changes in cell replication associated with EMF treatment ranged from 10-180%. Most members of the group felt that a change of greater than 10% could be followed in the laboratory for well-characterized, biological parameters. This is a practical limit, and it was agreed that larger changes are more desirable and easier to detect reliably.
2. There was discussion of how relevant it was to consider information from laboratories that were in the process of trying to replicate studies but had not yet completed their studies or not yet presented any data in abstract form at a public, scientific meeting. This information was considered interesting, but it did not carry as much weight as completed studies in which data was presented and discussed during a public, scientific forum or published in the peer-reviewed literature.
3. Agreement among members was variable about the significance of a study that was "not perfect." Most studies evaluated by the group were not perfect and some technical issues could invariably be improved by hindsight. In addition, only the Liburdy studies were currently replicated, so only this body of melatonin

data, which spans three laboratories, has the important benefit of increased credibility associated with replication.

4. The group discussed the importance of collecting data on cellular replication at different time points because cell growth is a dynamic process. EMF effects might be detectable at different time points in a growth curve; early lag phase, exponential growth phase, or early plateau phase.
5. Several members of the group expressed interest in chronic EMF treatment exposures. There are apparently no data available that directly address the issue of "chronic, long-term" EMF exposures or of the "reversibility" of any reported effects.

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EMF (50/60 Hz) Literature on Cell Replication (Tables 1-3)

The literature publications identified for review reflect scientific studies that were available in the peer-reviewed literature or were accepted for publication, prior to the breakout group meeting. Studies were reviewed by the group which represented investigations conducted using sinusoidal 50/60 Hz magnetic fields and which specifically employed mammalian model cell systems for assessing cell growth. For example, reports employing non-sinusoidal fields (e.g., pulsed fields), non-mammalian model systems (e.g., viruses, bacteria), or not investigating cell growth (e.g., gene activation) were deemed to lie outside the focus of our breakout group.

Studies at ≥ 10 Gauss

Rosenthal, M. and G. Obe. "Effects of 50Hz electromagnetic fields on proliferation and on chromosomal alterations in human peripheral lymphocytes untreated or pretreated with chemical mutagens." Mutation Research. 210:329-335, 1989.

Antonopoulos, A., B. Yang, A. Stamm, W.D. Heller, G. Obe. "Cytological effects of 50 Hz electromagnetic fields on human lymphocytes *in vitro*." Mutation Research. 346:151-157, 1995.

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Studies at <3 Gauss

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Wei, M, M. Guizzetti, L.G. Costa. "The effect of 60 Hz electromagnetic field exposure on the proliferation of human astrocytomas cells." Fundamental & Applied Toxicology. 36:126, 1997, Abstract #643.

Environmental-Level Studies at ≤ 12 mGauss

Liburdy, R.P., T.R. Sloma, R. Sokolic, P. Yaswen. "ELF magnetic fields, breast cancer, and melatonin: 60Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation." J. Pineal Res. 14: 89-97, 1993.

Harland, J.D., R.P. Liburdy. "Environmental magnetic fields inhibit the antiproliferation action of tamoxifen and melatonin in a human breast cancer cell line." Bioelectromagnetics. in press, 1997.

Blackman, C.F., S.G. Benane, D.E. House, J.B. Blanchard. "Independent replication of the 12-mG magnetic field effect on melatonin and MCF-7 cells *in vitro*." Abstract A-1-2. 18th Annual Bioelectromagnetics Meeting, Victoria, CN, June 9-14, 1996.

Luben, R.A., S. Saraiya, A.P. Morgan. "Replication of 12 mG EMF effects on melatonin responses of MCF-7 breast cancer cells *in vitro*." Abstract A-1. Annual Review of Research on Biological Effects of Electric and Magnetic Fields from the Generation, Delivery & Use of Electricity, San Antonio, TX, November 19-21, 1996.

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Harland, J.D. and R.P. Liburdy. "ELF inhibition of melatonin and tamoxifen action on MCF-7 cell proliferation: Field parameters." Abstract A-1-1. 18th Annual Bioelectromagnetics Meeting, Victoria, CN, June 9-14, 1996.

Afzal, S.J.M., M.Y. Lee, and R.P. Liburdy. "Human brain tumor cells, EMF, and tamoxifen: A preliminary study." Abstract A-4. Annual Review of Research on Biological Effects of Electricity and Magnetic Fields >From the Generation, Delivery, & Use of Electricity, San Antonio, TX, November 19-21, 1996.

Afzal, S.J.M., M.Y. Lee, and R.P. Liburdy. "Magnetic fields reduce the growth inhibitory effect of tamoxifen in human brain tumor cells." Abstract #L-7. Second World Congress for Electricity and Magnetism & Biology and Medicine. June 8-13, 1997.

Summary and Recommendations

A. Summary Points

- 1) There exists in the peer-reviewed literature approximately eight EMF (50/60 Hz) studies in which different investigators report alterations in cellular replication by magnetic field strengths $\geq$ about 10 Gauss. This database spans several different cell types and exposure treatments. None of these studies have yet been replicated. Little dose-response data is available. There are technical improvements that could be made in these studies.
- 2) There also exist several negative studies on cellular replication and these are associated with exposure field strengths of $\leq$ about 2 Gauss. None of these studies have been replicated. There are technical improvements that could be made in these studies.
- 3) There are a series of "environmental-level" experiments in which low-intensity magnetic fields of 12 mG (60 Hz) have been shown to "synergistically" interact with melatonin and block its growth regulatory effect on MCF-7 human breast cancer cell growth. These findings have been independently replicated in two laboratories; this replication data has been presented at scientific meetings, but it is not yet published.
- 4) Unpublished dose-response data is available for the melatonin studies. An apparent dose "threshold" exists between 6-12 mG. Unpublished exposure duration data is also available that suggests field treatment of longer than three days, or approximately one cell cycle, is required.
- 5) A second series of "environmental-level" experiments indicate that low-intensity magnetic fields of 12 mG (60 Hz) "synergistically" interact with tamoxifen and block its growth regulatory effect on MCF-7 human breast cancer cell growth (in press, Bioelectromagnetics). Follow-on studies indicate that the same effect is observed for ICI 182,780 a pure antiestrogen, suggesting estrogen receptor (ER) involvement. These studies have not yet been replicated.

B. Conclusion Statements

- 1) At magnetic fields above 10 Gauss there is laboratory evidence that EMF alters cellular replication. Although no findings have been independently replicated, this body of evidence represents data across a number of different laboratories, utilizing different cell types, and a variety of exposure durations. These studies could be technically strengthened by employing multiple techniques that are currently available to assess cell

replication such as quantitative flow cytometry and automated cell proliferation assays.

- 2) At magnetic fields below about 2 Gauss, available literature suggests there is evidence EMF alone does not alter cellular replication, but "synergistic" effects between EMF and melatonin have been reported and independently replicated.
- 3) "Environmental-level" magnetic fields of 12 mG block or inhibit the oncostatic action of melatonin, tamoxifen, and ICI 182,780 on human breast cancer cell growth. This field action involves co-treatment of cells with the field and the drug/hormone; the field alone does not influence cell growth. These melatonin findings have been independently replicated in two laboratories which adds significant weight to the credibility of these findings. An apparent dose "threshold" exists between 6-12 mG for the melatonin findings.
- 4) "Environmental-level" melatonin/tamoxifen studies may have potential for relevance to human health risk. Factors that play a role in this assessment are: a) melatonin is a naturally occurring hormone; b) tamoxifen is currently a drug of choice for management of breast cancer and is used by a large number of women; c) milliGauss field strengths are associated with these biological effects; and d) EMF is hypothesized to play a role in reducing available melatonin levels experienced at peripheral target cells such as human breast tissue via the "melatonin hypothesis."
- 5) Further research is recommended in a number of important areas. Further investigation is recommended to address a) the "generalization" of EMF effects on cell replication to a variety of different cell lines, including melatonin and tamoxifen sensitive cell model systems, utilizing multiple methodological approaches, b) the "synergizing" effect of EMF with other growth regulating hormones and drugs that are known to alter cell growth and replication, and c) the characterization of a dose "threshold" and an exposure duration "threshold" as well as studying the possible effects of chronic exposure and reversibility.

Table 1
EMF Effects on Cellular Replication
Magnetic Fields > 10 Gauss

Investigator	Freq/B	Cell Type	Observation
Obe (1989)	50Hz/1-75G	human lymphocytes	48-72 hrs ~10-15% Increase
Obe (1995)	50Hz/50G	human lymphocytes	48-72 hrs ~5-10% Increase
West (1994)	60Hz/11G	JB6	10 days 40-70% Increase
Johann (1993a)	20Hz/53G	MCF-7	8 days 180% Increase
Johann (1993b)	20Hz/up to 53G	MCF-7	6, 14 days ~31% Increase
Schimmelpfeng (1993)	50Hz/20G	mouse fibroblasts	60 min. ~10% Decrease
Blackman (1995)	50Hz/16G	mouse fibroblasts	30 min. Blocking of GJ/Melatonin

Table 2
EMF Effects on Cellular Replication
Magnetic Fields <3 Gauss

Investigator	Freq/B	Cell Type	Observation
Kwee (1995)	50Hz/0.8G	human AMA	15-90 min. 2-70% Increase
Roti Roti (1991)	60Hz/2.2G	CHO cells, human lymphocytes	24-96 hrs. No Effect
Fiorani (1992)	50Hz/2mG-2G	K562 cells	24 hrs. No Effect
Cohen (1986)	60Hz/1-2G	human lymphocytes	69 hrs. No Effect
Revoltella (1993)	50Hz/2G	Friend erythroleukemia cells	6 days No Effect

Table 3
EMF Effects on Cellular Replication
Synergistic Effects of 12 mGauss Magnetic Fields

Investigator	Freq/B	Cell Type	Observation
Liburdy (1993)	60Hz/12mG	MCF-7	7 days ~25% Increase + Melatonin
Blackman (1996) (abstract)	60Hz/12mG	MCF-7	7 days ~17% Increase + Melatonin
Luben (1996) (abstract)	60Hz/12mG	MCF-7	7 days ~29% Increase + Melatonin
Liburdy (1997)	60Hz/12mG	MCF-7	7 days ~38% Increase + Tamoxifen

Appendix 1

EMF Effects on Cellular Replication

submitted by Jack Sahl, Ph.D.

The peer-reviewed literature database for studies on cellular growth is broader than is cited in this breakout group report; therefore, the following reference list is provided as supplemental information for the report. Based upon the full body of scientific literature including information from studies listed below, it is my opinion that there is no basis to conclude that EMF can alter cellular proliferation.

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EMF Effects on Cellular Differentiation

Facilitator: Hiroshi Yamasaki, Ph.D.

Rapporteur: Kenneth J. McLeod, Ph.D.

Introduction

One goal of the EMF Science Review Symposium was to identify key cellular effects of electromagnetic field exposure on cellular differentiation processes *in vitro*. By attempting to identify consistent differentiation response patterns, dose requirements, and/or temporal responses patterns, the possibility of establishing a link to environmental exposures and *in vivo* health effects might be increased. Specific objectives of this review included a consideration of the quality of the available *in vitro* research in which an effect on differentiation has been reported, the gaps which exist in our understanding of EMF effects on cell differentiation, and the potential for eliminating these gaps through the conduct of additional, carefully designed *in vitro* experiments. In addition, the group discussed a related EMF effect, namely the alteration of intercellular communication.

The breakout group attendees assigned to discuss cell differentiation *in vitro* were enthusiastic about the decision to include this aspect of cell behavior in the symposium. The process of cell differentiation is closely tied to the carcinogenesis issue. One explanation for uncontrolled cell proliferation is that there is no inherent problem with cell growth or cell cycle control but that the cells are unable to proceed on to terminal differentiation. As importantly, alterations in the kinetics of cell differentiation are intimately tied to all tissue function characteristics and therefore to a broad range of health effects beyond carcinogenesis. Similarly, gap junctional intercellular communication is considered to play a key role in tissue homeostasis and its disturbance has been associated with various health problems including cancer. Overall, there was a consensus among breakout group participants that there were surprisingly few investigations of these aspects of cell behavior in the EMF literature. While each of the studies reviewed had redeeming features, it became clear that a robust effect on cell differentiation or intercellular communication in a study in which the cell preparation, exposure system, and quantification of results were beyond question, has not yet been accomplished. With minor improvements, several of these studies could be replicated with reasonable ease, and with the specific concerns of the review committee eliminated.

