U. S. Department of Transportation

Federal Railroad Administration

Safety of High Speed Guided Ground Transportation Systems

Office of Research and Development
Washington, D.C. 20590

An Overview of Biological Effects and Mechanisms Relevant to EMF Exposures from Mass Transit and Electric Rail Systems

DOT/FRA/ORD-93/32
DOT-VNTSC-FRA-93-19

Final Report
August 1993

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
August 1993

3. REPORT TYPE AND DATES COVERED
Final Report
October 1991 - July 1993

4. TITLE AND SUBTITLE
Safety of High Speed Guided Ground Transportation Systems; An Overview of Biological Effects and Mechanisms Relevant to EMF Exposures from Mass Transit and Electric Rail Systems

5. FUNDING NUMBERS
RR393/R4021

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8. PERFORMING ORGANIZATION REPORT NUMBER
DOT-VNTSC-FRA-93-19

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Department of Transportation
Federal Railroad Administration
Office of Research and Development
400 7th Street, S.W.
Washington, DC 20590

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
DOT/FRA/ORD-93/32

11. SUPPLEMENTARY NOTES
*Under contract to: U.S. Department of Transportation
Research and Special Programs Administration
John A. Volpe National Transportation Systems Center
Cambridge, MA 02142

12a. DISTRIBUTION/AVAILABILITY STATEMENT
This document is available to the public through the National Technical Information Service, Springfield, VA 22161

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The U.S. has implemented a national initiative to develop maglev (magnetic levitation) and other high-speed rail (HSR) systems. There are concerns for potential adverse health effects of the Extremely Low Frequency (3-3,000 Hz) electric and magnetic fields (EMF) produced by these systems. The Environmental Protection Agency's Office of Radiation Programs is assisting the Federal Railroad Administration address these concerns; this comprehensive review is part of that effort. It outlines magnetic field exposure measurements of the TR07 German maglev system compared with other HSR and conventional systems. Reported biological and health effects of EMF are reviewed, and their significance assessed in relation to the characteristics of fields generated by maglev and HSR systems. Among the conclusions: maglev emissions are unique in their patterns and time-varying intensities but flux densities fall within exposure guidelines; epidemiologic results suggest a low-level health risk for power-line frequency EMF, at present the most relevant risk to ascribe to maglev systems; bioeffects may occur at specific window frequencies and intensities; the most convincing effects ascribed to EMF are altered circadian rhythms of melatonin secretion, modulation of transmembrane calcium transport, slight increases in the risks for some rare cancers, and mild behavioral disturbances.

14. SUBJECT TERMS
Electric and Magnetic Fields (EMF); Extremely Low Frequency (ELF); Power Line Frequency; Static Magnetic Fields; EMF Analysis; Electrified Rail; Trains; Magnetically-Levitated Trains (maglev); Power Substations; Health Effects; Biological Effects, Animal; Biological Effects, Human; Biological Effects, *In Vitro*; Epidemiologic Studies; Experimental Studies; Literature Review.

15. NUMBER OF PAGES
176

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. 239-18
298-102

PREFACE

This review of the electromagnetic fields bioeffects literature