Questions Discussed by the Breakout Group

1. Can EMF alter cellular differentiation by itself or by acting synergistically with other agents?
 - a. What types of alterations are likely?

Differentiation effects are classically related to developmental progression or changes in the phenotypic expression of the cell. In the absence of obvious developmental progression, differentiation is usually identified by alterations in how the cell interacts with its environment. Common markers of cell differentiation include changes in cell morphology, mobility, shape, and cell attachment; changes in protein secretion (extracellular matrix molecules) or the activity of ectoenzymes that interact with the extracellular space; or changes in cell communication (alterations in gap junction characteristics, secretion of cell signaling molecules, i.e., autocrine, paracrine factors). (Note: A minority opinion was that the triggering of apoptosis more appropriately fell under the auspices of cell differentiation rather than cell proliferation and should have been discussed by this breakout group.)

The breakout group reviewed five papers which specifically addressed the potential for low level EMFs to affect various differentiation processes *in vitro*. Each of these papers was seen to contribute to the EMF/differentiation issue, though none of these papers was without readily identifiable limitations, which were of some, if not substantial, concern to the review participants. A brief review of the comments made on these papers include:

- i) Cameron et al. (1993), Delayed development in sea urchin and mouse embryos.

Clear markers for differentiation, but no formal concurrent sham exposure was utilized. (Note: A minority opinion in the breakout group was that formal shams are not always necessary in EMF studies). Extended observation in sea urchin studies, but long term effects unclear in mammalian studies.

- ii) Blackman et al. (1994), Neurite outgrowth in PC-12 cells.

While PC-12 cells are a well established model of terminal differentiation, the authors did not use an ATCC cell line so replication is problematic. No formal concurrent sham control was used, and a counting assay requires significant reader judgment. Inhibition was observed with one cell PC-12 sub-line but enhancement with another (PC-12D, this latter point may reflect intrinsic problems with the assay, the limitations of generalizations, or a serendipitous observation opening the door for mechanistic studies).

- iii) Spadinger et al. (1995), Motility and mobility changes in NIH-3T3 cells.

Excellent automated quantification scheme for analysis, but poor choice of cell line for differentiation studies [Note that NIEHS funded a replication trial using NIH-3T3 cells (Rodeman, 1989) for which the results have been negative (Nuccitelli), and the investigators are shifting their studies toward the study of keratinocyte differentiation]. Also, the short time course of the experiment results in its interpretation as a null response, or at best, a lower threshold level for response.

- iv) Rubin et al. (1996), Suppressed recruitment in murine marrow cultures.

Formal sham exposure system, well accepted, though difficult, terminal differentiation model, with long term exposure. TRAP positive multinucleated cell assay requires reader's judgment, but could be readily automated.

- v) McLeod (1997) Alkaline phosphatase activity reduction in a differentiating bone cell line.

Formal sham control, quantitative assessment, well established differentiating bone cell line. Long term implications unclear as cells were not followed to terminal differentiation.

- b. Are there dose-response data supporting these effects?

An underlying concern in all of the reviewed studies was the issue of whether the observed effect was due to the applied time changing magnetic field, or the induced electric field, or some combination of both. Two studies specifically addressed this issue (Blackman, McLeod); one provides dose-response data consistent with an electric field effect, while the other offers evidence suggesting a magnetic field dependence. The consensus of the breakout group was that this was a critical issue that remains unsolved. Magnetic flux exposure levels in the systems where responses were observed varied over a range of approximately 20 milligauss to 20 gauss. Correspondingly, and rather remarkably given the geometric differences in the exposure chambers, the maximum values of the induced electric field intensities in these various systems also covered roughly the three orders of magnitude range of 2-1000 microvolts/meter. All studies were undertaken at frequencies between 10 and 100 Hz. In the majority of the studies reviewed, the pattern of response to EMF exposure could be interpreted as a delay in the normal progression of differentiation.

- c. What are the temporal characteristics of the response?

Consistently, the reported results reflect a response to exposures of several hours to several days. Spadinger's work represents the shortest duration of the studies reviewed, utilizing a three-hour exposure duration; however, no effect or only a threshold level effect was observed. Studies in which a robust response was observed suggest a sustained exposure of 24 hours or more is necessary.

- d. How do these exposure patterns relate to long term effects in cells?

It is difficult to extrapolate these studies to long term effects. First, they largely deal with changes in the kinetics of differentiation processes rather than complete blocks to differentiation so that for long lived cells a terminal state may only be delayed. This is perhaps best exemplified in the developmental studies

where numerous other studies have shown that rather profound perturbations of the embryo at the blastula stage may still result in the development of a perfectly normal adult. Conversely, the robust effect on osteoclast recruitment may represent a significant response in these terms. A robust response is observed after eight days, yet this terminally differentiated cell line has a life expectancy of only ten days. Thus, for the population of cells exposed, the long term effect is profound.

- e. To what degree do these studies indicate strong support for an *in vivo* effect on cellular differentiation?

If the mechanism of interaction is due to the magnetic field, the observed responses are largely inconsistent with residential environmental exposures causing any long term effect, as the most robust effects on cell differentiation are associated with magnetic flux densities well above typical environmental exposure. (The PC-12 cell data would be a notable exception to this generalization with a cellular response above controls at 22 milligauss). If the observed effects are due to the induced electric field, all of these studies would support the possibility of an *in vivo* effect. As work presented at this symposium Dr. Om Gandhi's plenary lecture showed that peak induced electric field intensities on the order of 100-1000 microvolts/meter could be achieved in the adult human body at 60 Hz magnetic flux densities as low as 10 milligauss, a level consistent with residential and commercial exposures. Alternatively, assuming a coupling factor on the order of 10^{-6} , environmental electric fields of just 100 V/m would be sufficient to induce electric fields in the body that would be in the range of the electric field intensities utilized in these studies. The observation that sustained exposures lasting for hours to days is also consistent with human exposure patterns.

2. Does EMF alter cellular communication pathways related to tissue homeostasis?

Two recent publications related to this topic were discussed. Ubeda et al. (1995) showed that melatonin up-regulates gap junctional intercellular communication as determined by the scrape-loading method and that 50 Hz EMF abrogated such an effect in C3H 10T1/2 cells; and Shimmelpfeng et al. (1995) demonstrated that the communication, as determined by a dye-microinjection assay of NIH3T3 cells, was up-regulated after exposure to 50 Hz EMF and this was accomplished by elevated levels of intracellular cAMP. Recent unpublished work presented at this symposium by Dr. Carl Blackman suggests a magnetic field induced reduction in gap junctional coupling in rat liver cells (Clone 9 from ATCC). The lack of consistency in the published reports led the panel to conclude there was insufficient evidence to support the possibility of an effect *in vivo* or to extrapolate to potential health effects.

3. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on cellular differentiation *in vivo*?

Three suggestions were proposed by the panel. First, it was pointed out that additional studies following the "hen-house" protocol (i.e., a multicenter chick embryo development study using pulsed electromagnetic field exposure) have been completed both in the US and Europe subsequent to the last evaluation of that data base. Though this is actually a teratogenesis experiment, it would be both timely and a relatively short process to revisit this data, perhaps through a meta-analysis. Second, the NIEHS has funded ongoing studies on an *in vivo* model of endochondral ossification in Dr. Roy Aaron's laboratory. Though these studies would not be simple to replicate in a short time frame, additional data from this model system should be forthcoming over the next several months. Finally, it was noted that the most consistent set of observations appear to be associated with effects on bone remodeling, as numerous studies at both the cell level (osteoblasts and osteoclasts) and at the tissue level (in mouse, rat, rabbit, dog, turkey, and human), have been reported in which robust effects have been observed. This repair/regeneration response clearly depends on the orderly differentiation of several cell types. Because of the large number of labs actively involved in this research, short term studies on bone remodeling could readily be accomplished to resolved specific issues regarding frequency, dose or duration dependence of the EMF effects.

Additional Issues Discussed by the Breakout Group

Four additional issues were raised by the breakout group.

The first of these was the concern about extrapolating observations made at relatively high doses down to levels associated with environmental exposures. It was noted that such scaling has not worked well in the area of ionizing radiation, and because of the numerous mechanisms involved in non-ionizing EMF work, would certainly not work well in this area either. For example, at electric field intensities in the tissue above 10 V/m, thermal effects dominant; between approximately 0.1 V/m and 10 V/m, classical electrophysiological effects can explain most observed behavior; between 0.1 mV/m and 0.1 V/m consistent pure electric field responses (but which cannot be easily explained by electrophysiological theory) are evident; while below 0.1 mV/m the reported effects must arise through some much more complicated mechanism. This issue was seen as particularly problematic in the differentiation studies that were reviewed as these studies relied on a wide range of magnetic flux density exposures. While in the studies reviewed induced electric field intensities were primarily below 0.1 V/m, some of the experiments utilized flux densities greater than 10 G, reported high-field magnetic mechanisms such as alterations in free radical lifetimes, may become dominant at these flux densities, and yet would have little relevance to environmental exposure.

Another issue raised was that while a concerted effort is now being placed on carefully describing the exact EMF exposure conditions and cell line utilized, much less effort is being spent on characterizing or trying to optimize the growth substrate. Yet, integrin interactions with growth substrate are currently viewed as perhaps the principal factor controlling the differentiation and function of cells.

Related to both of these issues was the opinion that a move toward more physiologic or health related systems was going to be critical in making inroads on this problem. It was pointed out that there are now many well developed model systems for studying differentiation that may be more closely related to the health concerns of EMF exposure including mammary epithelium, keratinocytes, astrocytes, and lymphocyte progenitor cell lines.

Finally, there was a request that if future studies are to be funded that they should either be undertaken at environmental magnetic flux levels or they should utilize applied electric fields. The use of high flux magnetic fields, which have the potential to induce substantial electric fields (i.e., greater than 0.1 mV/m), was found to be confounding the entire debate on the implications of the reported bioeffects.

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Summary and Recommendations

An understanding of EMF influences on differentiation will undoubtedly be critical to establishing an EMF/health effects relationship, but to date, there are a limited number of studies available to guide this work. Nonetheless, it was felt that particularly in the area of bone cell biology, there was sufficient evidence at the cell and tissue level to believe that credible effects on cell differentiation have been established. Of the studies reviewed, it was generally felt that the murine marrow culture system provided the most useful data from a health effects perspective (Rubin et al., 1996). This work utilized a well-controlled exposure system, the cell system undergoes a true terminal differentiation, this differentiation requires hormonal stimulation to progress, and the system relates to some degree to concerns about marrow cavity exposure and leukemia. Importantly, this study showed a significant suppression of differentiation on a time scale that was long with respect to the life span of the differentiated cells under study. All this study needs is the development of an automated assay. Unfortunately, this work was not undertaken by an EMF researcher and so further developments from this lab are unlikely. Extension of this work in other laboratories, however, is a possibility that should be considered. In addition, NIEHS funded *in vivo* work on endochondral ossification (Aaron laboratory) may provide some insight on the longer term implications of these *in vitro* bone cell studies.

NIEHS has already initiated at least one attempt at replicating a differentiation study on fibroblasts (Nuccitelli and colleagues), though the investigators have met with little success in replicating the reported results and will now be moving on to the study of keratinocytes. Other ongoing replication trials include an attempt by Oak Ridge National Labs (Dr. Paul Gailey and colleagues) to replicate some of the PC-12 neurite outgrowth studies recently reported. The weak response of the cells and the extensive observer training required have been serious detriments to completing these studies. While few gap junction studies have been reported to date, both NIEHS-funded and Electric Power Research Institute-funded research directed toward this question are ongoing, and results should soon be available.

Finally, it is suggested that a summary statement reviewing the "hen-house" data be compiled, but that future experimental designs concentrate on simpler exposure systems, which may permit a consensus agreement on the magnetic vs. electric field question, at least over certain exposure ranges.

EMF Effects on the Molecular Biology of the Cell

Facilitator: J. Carl Barrett, Ph.D.

Rapporteurs: David M. Binninger, Ph.D. and Gayle E. Woloschak, Ph.D.

Introduction

The purpose of the breakout group was to evaluate the available scientific data concerning cellular interactions with EMF at the molecular level. The majority of work in this area has focused on effects on gene expression, especially at the level of transcription. This literature has recently been reviewed (1). In general, *in vitro* assays are not directly applicable toward statistical quantification of a human health risk. However, understanding the molecular mechanisms and the target genes lends insight into the biological endpoints that should be evaluated in both epidemiological and *in vivo* models. Additionally, genetically predisposed subpopulations at risk might also be identified.

Questions Discussed By the Breakout Group

1. Does EMF alter gene activation by itself or by acting synergistically with other agents?
 - a. What types of alterations are likely?

DNA damage

It was generally accepted by the group that EMF has no directly clastogenic effect on cellular DNA. There are numerous negative reports in the literature [for a recent example, see (2)]. However, there are several reports indicating possible mutation induction following EMF exposure. Single-strand DNA breaks have been reported by Phillips et al. (5). Ahuja et al. (19) also reported a dose-dependent increase in DNA single strand breaks in human leukocytes exposed to a 50 Hz magnetic field (0-2 μ T range, 1 hour exposure). Lai and Singh have recently published data to support the conclusion that DNA damage occurs in rats exposed to 1 gauss (1G, 100 μ T) EMF (3). A paper was mentioned by Nordenson et al. (4), which reports that intermittent exposure (15 sec. on, 15 sec. off) of amniotic cells to 50 Hz, 300 mG (30 μ T, rms.) magnetic fields for 72 hr. results in increased chromosomal aberrations. Similarly, chromosomal damage including gaps was observed when cells were exposed in a similar manner except the fields were on for 2 sec. and off for 20 sec. Interestingly, increases in chromosomal aberrations were not observed when exposure was continuous for 72 hr. at a 10-fold higher field strength [3 G (300 μ T)] (4). Preliminary work by Phillips and colleagues suggests that exposure of Molt 4 T-lymphoblastoid cells to a 1 G (100 μ T) 60 Hz sinusoidal magnetic field leads to decreased activity of the DNA repair enzyme,

poly (ADP-ribose) polymerase, and increased DNA fragmentation in the presence of the DNA damaging agent, etoposide (VP16) (5). The reports described above of effects of EMF on mutation were not considered conclusive, and the group decided to focus this session on discussing the effects of EMF on gene expression.

Gene expression

During the morning's plenary session, Dr. Gayle Woloschak presented some of her work on the identification of several novel genes induced following EMF exposure in HeLa cells. The gene modulation events described by Dr. Woloschak were small (no greater than 2.1-fold). Dr. Jeffrey Saffer noted that these initial findings using differential display are interesting but further experimentation and confirmation of the results (e.g., sham-sham exposures) are needed. Dr. David Binninger noted that in his yeast system he has found induction and repression of several genes in response to EMF exposure; in some cases the changes were over 2-fold (6). Dr. Reba Goodman discussed some of her work demonstrating changes in the SSA1 heat shock gene in yeast (7), some heat-shock genes, and other cellular stress genes in mammalian cells following EMF exposure (8). Dr. Saffer noted that he was not able to replicate the original work of Dr. Goodman (9, 10). Dr. Russell Owen commented that work by Lacy-Hubert (11) reports findings not in support of those by Dr. Goodman. Secondly, Dr. Saffer expressed concern that proper experimental controls, positive and negative, were lacking in most of Dr. Goodman's studies. He predicted that with a more carefully controlled, randomized, experimental approach most of the changes observed by Goodman would have "fallen out" as experimental background and noise.

Dr. Goodman presented her perspectives on replication experiments. She presented data she interprets as indicating that HL60 cells from different sources (ATCC vs. Columbia University's Cancer Center) can be distinguished microscopically, have very different growth characteristics, and exhibit different responses to *c-myc* gene induction by TPA. Dr. Goodman also discussed the issue of experimental protocol and commented that differences (among laboratories) in RNA preparations and culture conditions could all contribute to different experimental results. She also noted that the EMF exposure system used in HL60 replication studies reported by Metcalfe et al. (11) may not have effectively shielded the sham-exposed control cells from electric and/or magnetic fields. A manuscript describing cell line differences has been accepted for publication (26). The group generally agreed on the great difficulty in performing replication experiments. Dr. Owen noted that several laboratories have been unable to confirm the original work of Goodman (20,21) including his own (22). His own experiments have failed to support the conclusion that differences among laboratories in experimental conditions including cell line source, cell morphology and growth, RNA preparation methodology, culture conditions, or effectiveness of shielding can account for why researchers have been unable to confirm the gene expression results of Goodman. Dr. Saffer also pointed out that many, if not all, of the design issues raised by Dr. Goodman have been worked out experimentally by others. Dr. Saffer explained his own experiments, with findings that differ from

those of Goodman, and emphasized that support for an effect of EMF depends upon consistency and generalization of the measured response across labs and without requiring peculiarities in the experimental system. It was noted by one attendee that in other fields consistency is more important than direct replication -- when several groups have findings which make a consistent picture (for example, a gene is induced, other genes with the same response elements are induced, the protein is affected, the binding of a protein to its consensus element is affected, etc.), all provide supporting evidence for a finding. Dr. Owen noted that in the case of magnetic field induction of gene expression, this type of consistency of findings has not been demonstrated. It was also commented by an attendee that responses below five-fold in magnitude are somewhat questionable. In response to this, Dr. Martin Blank noted that setting an arbitrary criterion without full statistical and biological evaluation of the experimental system is not reasonable. The significance of a response always depends upon the magnitude of the dispersion of the measurements (usually standard error), which varies with the technique. The issue of "what is a robust response" was raised within the group -- all labs are looking for it, but it is not really defined.

Synergistic interactions with EMF

This is still a somewhat open question, but there are some examples in the published literature for possible synergistic interactions with EMF. The reports by West et al. of promotion of JB6 cells in the presence of EMF and TPA was mentioned (23,24); however, these findings could not be replicated (25). Studies in which cAMP and EMF exhibited a synergistic increase in ornithine decarboxylase (ODC) activity were mentioned (27) as was a paper describing a synergistic effect of forskolin and EMF exposure on expression of a beta-galactosidase gene using PC12-VG cells (12). Dr. Blank referred to studies suggesting additive effects of ac magnetic fields and applied electric fields on *c-myc* expression (18), Na,K-ATPase (14), and ornithine decarboxylase (14). Dr. Blank explained that under conditions of mixed electric and magnetic fields, an electric field decreases ODC activity while the magnetic field increases enzyme activity. He interprets the data as showing that the result was approximately the arithmetic sum of the two effects (18).

Types of genes likely to be affected

The categories of genes likely to be important in EMF-induced responses are stress-induced genes (*hsp*, poly ADP ribosylase, etc.), immediate early genes, genes affected by calcium changes, NFKB/AP-1, and novel genes. Dr. Woloschak commented that from her studies only a small fraction of the genome is likely to be EMF-responsive. Dr. Binnering has found similar results in his yeast system. Overall, effects on gene expression are reported to be no more than two-fold and show variation among cell strains. It was noted that to understand and to interpret the significance of reported EMF effects will require demonstration of consistency among the findings, particularly gene expression effects in different cell lines and

at multiple levels (transcription, accumulation of mRNA, accumulation of protein, etc.), and as yet, this has not been observed.

Dr. Blank discussed data from his lab and that of Dr. Goodman documenting effects of EMF at multiple levels -- mRNA, protein changes, and binding of transcription factors. It was also noted that Phillips et al. (15) have reported changes in the DNA binding activity of specific transcription factors in the presence of EMF. Mutational analysis of the promoter region of the *c-myc* gene has identified a putative EMF-responsive element located between -353 and -1,257 relative to the P-1 promoter using CAT as a reporter gene (13). Dr. Owen noted that this sequence element has not been shown to be capable of conferring EMF-responsiveness on a heterologous promoter, and Dr. Saffer pointed out that the report of an EMF-responsive sequence element in the *c-myc* promoter was inconsistent with data from the majority of labs investigating possible EMF effects on *c-myc* expression.

- b. Are there dose-response data supporting these effects? What are the temporal characteristics of the response? How do these exposure patterns relate to long-term effects in cells?

It was noted that only a few studies have examined dose-response and dose-metrics, making generalization about dose effects impossible. One example given was the previous reference to a study on the synergistic effect of forskolin and EMF on beta-galactosidase expression (15). This report contains data that the authors interpret as indicating a magnetic field strength dose-response. Dr. Blank summarized his published data showing dose-dependent changes in the percentage of proteins in broadly defined bands of molecular weight range of less than 100 kD. He noted that the most prominent changes were in the "30 kD" and "70 kD" proteins (14). The changes in these protein profiles as a function of applied field strength, paralleled the type of effect seen with temperature changes. The apparent similarity in responses to the two stresses suggest that the cell reacts to increasing energy of stresses in apparently the same way, independently of the nature of the stress or the energy range. Dr. Blank commented that he interprets this data to suggest that the scale of the EM energy necessary to cause an effect is orders of magnitude weaker than thermal, but the receptor apparently adjusts and responds similarly. He noted that these data do support an EMF effect on protein synthesis. There was some discussion on the unconventional analysis used in these 2D-PAGE experiments and hence a reluctance to accept the interpretation at face value. The entire group agreed that extant EMF effects are transient. How these exposure patterns relate to long-term cellular effects cannot be predicted by the available data.

- c. To what degree does this indicate strong support for an *in vivo* effect?

Studies of gene expression changes *in vivo* have not been done and would be useful in evaluating results particularly where such studies are designed to

compliment confirmed *in vitro* effects. It is not clear if the "two-fold" effects which have been generally observed, are biologically relevant.

2. Are the observed (if any) EMF alterations in gene expression at the level of transcription (message abundance, transcription rate, message turnover, etc.) or translation (protein synthesis rate, protein processing, protein turnover, etc.)?

The majority of studies on gene effects have focused on changes in mRNA level (see Gene Expression). It was noted that Phillips et al. (15) have reported nuclear run-off experiments that indicate EMF effects at the level of RNA synthesis. Greene et al. (20) also reported effects on rRNA transcription rate and processing/degradation, but with no net effect on steady state levels. Effects on mRNA stability have not been extensively evaluated. Greene et al. (20) reported no effect on *c-myc* and β -actin transcription rates or steady state levels in HL60 cells exposed to 60 Hz EMF. EMF studies where the biological endpoint is at the level of translation are more limited. Dr. Blank reported data on changes in proteins due to various temperatures and magnetic field strengths. Early work by Goodman and Henderson reported changes in the 2D protein profile from salivary glands of *S. coprophila* in response to EMF (16). E. Goodman and colleagues have also observed EMF-associated changes in protein profiles using *E. coli* (17). Effects of EMF on protein activities (such as ODC, protein kinase C, etc.) were not discussed since they were being covered in other breakout groups.

a. Are there *in vitro* studies that could be proposed to demonstrate and/or strengthen the evidence for effects of EMF on gene expression?

It was suggested that experimental approaches should more extensively exploit differential display and related technologies toward genome-wide surveys that exploit the highly sensitive technologies that are becoming available. These surveys should concentrate on field exposure conditions and biological systems for which there are confirmed biological responses. The preponderance of the work done to date has focused on gene-specific assays.

3. Are there studies that could be proposed to clarify potential EMF effects on gene expression and/or control?

a. Would the use of nonmammalian systems (i.e., *E. coli*, yeast, *Drosophila*, etc.) serve as good model systems for understanding EMF effects?

It was agreed among the entire group that nonmammalian systems such as *E. coli*, yeast, and *Drosophila* would be valuable models toward understanding molecular mechanisms because of the excellent genetics and molecular cloning technology that is available for each. The PC-12 neurite outgrowth model was also suggested as a model system. Investigators with interesting biological responses should focus on the molecular biology of the specific responses. It was noted that

identification of any biomarker for EMF effects would be useful in providing a means for characterizing a pathway important in EMF effects on cells.

Additional Issues Discussed by the Breakout Group

Replication experiments

The group discussed the issue of replication experiments, which have played an important role in this field. Concerns about how to determine whether failure to replicate is due to difficulties in reproducing biological and physical parameters or due to the effect not being real were discussed without resolution. Current attempts to resolve this issue include the independent analysis of RNA samples by involved laboratories using blinded samples.

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Summary and Recommendations

As shown in the above comments, a general consensus about EMF alteration of gene expression was not obtained. This work still requires additional studies with sensitive genome-wide (and not just gene-specific) assays. In

addition, it would be useful if investigators with biological effects would begin to examine the molecular biology of their particular responses. The use of nonmammalian systems (especially yeast, *E. coli*, and *Drosophila*) is particularly valuable in providing mechanisms of EMF responses, primarily because of the well-developed genetics and molecular cloning technologies available for these systems.

All agreed that studies done to date cannot be used to extrapolate to *in vivo* effects of EMF. Some members concluded that EMF-associated modulations of gene expression exist and may be a generalized cellular response since effects have been reported in organisms as diverse as *E. coli*, yeast, slime molds, diptera (fruit flies) and mammalian cells including humans. However, others concluded that this is not supported by the contradictory evidence and by the apparent sensitive dependence on the particular cell line or even particular subline used in reports of positive effects experiments. Much more work is required before those types of assessments can be made. Several investigators commented that EMF studies are in a very early stage -- similar to that of ionizing radiation in the 1920's. Although biological phenomena have been observed, little is known about the molecular and cellular effects of EMF exposures; however, scientists are put in the position of having to develop mechanisms without the needed experimental base being available.

The Role of *In Vitro* Assays in Clarifying and Quantifying Human Health Risks

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Rapporteurs: Richard A. Luben, Ph.D. and Michael Galvin, Ph.D.

Introduction

Recent advances in cell biology have led to the use of a broad array of *in vitro* assays for studying the mechanisms by which toxicological agents alter cellular physiology. While the investigation of biologic processes using isolated systems is often a useful and cost effective research tool, it takes a tremendous leap of scientific intuition to move from *in vitro* findings to potential *in vivo* health effects. This is especially true for toxicological agents for which there are epidemiological findings, *in vitro* findings, but very little *in vivo* work. Some areas of electric and magnetic fields (EMF) research fall into this category.

There are numerous references in the literature in which individuals have used *in vitro* assays to identify qualitatively potentially hazardous agents; however, quantitative analyses of human health hazards incorporating *in vitro* results are rare. The most common use of *in vitro* assays for quantification of risks is to identify key biological and physical components, which are involved in the hazardous insult, and to quantify the effects of these key components *in vivo*. The basic question considered in this group was to determine the extent to which *in vitro* findings can be used to clarify human health risks from exposure to EMF and where possible, to directly aid in quantifying those human health risks.

One key goal of this first science review symposium was to evaluate the quality of the *in vitro* scientific data relating to health affects from EMF. Pertinent to the discussion of this topic is the utility of *in vitro* assays pertaining to cellular replication, differentiation, and functional control for predicting *in vivo* responses.

1. Definitions agreed upon by the breakout group

- Genotoxicity - a change in genetic information that is permanent. Genotoxic events may be random, rare, and unique
- Epigenetic - an alteration in expression of genes in a cell that may not be permanent. Epigenetic events are usually general within a population of cells. Epigenetic changes may be normal, reversible, and not necessarily toxic to the cell.
- Cell communication - 1) extracellular, e.g., hormones; 2) intracellular, e.g., second messengers; 3) intercellular, e.g., gap junctions, cell adhesion molecules (paracrine factors)

2. *In vitro* systems allow for careful control of experimental parameters and examination of physiological responses. Examples of phenomena for which *in vitro* assays can be used appropriately for study of:

- Mutations

- Cell death - apoptosis, necrosis
 - Cellular proliferation and cellular differentiation
 - Adaptive responses of differentiated cells
 - Extracellular, intracellular, and intercellular communication
3. Particular caution should be given when conducting and interpreting experimental data from *in vitro* assays including these issues:
 - *In vitro* systems can generally measure only a single type of event. The functional aspects of the event being measured *in vitro* should be studied using *in vivo* systems.
 - All *in vitro* assay systems have limitations and potential artifacts must be kept in mind during interpretation of results.
 - The experience and expertise of the researcher are important for proper conduct of *in vitro* experiments.
 - The type of cells and the cell line being used must be carefully chosen. Primary cells are preferable for some studies; however, for others homogeneity of the cell lines is necessary. In some incidences, a cell free system may be appropriate.
 - *In vitro* experiments must be hypothesis driven.
 4. *In vitro* systems have advantages in controlling parameters, cost, and data replication, and in examining physiological responses, not limited to cancer. In addition, mechanistic studies, underlying the biological endpoints being measured, can often be optimally performed.

Questions Discussed by the Breakout Group

1. Discuss the *in vitro* experimental basis for potential physiological effects of EMF.

The data from *in vitro* systems are limited. Generally the information can be used only to answer very specific questions. *In vitro* systems have limitations as models for *in vivo* processes. Individuals applying *in vitro* systems to study physiological events must have appropriate expertise. When negative experimental results are obtained they must be examined with the same degree of statistical rigor as positive results. The type of cells chosen for *in vitro* studies must be carefully selected and the experiments must be hypothesis driven. In all incidences, there needs to be a valid basis for comparison between *in vivo* observations and *in vitro* experiments.

- a. What systems are likely targets?

Although *in vitro* experiments cannot be used to directly predict human health hazards, certain experimental areas, which can be studied *in vitro*, have

been postulated to participate in the disease process *in vivo*. In general, the group agreed that there are several areas that can be studied *in vitro* and these are listed below along with the likelihood that EMF would have reproducible effects. The group differed over whether sufficient evidence exists now to draw strong conclusions from any or all of these areas:

- i. Mutations - probably not
- ii. Necrosis - probably not
- iii. Apoptosis - yes, probably relevant
- iv. Cellular differentiation and cellular proliferation- yes
- v. Adaptive responses - can be done, but may not be relevant

b. Are EMFs adverse to health?

In vitro experiments cannot answer this question, but may provide guidance for the selection of reasonable field characteristics to apply to *in vivo* and epidemiologic studies.

2. What experimental evidence from *in vitro* systems is lacking for support of EMF as a human health risk?

See answer to 1. b. above.

- a. Are there *in vitro* studies that could be proposed to demonstrate and/or strengthen the evidence for potential human health risks from EMF exposure?

The types of experiments that should have the highest priorities include:

- Systems that have been suggested by specific *in vivo* or epidemiological observations.
- Investigations of specific mechanistic questions related to *in vivo* observations.
- Dose-response questions
- Threshold questions
- Sensitivity questions
- Studies on effects of different EMF characteristics and co-factors (e.g., frequency, dc field magnitude and orientation, and chemical co-factor)

Investigations of low priority would be those for which sound, objective reasoning for pursuing a new direction is not clearly defined.

Specific *in vitro* systems that may be related to physiological effects include:

- ODC, PKC, and other tumor promotion related models
- cell transformation assays
- gap junction assays

- cAMP, Ca²⁺, and other second messengers
- gene expression studies
- *in vivo* versus *in vitro* comparisons of similar systems (e.g., co-promotion, tumor cell growth rates)
- cell differentiation and proliferation

3. Is there scientific evidence from *in vitro* studies for EMF affecting biological systems directly or is it likely a co-factor interaction of EMF with another physical or chemical agent?

Most of the data suggest that EMF acts with other agents, whether as an antagonist, an agonist, or a co-promoter. There is very little evidence for EMF producing specific effects absent some experimental co-variable.

4. At the EMF exposure levels observed *in vivo*, would cellular effects (growth, differentiation, control of gene expression, etc.) be observable *in vitro*?

- mutations - probably not
- necrosis - probably not
- apoptosis - yes, this is relevant
- cell proliferation and differentiation - yes, this is relevant
- adaptive responses - can be measured, but are they relevant?

For each of these systems there are probably considerable differences between *in vitro* and *in vivo* systems in dose-response properties, threshold doses, and sensitivity of responses. There needs to be a valid basis for comparison between *in vivo* and *in vitro* experiments, and thus far, no such valid basis for comparison has been developed for any of the systems listed above with the exception of mutations.

5. Discuss the feasibility of EMF as a health risk at its experimentally observed levels.

This question cannot be answered directly based on available *in vitro* studies.

6. How is our ability to detect health risks from EMF affected by the types of measurements that have been done or not done?

Specific *in vitro* systems that probably are related to *in vivo* physiological effects:

- ornithine decarboxylase/tumor promotion
- cell transformation
- gap junctions
- cell proliferation

- cAMP
- Ca^{2+}
- bone function (is not known to be pathological, only therapeutic)
- melatonin changes - this needs more investigation *in vivo* as well as *in vitro*

None of these has been directly linked with a disease or pathological process. The data cannot be extrapolated any better or worse than with some other known *in vitro/in vivo* comparisons, e.g., drug or toxin effects. Nothing that has been done can be considered unequivocally established as a disease process, especially when it comes to *in vivo* predictions derived from *in vitro* findings.

Additional Issues Discussed by the Breakout Group

1. What experiments are lacking?

Examples of studies that would provide needed experimental data include: *in vitro/in vivo* co-promotion studies and *in vitro/in vivo* stem cell studies.

Summary and Recommendations

The breakout group concluded that *in vitro* experiments are a useful, although limited, means of pursuing investigations on the possible effects of EMF on health. *In vitro* experiments provide a means for detecting changes in cellular function, in particular, cell division, cell death, and signal transduction processes (specific experiments are summarized above). They can also be cost effective in establishing critical EMF exposure parameters. On the basis of existing *in vitro* evidence, the breakout group concluded that genotoxic effects of EMF are unlikely to contribute to any possible *in vivo* health risk. However, consensus conclusions were not obtained regarding *in vitro* experiments, which are related to cellular processes, that do not directly alter the genome, but do alter cellular function or the expression of genetically determined characteristics of cells (epigenetic mechanisms). A majority of the group felt that findings do provide support for EMF-related effects while a minority felt further research is needed (especially at lower exposure levels) before conclusions can be reached.

The interpretation of experimental results from *in vitro* assays must be done carefully; in particular, it is not yet possible to extrapolate reliably from *in vitro* experiments to any definitive EMF effect *in vivo* or to the contribution of EMF to any specific disease process in human beings. In addition, no quantitative risk assessments, which can predict a correlation between EMF exposure and any known disease process, are currently possible based upon reported *in vitro* experiments.

Future *in vitro* experiments should be designed to clarify further the impact of known effects of EMF on cells (e.g., signal transduction pathways, cellular proliferation, apoptosis, cellular differentiation, or gene expression) and to elucidate underlying molecular/biochemical mechanisms that will aid in our understanding of how EMF can impact on human health or disease processes. In formulating these new lines of research, the data from *in vivo* and/or epidemiological studies may be used to guide these new directions and the development of novel *in vitro* assay systems.

EMF Effects on Cellular Calcium

Facilitator: Elizabeth Murphy, Ph.D.

Rapporteur: Kjell H. Mild, Ph.D.

Introduction

This breakout group evaluated the data addressing EMF effects on cytosolic free calcium and other ions as well as the effect of EMF on calcium signaling pathways. A literature search of papers published from 1990 to 1996 showed approximately 25 manuscripts directly related to the topic (see Table 1 and References; note these searches are inevitably incomplete). The breakout group discussed several of the manuscripts including some that report that EMF can alter intracellular Ca^{2+} regulation and some that reported no effect.

We evaluated the methods used to measure calcium, the time course of any response, and if available, the dose response. Dr. Kenneth McLeod suggested that we should also evaluate the induced electrical field. Taken together, the available data indicate that in some cell types under certain conditions, EMF may alter cytosolic free calcium; however, EMF effects on cytosolic free calcium do not appear to be a universal response. The effects of EMF are highly specific regarding both cell type, requirements for priming (e.g., ConA), and the amplitude and frequency of the applied fields.

Questions Discussed by the Breakout Group

1. Does EMF alter cytosolic free calcium within cells?

The group concluded that there was evidence that EMF altered calcium in some cell types under defined conditions (e.g., Liburdy et al., 1993; Lindström et al., 1993), but that this was not a universal response (see Garcia-Sancho et al., 1994). The majority of studies and virtually all studies which report an effect of EMF, are performed on immune cells. In studies in which EMF effects have been described, some indicate that activation by mitogens is required (e.g., Liburdy et al., 1993) whereas others indicate that this is not necessary (e.g., Lindström et al., 1993). Alterations have been reported to occur in transformed cells, non-transformed cells that have been primed, and in non-primed non-transformed cells. In addition, different groups used different measures of calcium (e.g., fluorescent indicators vs. ^{45}Ca) making it difficult to compare results. For example, most of the studies employing ^{45}Ca measured changes over 30 to 60 minutes whereas studies with fluorescent indicators measured more rapid changes over the time course of 1-2 minutes. The exposure conditions were variable. Also, in some studies cells were in suspension, and in others, cells were attached.

In discussing the literature, it was noted that EMF has been reported to both stimulate and inhibit ⁴⁵Ca uptake depending on the conditions. Yost and Liburdy (1992) reported that EMF inhibits ⁴⁵Ca efflux, which is induced by addition of 1-3 µg/ml of ConA, whereas Liburdy et al. (1993) reported that EMF stimulates ⁴⁵Ca efflux induced by the addition of 1 µg/ml of ConA, which in this study is a suboptimal dose. As discussed by Walieczech and Budinger (1992), in some cells ConA stimulates ⁴⁵Ca uptake while in other cells ConA has no effect. EMF tends to inhibit ⁴⁵Ca uptake induced by ConA, but in cells in which ConA alone has no effect, addition of EMF + ConA can stimulate ⁴⁵Ca uptake.

2. How do these exposure patterns relate to long-term effects within cells?

The consensus of the group was that the effects of calcium in cell biology are too complex to draw meaningful conclusions with current knowledge. However, it was noted by some members of the group that the response pattern observed (e.g., EMF altered calcium in activated transformed cell) is similar to the response pattern reported by the cellular replication breakout group. In addition, the variability of the responses also make interpretations difficult. It was pointed out by a member of the group that the question refers to "exposure patterns" and the answer refers to "response patterns."

3. What is the biological significance of "modulation and intensity windows" for EMF effects on calcium?

Although frequency dependence was observed in some cases, the explanation for this observation is unclear. "Modulation and intensity windows" have been reported, although some panel members expressed reservations about whether they are real or just experimental artifacts.

4. To what degree does this indicate strong support for an *in vivo* effect of EMF on processes mediated by cellular calcium?

Because of the complexity of calcium mediated processes and the inconsistencies in the data, there is not strong support for *in vivo* effects at this time.

5. What health effects might be associated with these types of *in vitro* effects?

Without information on how long calcium is elevated and what other signals are also altered, it is difficult to draw conclusions. However, the group noted that alterations in calcium have been associated with an increase in β -amyloid production as well as an increase in cell proliferation, and therefore, an increase in calcium might have an effect on these processes.

6. Does EMF alter cellular pathways related to calcium's role in signaling?

There are very few studies on this topic, and because of the limited nature of these studies it is difficult to draw conclusions about *in vivo* effects. There is a study reporting that EMF causes an increase in IP3 (Korzh-Sleptsova et al., 1995). This is an important study because it would suggest that multiple signaling pathways (e.g., calcium and PKC) are activated. There is also a study reporting that calmodulin binding of calcium is not altered by EMF (Lednev et al., 1994). This is an important study because Lednev has suggested that calcium binding to calmodulin might be altered by EMF, and given calmodulin's central role in calcium signaling pathways, this would be an important finding. However, a careful study by Bruckner-Lea et al (1992) found no effect of EMF on calmodulin binding to calcium.

7. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on processes mediated by cellular calcium?

It was suggested that one could investigate whether EMF alters β -amyloid via changes in calcium. It was also suggested that one could investigate whether EMF has effects on rapid calcium oscillations such as those that occur in the heart or islets of Langerhans. On reviewing this report several members of the group expressed the concern that these particular studies mentioned at the session reflect the particular research interest of some of the breakout group participants.

8. At the EMF exposure levels observed *in vivo*, would cellular effects on calcium and other ions be likely to be observable *in vitro* ?

The answer depends on whether effects are due to electric or magnetic fields and the temporal characteristic of exposure (time-average or peak); these effects have not been sufficiently studied. Typical exposure conditions in the calcium experiments were in the 1G range.

Additional Issues Discussed by the Breakout Group

There was a discussion of defining typical "EMF exposures observed *in vivo*." Should one consider time-averaged exposures (typically low mG) or should one consider peak exposures (~1 G) as typical *in vivo* exposures? There was also a discussion of the role of magnetic vs. induced electric fields.

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Summary and Recommendations

The available data suggest that EMF might cause an increase in cytosolic calcium in some, but not all cells. However, given the complexity of calcium mediated processes, the biological effect of any such increase is unclear.

Table 1
Summary of Literature Related to EMF Effects on Calcium
(Published in English 1990-96)

Name	Journal	Cell type	Does alter Ca ²⁺ ?
1. Yost and Liburdy	FEBS Lett. 296:117-22, 1992	lymphocytes	yes
2. Liburdy	FEBS Lett. 301:53-9, 1992	lymphocytes	yes
3. Liburdy et al.	FEBS Lett. 334:301-8, 1993	lymphocytes	yes
4. Lindström et al.	J. Cell. Physiol. 156:395-8, 1993	Jurka/lymph.	Yes
5. Lindström et al.	Bioelectromagn. 16:41-7, 1995	Jurkat	yes
6. Korzh-Sleptsova et al.	FEBS Lett. 359:151-4, 1995	Jurkat	yes
7. Lindström et al.	FEBS Lett. 370:118-22, 1995	Jurkat	yes
8. Walieczech et al.	FEBS Lett. 314:351-55, 1992	lymphocytes	yes
9. Walieczech	FASEB J. 6:3177-85, 1992	immune cells	yes
10. Rosen	BBA 282:149-55, 1996	GH3 cells (static field)	yes
11. Lyle et al.	Bioelectromagn. 12:145-56, 1991	immune cells	yes
12. Carson et al.	Am. J. Physiol. 259:C687-92, 1990	HL-60	yes
13. Garcia-Sancho et al.	Bioelectromagn. 15:579-88, 1994	immune/RBC/HL-60	no
14. Bruckner-Lea et al.	Bioelectromagn. 13:147-62, 1992	calmodulin	no
15. Hojevik et al.	Bioelectromagn. 16:33-40, 1995	RINm5F	no
16. Schwartz and Mealing	Bioelectromagn. 14:521-33, 1993	frog heart	no
17. Fitzsimmons et al.	Calcif. Tissue Int. 55:376-80, 1994	bone cells	yes
18. Coulton et al.	Phys. Med. Biol. 38:347-60, 1993	lymphocytes	no
19. Barbier et al.	Bioelectromagn. 17:303-317, 1996	rat pituitary cells	yes
20. Galvanovskis et al.	Sci. Total Environ. 180:19-33, 1996	leukemetic-T cells	yes with poly-lys no w/o poly-lys

Related Studies---but these studies do not measure cytosolic calcium

Name	Journal	Cell type
21. Blackman et al.	Bioelectromagn. 12:173-182, 1991	temperature
22. Karabakhtsian et al.	FEBS Lett. 349:1-6, 1994	need extracellular Ca
23. Ayrapetyan et al.	Bioelectromagn.15:133-42, 1994	solutions
24. Eichwald and Kaiser	Biophys. J. 65:2047-58	model
25. Prasad et al.	Health Phys. 66:305-12, 1994	diatom/positive effects
26. Parkinson and Sulik	Radiat. Res. 130:319-30, 1992	diatom/negative

Appendix 1

Specific Comments on Calcium Metabolism Summarized by Dr. Kjell H. Mild from his Plenary Lecture, “EMF Effects on Calcium Metabolism”

Many of the earlier studies of calcium and EMF were looking at calcium efflux from chick brain tissue and “frequency and intensity windows” were observed—that is, the response of the biological system depended on particular combinations of the dc magnetic flux density and the ac frequency. By changing the ac frequency over a wide range, they observed that the active frequencies for a given dc flux density were integer multiples of a fundamental. This work provided the impetus for the subsequent development of the Cyclotron Resonance (CR) model. CR phenomena has as a basis the interaction force between a charged particle and a magnetic field. The condition for a circular movement is that the velocity or the number of turns, frequency, follows the relation: $f = q B / 2\pi m$. While the model has been criticized on theoretical grounds, the ‘harmonic’ relation observed in the data seems to be real and persists over quite a spectrum of frequencies.

A complex, but different, interrelation of the independent variables, ac flux density, ac frequency, and dc flux density was identified by the ion parametric resonance (IPR) model. (The only point of commonality between the IPR and CR models is at their fundamental frequencies. The harmonics identified by each model are inverse of each other.) Another model is the Parametric Resonance (PR) Model where interference of the vibrational energy sublevels of ions bound in calcium-binding proteins is the basis for the interaction of weak magnetic fields with the biological systems.

None of these models have full experimental support today, but the data found in the literature show that many of the EMF biological effects seem to fulfill the basic formula for the frequency and static magnetic field. Very often, a non-linear extremely low frequency amplitude response is also seen.

Changes in intracellular calcium concentration have been reported from several laboratories using different cell models. Since calcium is a general messenger molecule, this means that the possibility exists for many diverse responses from the cell system. Our own work has shown that the primary interaction site for the EMF is at the membrane level, and thus, the effect is not primarily on the calcium ion. We have used a fluorescent microscope with the possibility to study single cells and Fura-2 as an indicator for the intracellular free calcium. Using the T cells Jurkat, an acute response was observed with oscillatory increases in calcium concentration, which subsided when the field was turned off (Lindström et al., 1993, 1995 a,b). The response at 0.15 mT was over a frequency range from 5 to 100 Hz with a fairly broad peak having its maximum at 50 Hz. The result of testing with increasing flux densities at 50 Hz was a threshold response with no effect below 0.04 mT and a plateau at 0.15 mT. By using different CD45 mutant clones of Jurkat, we found that cells with a chimeric CD45 responded to the magnetic field (B field) in the same manner as when treated with

anti-CD3, but when the cytoplasmic part was missing, no effect of the B field was seen. This showed the necessity for an intact signal transduction pathway for the increase in intracellular calcium to occur.

Our laboratory has also measured the inositol triphosphate (IP3) level in the Jurkat cells after exposure to B field, 50 Hz, 0.10 mT (Korzh-Sleptsova et al., 1995). The exposure resulted in significant increases of IP3 levels, and this peaked after two minutes. When cells were treated with anti-CD3, the peak came first after six minutes. Combining the two shows an additive effect.

The conclusion from our experiments with Jurkat cells is that the ability of EMF to activate Jurkat cells is comparable to anti-CD3 crosslinking antibodies, the target molecules are localized at the cell membrane and the field effect is reversible and has a threshold.

Bioelectromagnetic research has much more to learn from a basic understanding of calcium regulation. For instance, it has been seen that in stimulated cells, calcium concentration rose as predicted, but did so only transiently, and fell back to its base level within a few minutes while the cells continued to respond for hours. Another example is the recently demonstrated causal relationship between (Ca²⁺) transients that occur during the cell cycle. These things throw light upon the discussion of "what is exposure," as we will see magnetic fields effect the calcium concentration and thus a short term - maybe high peak - exposure might be all that is needed and the long duration might not be needed. More research along the line of different exposure regimes with respect to the timing is needed, and things like biological reset time need to be introduced into this research.

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Effect of EMF on Enzymes and Polyamines

Facilitator: Theodore A. Litovitz, Ph.D.

Rapporteurs: Martin Blank, Ph.D. and Charles N. Rafferty, Ph.D.

Introduction

Elevated levels of enzyme activity have been consistently correlated with cell transformation and tumorigenesis. Research reported in recent years has made it clear that this enhanced enzyme activity can have a causative role in tumorigenesis [e.g., transformation of mammary epithelial cells, Manni (1997); neoplastic transformation in prostate tissue, Magi-galluzzi (1997); tumorigenesis in mice, Moshier (1993)]. For this reason an evaluation of reports concerning the modification by electric and magnetic fields (EMF) of cellular enzyme activity (both in cell cultures and intact tissues) is important in any EMF hazard identification.

Our major goals were to establish whether or not there was sufficient credible *in vitro* evidence to determine:

- 1) If electromagnetic fields can alter enzyme activity,
- 2) If the magnitude of the change in activity is biologically significant,
- 3) If the fields at which these changes occur have magnitudes of importance in environmental exposures, and
- 4) If the *in vitro* changes are consistent with the available *in vivo* data.

The opening question in the discussion suggested that the group address the issue of whether effects on enzyme activity at low field strengths were possible. This issue was discussed to a limited degree at various points in response to some of the questions below. However, the primary focus of our discussion was reviewing the enzymatic evidence for EMF effects. Many participants thought that the evidence for weak 60 Hz magnetic field effects was strong. Those that raised the biophysical plausibility argument felt that the experimental standard has to be higher when there is no biophysical model to explain the effect. Both physicists and biologists share the burden of advancing our understanding in the area; biologists by extending the amount and quality of the experimental data, and physicists by pursuing models of field-cell interaction that are realistic and thus appropriate for enzyme reactions.

Questions Discussed by the Breakout Group

EMF effects on enzymes

1. To what extent has it been shown that extremely low frequency EMFs can alter cellular enzymes?

A number of studies of electromagnetic field effects on cellular enzymes have been reported, including effects on ornithine decarboxylase (ODC), Na,K-ATPase, lyn kinase, cytochrome C oxidase, ethanolamine ammonia lyase, and horseradish peroxidase. The breakout group focused on ODC, Na,K-ATPase, and enzymes (ethanolamine ammonia lyase, and horseradish peroxidase) that are affected through radical pair mechanisms. There is substantial evidence from several labs that EMF can alter the activity of ODC (23 cell lines or tissues, 7 labs). Both electric and magnetic fields have been studied over a large range of exposures and frequencies [extremely low frequency (ELF) to modulated microwave fields]. At ELF the direct applied electric fields ranged from 0.1 mV/cm to 50 V/cm; the magnetic fields ranged from 2 mG to 1500 mG; the electric fields induced by these magnetic fields ranged from 0.1 V/cm to over 100 V/cm.

Both *in vivo* and *in vitro* studies have been reported. Many cell types have been investigated. Most *in vitro* studies have indicated a two to four-fold enhancement of ODC activity; one study reported a modest suppression, and one study failed to show an effect. Studies of ODC activity in animals are more variable. One explanation for this variability is that the effect of the EMF on ODC activity may be tissue dependent. In one *in vivo* study (El Kanza, 1996), examination of the brain, intestine, spleen, spinal cord, and kidney showed a two to five-fold increase in ODC activity. In another study on a different strain of rats (Mevisson, 1995), mammary and spleen tissue showed a 1.4 to two-fold increase in ODC activity, but the liver, intestine, bone marrow, and ear skin showed no effect. Studies of skin cancer in mice show no enhanced ODC activity in the epidermis after either acute (24 hr) or prolonged (weeks) exposure. No studies on ODC gene induction have shown an EMF induced effect, suggesting the possibility that the changes found above are due to alterations in the degradation rate of ODC.

The preponderance of evidence clearly supports the existence of ELF EM field effects on ODC. Efforts to replicate specific studies using the same cell types and exposure conditions have yet to be reported. It appears that *in vitro* confounders exist. It has been noted that cell sub-line, confluence, and passage number can play a role in determining whether or not there is an *in vitro* response to EMF exposure. (Table 1 a tabular summary of studies and reference list based on one prepared by Dr. Craig Byus.)

Two laboratories have investigated effects of ELF electric or magnetic fields on the Na,K ATPase activity in two different preparations, one cellular and the other consisting of vesicular membrane fragments. Consistent observations of enhanced activity were reported for high (>1 V/cm) electric fields. One laboratory has reported enzyme effects for extremely weak (< 20 mG) magnetic fields.

There is strong evidence (Grissom, 1995; Harkins, 1994; Taraban, 1997) of magnetic field effects on the activity of two enzymes, ethanolamine ammonia lysase and horseradish peroxidase, via radical pair mechanisms at high dc magnetic field levels exceeding 10 G. The specific mechanisms underlying these effects are well established; however, because of the high magnetic fields required, it is uncertain that this radical-pair mechanism is relevant to environmental considerations. Many other enzymes have also been investigated with dc magnetic fields but with no effects reported (See Table 2).

2. Are there dose-response data supporting these effects?

Dose-response data have been reported for three experimental enzyme systems. Sigmoidal magnetic field dose-response patterns have been reported for two ODC systems and one Na,K-ATPase system. ODC data from one *in vivo* study were consistent with a linear magnetic field dose-response. Complex dose-response patterns have been reported for enzymes affected through a radical pair mechanism.

- ODC *in vitro* (murine fibroblasts) effects detected over a 20-100 mG range (at 60 Hz) with a sigmoidal response and an inflection at ~40 mG.
- ODC *in vivo* (ENU treated rats) detected over a 20-2000 mG range (at 60 Hz) with an approximately sigmoidal response (i.e., linear response at low fields but appearing to saturate at high field strengths) with an estimated inflection point at (i.e., 1/2 way to saturation) ~20 mG.
- Na,K-ATPase (micellar preparation) detected over a 2-500 mG range (at 60 Hz) with a sigmoidal response and an inflection at 4 mG.
Na,K-ATPase (micellar preparation) studied with applied electric field gave a sigmoidal response with inflection at 6 mV/m.
Na,K-ATPase (micellar preparation) studied with combined electric and magnetic field (an induced electric field together with a stray magnetic field). Since under these conditions, the electric field decreases enzyme activity and the magnetic field increases enzyme activity, it is possible to show that the result (a sigmoidal response with inflection at 35 mV/m) is approximately an arithmetic sum of the two effects.
- Ethanolamine ammonia lyase effects detected over a 100-1500 G range with a complex dose-response.
- Horseradish peroxidase effects detected over a 10-1500 G range with a complex dose-response.

It must be emphasized that for the data above where the magnetic fields are time varying (e.g., 60 Hz), there is an associated induced electric field. It is not known whether the field-cell interaction is electric or magnetic. If it is the induced electric field, then care must be taken in extrapolating the threshold results found *in vitro* with anticipated thresholds in humans. Because of geometric considerations, the induced electric fields will be larger in humans for a given 60 Hz, magnetic field and thus predicted threshold magnetic fields will be lower.

In the group discussion, concern was expressed regarding the credibility of low level effects, especially for levels of magnetic fields below 100 mG, for the ODC and Na,K-ATPase systems. The reasons given were

- a) the lack of a biophysical plausibility of magnetic field effects that induce electric fields at levels less than the thermal noise level of membranes,
- b) the lack of precise replication for any given set of results,
- c) the preliminary nature of some of this data that may be reported only in abstract or work in progress form.

On the supportive side is:

- a) the internal consistency of the individual data sets and the tightness of the reported error bars,
- b) the broad range of systems studied,
- c) the general agreement between the reported *in vivo* and *in vitro* results, and
- d) the consistency between the reported ODC and polyamine results.

3. In what way is enzymatic activity affected?

Most *in vitro* studies on ODC activity have indicated a two to four-fold enhancement of the observed enzymatic activity; one study reported a modest suppression. Based upon his research, Dr. Craig Byus suggested that the increases in activity are due to increases in concentration of the enzyme possibly caused by a decrease in the degradation rate. No studies on ODC gene induction have showed an effect.

Studies of ODC activity in animals have, except for those using skin models, indicated an enhancement at least in some tissues when 50 or 60 Hz magnetic fields were applied.

Experiments on Na,K-ATPase show increases of activity of up to two-fold in either electric or magnetic fields. There are some differences in the responses to the two fields, but in both cases, there is a relation between field interaction and basal enzyme activity. The differences in the effects may arise because the two fields penetrate to different extents.

V_m/K_m decreases 25% when ethanolamine ammonia lysase is exposed to 1000 G dc magnetic fields and 60% when exposed to 1500 G dc magnetic. V_m is not sensitive to magnetic field exposure. In this system (as well as in horseradish peroxidase) the observed kinetic rate constants are changed by the magnetic field. One of the rate constants, k_2 , in the reaction scheme for horseradish peroxidase is increased by about 20% due to exposure to 10 G dc magnetic fields and decreases by about 60% when exposed to 750 G dc magnetic fields.

4. Are there long-term effects of EMF exposure in cells?

There is little or no experimental data to address this question depending on the enzyme system examined. Very limited adaptation studies have been carried out on ODC and Na,K-ATPase in cell systems. However, adaptations to electric and magnetic field exposure in animals have not been investigated. Long-term animal studies in which ODC has been measured demonstrate that ODC enhancement can be sustained. There are no studies on either cell or animal systems in which radical pair mechanisms have been investigated.

5. Do EMFs affect polyamine levels?

The limited evidence available suggests that EMF can affect polyamine levels. Only two relevant studies have been carried out, one *in vitro* and the other *in vivo* (Valtersson et al., 1997 in Jurkat cells; Juutilainen et al., 1996 in mice). In both of these studies, the polyamines, putrescine and spermidine, were found to increase by a factor of ~2 following ELF magnetic field exposure. The initial and rate limiting step in polyamine synthesis is catalyzed by ODC. Belief that ODC activity can be enhanced by EMF is strengthened by these experimental findings. In the *in vivo* study by Juutilainen et al. (1996) polyamine levels were higher after 24 hours of exposure whereas ODC activity was lower than the initial activity. This can be explained by assuming that (as in the *in vitro* studies) acute exposure causes a transient increase in ODC activity (peak in 4-6 hr) followed by rapid degradation even in the presence of the field. The complexity of these temporal relationships emphasizes that one must not only look at ODC levels, which may or may not appear to change depending on the duration of the exposure and the time of measurement of ODC activity, but that the amount of polyamine produced by the transient enhancement of ODC activities should also be determined.

6. Is the magnitude of the observed EMF-induced effects on enzymes and polyamines large enough to be biologically significant?

The magnitude of the observed EMF-induced effects on the enzyme ODC is large enough to be biologically significant. *In vivo* evidence shows that the magnitude of the EMF-induced increase in the enzyme ODC is large enough to increase tumorigenesis. However, these findings do not prove that these EMF-induced alterations in ODC activity actually cause either adverse or beneficial downstream effects. It simply means that EMF induced effects can not be ruled out on the basis of the magnitude of the change in ODC activity. Most of the ODC studies reviewed indicate that EMF can cause an approximately two-fold increase in ODC activity.

This appears to be a small (and possibly insignificant) change in such a highly inducible enzyme such as ODC. However, the work of Moshier et al. (1993) bears directly on this subject. They have studied the transformation of NIH/3T3 cells by ODC over-expression using an expression construct. They demonstrated that when the ODC activity levels were elevated by three - six-fold, the cells were no longer contact inhibited, exhibited anchorage independent growth, and induced tumors in nude mice more efficiently and rapidly (e.g., a five-fold increase in tumors relative to controls). The data indicate that a three-fold increase is not the smallest increase needed for enhanced tumorigenesis.

Two lines of *in vivo* experimental evidence pertain to a possible relationship between EMF effects on polyamines or ODC activity on the order of two-fold and tumorigenesis: the preliminary results of Juutilainen et al. (1996) using a UV potentiated mouse skin tumor model and the published results of Mevissen et al. (1995) using the DMBA rat mammary tumor model. Both groups report positive effects of magnetic field exposure on various measures of tumorigenesis.

Juutilainen et al. (1995) reported that the measured effect of the magnetic field is the same as that of being ODC transgenic (i.e., in the presence of UV, both conditions cause an approximate doubling in the average number of tumors per animal). The work of Mevissen further substantiates the significance of a two fold increase in ODC. They found that exposure of this strain of rats to EMF caused a significant increase in the number of mammary tumors despite only a two fold increase in the activity of ODC. (Note: the ODC studies were done in a separate experiment on the same strain of rats as in the tumor study).

Having suggested that a small change in EMF induced ODC activity can be significant, one must offer caveats:

- a) The homeostatic mechanisms of organisms will tend to minimize the magnitude and duration of such changes in enzyme activity.
- b) The experimental conditions and possible biological effects of EMF exposure are quite different from the stable transfection of a cell with an expression construct containing ODC genes, as in Mosier et al. (also true for chemical carcinogens).
- c) Care should be taken in inferring a cause-effect relationship between increased ODC activity and tumorigenesis because of the limited nature of these experiments and the weak statistical strength of the tumor findings (McCann et al., 1997). (See Table 1 for details.)

For **Na,K-ATPase** and **radical pair** enzyme effects, no conclusions can be drawn at this time.

7. What health effects might be associated with these types of *in vitro* effects?

There was no agreement on the answer to this question. Most discussion focused on the potential role of ODC in the promotion of cancer. It is recognized that over-expression of ODC is known to enhance tumorigenesis. (Moshier, 1993; Manni, 1997). If we accept that EM fields do enhance ODC activity by a factor of about two to four, then we are faced with two views. The traditional view is that large changes in ODC are needed when caused by the high chemical concentrations of the usual tumor promoters (e.g., TPA). Another view is based upon the recent literature which indicates that a ~3-fold change in ODC can be sufficient to play a causative role in tumorigenesis (Moshier, 1993).

During the discussion it was emphasized that it is not the magnitude of ODC change which is important but the magnitude of the downstream polyamine changes that are crucial. A further consideration is that it is not the percentage change that is important but the absolute change in concentration; for example, if the basal concentration of ODC is high, then a two-fold increase might be significant with respect to cancer. The participants, in general, felt that based on the extent of the available information, it was premature to draw conclusions about the relationship of EMF effects on ODC to cancer. However, a number of participants felt, after reviewing the *in vivo* and *in vitro* enzyme and polyamine data, that a relationship between EMF-induced ODC and cancer has become more likely, as the data has become more extensive and refined.

8. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on enzymatic activity?

Several studies were proposed:

- a) Evaluate frozen sections archived in current and recently completed long-term EMF animal studies. Samples from both promotional animal studies and chronic bioassays should be available.
- b) Conduct a new rodent study in which ODC and polyamine levels are measured at 1,2,3,4 and 5 weeks of 60 Hz magnetic field exposure. Following cessation of field exposure, ODC and polyamines should also be assayed over a 0-24 hour period to evaluate reversibility of responses.
- c) Repeat study by Mandeville (Kanza et al., 1996) in which ODC was evaluated in multiple tissues at multiple magnetic field levels in a rat brain cancer model in which ethylnitrosourea (ENU) was administered in utero.

9. At environmental EMF exposure levels would cellular effects on enzymes and polyamines be likely to be observable *in vitro* ?

While there was no difficulty obtaining a consensus of the discussion group that this is possible for high occupational and residential exposures, the feeling about lower exposures was divided. The differences reflected the level of comfort one felt with the results of enzyme studies. Those actively involved in experiments with enzymes felt that examination of the data required a definite yes answer to this question.

Transients were mentioned as a possible, though fairly uncommon (especially in residential environments) source of high field magnetic field levels. However, the ODC coherence experiments suggest that EM field exposure with field constancy less than ~ 10 seconds should not cause any bioeffects.

In ensuing discussion, support of new *in vitro* studies was also suggested. One participant suggested that a cost-benefit analysis be conducted to evaluate the relative merits of animal and cell studies. There was also support for more basic research to find mechanisms to account for observed effects at low magnetic field levels (e.g., less than about 100 mG). This last point is especially important since the credibility of weak field results will depend on improved understanding in this area.

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Summary and Recommendations

Electric and magnetic fields can alter enzyme activity in such enzymes. The preponderance of evidence clearly supports the existence of ELF em field effects on ODC both *in vivo* and *in vitro*. The *in vitro* changes in ODC are consistent with the available *in vivo* data. There is credible data that indicates effects on other enzymes, such as Na, K-ATPase and horeseradish peroxidase. It appears that *in vitro* confounders exist. It has been noted that cell sub-line, confluence, and passage number can play a role in determining whether or not there is an *in vitro* response to EMF exposure. The magnitude of the observed EMF-induced effects on the enzyme ODC is large enough to be biologically significant. *In vivo* evidence shows that the magnitude of the EMF-induced increase in the enzyme ODC is large enough to increase tumorigenesis. However, these findings do not prove that these EMF induced alterations in ODC activity actually cause either adverse or beneficial downstream effects.

While there was no difficulty obtaining a consensus of the discussion group that effects on enzymes are possible for high occupational and residential exposures, the feeling about lower exposures was divided. The participants in general felt that based on the extent on the available information, it was premature to draw conclusions about the relationship of EMF effects on ODC to cancer. However, a number of participants felt, after reviewing the *in vivo* and *in vitro* enzyme an polyamine data, that a relationship between EMF-induced ODC and cancer has become more likely, as the data have become more extensive and refined.

Table 1
EMF-Induced Alterations in Ornithine Decarboxylase (ODC) Activity and Polyamines
(prepared by Dr. Craig V. Byus, University of California at Riverside)

Cell/Tissue	EMF Exposure	ODC Response		Laboratory (PI) Reference (*abstract)
		E/C	Time	
Primary rat bone cells (collagenase calvaria)	Pulsed electric field (25 msec, 3 Hz repet.) 5 min. exposure			R. Korenstein (Sömjen et al., 1983)
	12 V/cm	2	4 h	
	22 V/cm	1.2	4 h	
	55 V/cm	3	4 h	
Primary mouse bone cells (collagenase calvaria)	Pulsed electromagnetic field (PEMF)	1.7 (6 d basal)	3.5 h	C. Cain (Cain et al., 1985)
	(+ 100 msec, -2 msec, 15 Hz) 1 h exposure	5.0 (8 d basal)	3.5 h	
	Induced magnetic field 8 G	1.5 PTH	3.5 h	
	E-field 0.6 V/cm Current 20 mA/cm ²			
Reuber H35 hepatoma	450 MHz RF-field			C. Byus (Byus et al., 1988)
	AM 16 Hz	1.5 (basal)	1-4 h	
	1.7 watts PEP 1.0 mW/cm ² field intensity	2.6 (+ TPA)	4 h	
Chinese Hamster Ovary	SAR 0.08 w/kg	2.0 (basal)	1-2 h	
	1 hr exposure	2.0 (+ TPA)	4 h	
294T melanoma	2.0 (basal)	0-1 h		
CEM (human lymphoma)	60 Hz electric field	3-5	1-2 h	C. Byus (Byus et al., 1987)
P3 (mouse myeloma)	10 mV/cm - 0.1 mV/cm	1.75	2 h	
Reuber H35 (rat hepatoma)	(nonlinear d.r.) 1-3 h	1.5	1 h	

Table 1, continued
EMF-Induced Alterations in Ornithine Decarboxylase (ODC) Activity and Polyamines
(prepared by Dr. Craig V. Byus, University of California at Riverside)

Cell/Tissue	EMF Exposure	ODC Response		Laboratory/P.I. Reference (*abstract)
		E/C	Time	
L929 mouse fibroblasts	60 Hz, 10mT _{rms} magnetic field 4 h exposure sinusoidal signal coherent for 5-10s; 30-90 Hz incoherent (noise) field blocks ODC response	2.1	4 h	T. Litovitz (Litovitz et al., 1991) (Litovitz et al., 1994)
L929 mouse fibroblasts	Amplitude-modulated RF 915 MHz, AM 50, 60, 65 Hz S.A.R. 2.5 w/kg (coherence effects similar to ELF)	2	8 h	T. Litovitz (Litovitz et al., 1993)
Jurkat (human lymphoblastoid) HL 60 ELD F9 (embryonal)	50 Hz, 0.10 mT _{rms} vertical polarization 30 min-4 h	1.5 (P,SD also increase $\bar{o}$)	3 h	M.-O. Mattsson (*Valtersson et al., 1995) (*Mattsson et al., 1992) (Valtersson et al., in press)
C3H/10 T 1/2 fibroblasts	60 Hz,			C.D. Cain
	50 mG	0.84	3 h	(*Cain et al., 1993)
	100 mG	0.53	3 h	(*Cain et al., 1995)
	200 mG	0.75	3 h	
Chicken embryo (26 h)	60 Hz sinusoidal		2.0	15 h T.A. Litovitz
	4 mT	0.5	25 h	(Farrell et al., in press)

Table 1 - continued
EMF-Induced Alterations in Ornithine Decarboxylase (ODC) Activity and Polyamines
(prepared by Dr. Craig V. Byus, University of California at Riverside)

Cell/Tissue	EMF Exposure	ODC Response		Laboratory (PI) Reference (*abstract)
		EC	Time	
ODC transgenic (K2) mouse-epidermis	50 Hz MF 100 mT continuous	0.75 (2-3 increase in P & SD at 24 h)	24 h	*Juutilainen et al., 1996
SENCAR mouse epidermis, papillomas	60 Hz MF 2 mT 6-7 h/d, 5-7 d/wk	1.0 (3 doses TPA)	1, 2, 5 wks	*DiGiovanni et al., 1996
		1.0 papillomas, epidermis	43 wks	Byus et al. (submitted)
Sprague-Dawley Rat (various tissues)	50 Hz MF 50 mT 24 h/d, 7 d/wk	2.0 (mammary tissue)	6 wks	W. Löscher (Mevissen et al., 1995)
		1.42 (spleen)	6 wks	
		1.0 (liver, (small intestine, bone marrow, ear skin)	6 wks	
Rats (Fischer) various tissues	60 Hz 2 mT 20 mT 200 mT 24 h/d, 6 d/wk (<i>in utero</i> , day 13 pregnancy)	linear dose/response increase ODC, up to 5.0 time/flux density 5, 15, 32 wks liver, brain, intestine, spleen, spinal cord, kidney		R. Mandeville (*Kanza et al., 1996)

Table 2
Enzymes Investigated Using Strong Dc Magnetic Fields That Do Not Show
Magnetic Field Effects Due To Radical Pair Mechanisms
(Provided by Dr. Charles Grissom, University of Utah)

Note: These fields do not induce an electric field and are clearly not typical of environmental fields.

- B₁₂-Dependent Methylmalonyl-CoA Mutase (Human)
- B₁₂-Dependent Methylmalonyl-CoA Mutase (Bacterial)
- Momoamine Oxidase B (Human)
- Lipoxygenase (Soybean)
- Tyrosine Hydroxylase (Human)*
- Hexokinase (Yeast)
- Catalase (Bovine)
- Luciferase (Bacterial)
- Methane Monooxygenase (Bacterial)*
- Nuclease (Staphylococcus)*
- Chymotrypsin*

* Not published, but reliable report

EMF Effects on Early Signal Transduction Events at the Plasma Membrane

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Rapporteurs: Deborah McK.Ciombor, Ph.D. and Gayle E. Woloschak, Ph.D.

Introduction

There have been many studies in the literature reported by a variety of investigators using a large number of different *in vitro* systems which have suggested that cellular signal transduction pathways are affected by EMF. This finding is quite expected since the signal transduction pathways receive both physical and biological information from other cells, as well as the environment, and transmit them in a cellularly appropriate fashion to produce the biochemical signals that regulate cellular functions. The electric field is attenuated within the cell but is amplified across the plasma membrane; therefore, in the absence of a clear indication that the magnetic field alone is responsible for all observed EMF effects, we must hypothesize that recognition and amplification of the information carried in the EMF signal is the domain of the plasma membrane and its signaling systems.

There is reasonable agreement as well on which early signaling pathways may be affected. The agreement, however, does not extend to a consensus regarding whether or not EMF effects at the *in vivo* level can be directly ascribed to any one pathway. Lively discussions notwithstanding, the group feels fairly strongly that the interaction of the affected pathways is a critical and potentially fruitful area of future research.

Questions Discussed by the Breakout Group

1. Has EMF exposure been associated with alterations in early steps in signal transduction?

As an overall response, it was the considered opinion of the group that there was a positive association of EMF effects and signal transduction. The group also feels, however, that the earliest steps have not yet been identified, including the recognition step (or steps).

a. If yes, identify the signaling systems affected.

The following list is meant to indicate those areas the group felt were the most well accepted and well represented in the literature. The final two items on the list are in parentheses since the group felt these were possibly the province of another breakout group; we list them only for completeness.

- i. Protein kinase C (PKC, work of Dr. Richard Luben)
- ii. Syk and syk-related pathways (work of Dr. Fatih Uckun)

- iii. cAMP
- iv. IP3
- v. (Na⁺ / K⁺ ATPase)
- vi. (Voltage sensitive channels)

Dr. Richard Luben suggested that the group note that *in vitro* responses were frequently dependent on cell specificity and heterogeneity and this should always be considered in evaluating data. His example: In B lymphocytes--PKC is elevated in response to EMF while exposure of osteoblasts to a comparable EMF shows little or no effect. Others noted that there is also evidence of Ca⁺⁺ efflux from EMF-exposed cells.

b. At what level are these effects observed?

The following is the consensus list of the group:

- i. Ligand - Receptor binding
- ii. Enzymatic Activity
- iii. Phosphorylation
- iv. Secondary messenger

c. Are there dose-response data supporting these effects?

The group felt that little dose-response data exist in the literature. Few references could be recalled. This may be due in part to the difficulties in constructing a "dose-response." Several suggestions were put forth for promising experiments to fill the perceived gaps in the literature. They include: 1) a study of coherence to include physical and biological coherence; 2) time sequence to include intermittence and transients; and 3) amplitude, frequency and co-factors (although difficulties with interpreting the cAMP studies in the literature were cited).

d. What are the temporal characteristics of the response?

The group felt that this was an important question that has not been examined in adequate detail, particularly the definition of "early," i.e., microseconds versus minutes. Additionally, everyone expressed the belief that the characterizations were entirely dependent on the biological systems studied and that attempts to generalize across systems were unproductive pursuits. Fundamentally, however, it was believed that the current literature does not indicate that the time constants of the pathways mentioned above were changed significantly.

e. How do these exposure patterns relate to long-term effects within cells?

It was deemed biologically plausible that long-term effects would result from changes in the signaling pathways mentioned, but detailed accounts are limited. It was noted by one attendee that ornithine decarboxylase (ODC) was to date

probably the only system that could be assessed in this manner. Field effects on the epidermal growth factor (EGF) signaling pathway were also mentioned as likely to result in significant long-term effects within a given tissue, and that further study in this area, if it were to replicate the preliminary results, would be important. Two other studies were mentioned as possibly indicating the potential for these types of effects: the Litovitz coherence studies with ODC and the Schimmelpfeng study of cAMP.

- f. To what degree does this indicate strong support for an *in vivo* effect on signal transduction?

There is not yet strong support in the literature for an *in vivo* effect on signal transduction. More experiments are needed to assess whether an *in vivo* effect occurs.

- g. What health effects might be associated with these types of *in vitro* effects?

It was believed that it would be premature to attempt to answer this question at this time.

2. Do short-term exposures to EMF lead to long-term changes in factors involved in early signal transduction events?

Basically the group answered this question in an identical manner to the previous question; however, it was noted that experiments designed to answer this question are underway in several laboratories, including experiments with 10 T 1/2 cells treated with TPA and continued EMF exposures.

3. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMF on early signal transduction activity?

There are numerous possibilities and some studies are currently underway, including studies on cell signal transduction molecules/pathways known to be important in stress and mitogen responses. Since induction of signal transduction can bring about several cellular responses, the cellular effects would include:

- a) cell proliferation
- b) cell differentiation
- c) programmed cell death or apoptosis
- d) adaptive responses of differentiated cells, such as secretion of hormones, contraction of cells; induction of calcium waves between cells.

4. At the EMF exposure levels observed *in vivo*, would cellular effects on the early steps in signal transduction be likely to be observable *in vitro* ?

The group answered with an unanimous and resounding - YES.

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Summary and Recommendations

In general, there are several demonstrations in the literature of an effect of EMF exposure on cellular signal transduction. These effects have been shown in particular cell types, but experiments demonstrating broad applicability to other cellular systems are needed. In addition, further work to uncover specific pathways involved, as well as activation mechanisms, will be important.

EMF Effects on the Signal Transduction Cascade

Facilitator: Steven C. Miller, Ph.D.

Rapporteurs: Henry C. Lai, Ph.D. and Jeffrey D. Saffer, Ph.D.

Introduction

Considerable advances in research on intracellular signal transduction pathways have been made in recent years. Such pathways amplify signals initiated at the cell surface, e.g. ligand-receptor interaction, and affect cell functions via enzyme activation and affect genetic functions.

Though biological systems have evolved an efficient signal transduction system to respond appropriately to chemical, biological, and environmental stimuli, the mechanism whereby the energy in low level extremely low frequency electromagnetic fields (EMF) may be coupled to a biophysical process is unknown. There are speculations and experimental data that suggests that EMFs act through an interaction mediated at the cell membrane that affects enzyme activities, gene expression, and ultimately cellular functions. The signal transduction processes link these cellular events. Studying changes in signal transduction pathways *in vitro* can be a powerful means to understand the mechanisms of interaction of EMF and to aid in the further assessment of a possible EMF hazard to human health.

Questions Discussed by the Breakout Group

1. Has EMF exposure been associated with alterations in the signal transduction cascade?

Several examples in the literature were named suggesting changes in the signal transduction cascade after EMF exposure. These include: receptor-mediated tyrosine phosphorylation, second messengers, enzymes such as ornithine decarboxylase (ODC), and amplification through protein kinase C (PKC).

There was a general feeling that the signal transduction processes are dynamic and complicated, involving multiple pathways and steps, and that present knowledge is limited. The question of where to start looking for effects of EMF on signal transduction pathways was raised. Clearly, significant publications in the literature provide evidence for mechanisms of interaction that should be examined.

The literature base for discussion in this breakout group focused upon two papers. One, a paper by Uckun et al. (1995) was discussed as a significant publication with evidence for effects of a 0.1 mT 60 Hz EMF on tyrosine phosphorylation induced signaling at multiple levels. This paper was compared to another paper by the same first author (1992) on ionizing radiation in which a similar magnitude of effects was observed at 1 Gy. The papers illustrate the complex protein interactions taking place at the level of the cell membrane in response to a radiation-induced signal. The issue of the similarity of the signaling

responses, which suggests a common mechanism, was raised. Getting essentially the same signaling response from a known stimulus (1 Gy of ionizing radiation) and EMF was pointed out as “curious.” Since signaling responses to ionizing radiation are generally robust, the discussion centered on the question of why EMF effects are not that obvious. During discussion it was pointed out that both Dr. Steven Miller’s and Dr. Richard Luben’s laboratories have had difficulties maintaining the Nalm-6 human pre-B leukemia cell line used by the Uckun laboratory. Thus, an exact replication effort has not been attempted by either of the laboratories. The actual details of this effort were not discussed or data presented for evaluation by the breakout group. It was also commented during the session that using a different human B leukemia cell line (Daudi), Miller’s laboratory has been unable to detect an effect of a 0.1 mT 60 Hz field on phosphotyrosine profiles of proteins under conditions where anti-IgM treatment demonstrated significant and reproducible changes in phosphotyrosine protein profiles as compared to controls.

A question was raised on whether a model system exists with well known pathways such that each step can be dissected out for study. The Jurkat T cell leukemia model was suggested since multiple levels of evidence have now been presented.

2. Are there dose response data supporting these effects of EMF exposure on alterations in the signal transduction cascade?

Several experimental issues were raised, but no strong conclusion was made on this question. It was pointed out that no reliable dose-response data on EMF effects on signal transduction processes are available at this time. Dose-response study of bone healing was mentioned, but it was pointed out that it was not related to cancer and health risk and that unique and pulsed EMF wave-forms were used in those studies. The implication of effects of these fields relative to effects of sinusoidal fields was questioned. With regard to the time-course of response, evidence suggests that rapid changes can occur after EMF exposure (e.g., Uckun et al. reported changes in tyrosine phosphorylation of multiple proteins and protein kinase C activation within ten minutes of exposure to a 60-Hz magnetic field at 0.1 μ T).

3. Do short-term exposures to EMF lead to long-term changes in factors involved in the signal transduction cascade?

It was agreed that there were no data available and therefore, not much discussion on this question was made.

The effect of exposure time was briefly discussed. It was mentioned that in ionization radiation studies it is known that the effect is more pronounced if a dose is given in one bolus than if it is fractionated into smaller ones and given over time. However, it was mentioned that this may not be true for EMF effects.

Another concern of exposure time is that the cellular re-set time may limit the responses of cells to EMF. For example, cells may be only responsive to EMF

at a certain phase of their cell cycle. This could be an important issue in *in vitro* experiments.

4. Are there short-term *in vivo* studies that could be proposed to demonstrate effects of EMFs on the signal transduction cascade?

Several models were proposed: (1) cells used in an *in vitro* model can be implanted in animals for an *in vivo* exposure; (2) transgenic animals with appropriate transduction pathways hypersensitive to a ligand-induced signaling can be used; (3) if a field sensitive pathway is identified, one can evaluate the significance of EMF exposure in cells initiated *in vivo*.

5. At the EMF exposure levels observed *in vivo*, would cellular effects on the signal transduction cascade be likely to be observable *in vitro*?

It is generally agreed that this question is context dependent, e.g., on the processes studied and the parameters of exposure. However, it was pointed out that cellular effects from EMF exposures could be tested by the implantation of the cells from the *in vitro* model into an *in vivo* animal model. Otherwise no answer is possible at this time.

Additional Issues Discussed by the Breakout Group

There was a long discussion on the logistics in the replication of an experiment. It was generally agreed that it is necessary to recognize the sources of possible artifacts in an experiment. Since this research involves studying small changes in cell functions in response to EMF, protocol design and experimental details may be critical. It was pointed out that cooperation of and sharing of important reagents and methods from the original investigator are important in an attempt to replicate an experiment. To illustrate these points, the facilitator presented a "rigor to risk pathway" (rigor, robustness, replication, requirement, response, and risk). The group expressed consensus that the study of the bioeffects of EMF begins with **rigor** to define an exposure environment, field conditions, and biological conditions that results in a **robust** biological response. To facilitate **replication** the original investigator must define the physical and biological **requirements** responsible for a given **response**. Thus, careful attention to exposure assessment and protocol design is important for an EMF-induced signaling pathway to be 1) recognized, 2) understood mechanistically, and 3) evaluated for human health **risk**.

It was pointed out that there is a significant difference between research that is hypothesis-driven versus replication-driven. The former may require flexibility in protocols whereas the latter requires the rigid adherence to predetermined protocols.

Use of simpler model systems such as yeast was suggested because they have signal transduction pathways similar to higher species and their genome

has been mapped. These features would be useful in investigating the mechanisms of EMF interaction in robust signaling effects observed in mammalian systems.

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Summary and Recommendations

It is generally agreed that the effect of EMF on signal transduction processes is an important area of research both in the understanding of the mechanisms of bioeffects and human health risk assessment. Although definite effects are observed in experiments and therapies using pulsed EMF waveforms to initiate bone healing in recalcitrant fractures, there is not enough information at this time to conclude whether EMF exposure conditions, which are of concern for human health risk, affect signaling cascades. Therefore, more research is needed. The further assessment of the possible human health hazards of EMF exposure depends, in part, upon establishing a causal connection between the existence of robust, replicable biological effects and the precise dosimetric parameters of EMF exposure upon which these effects depend.

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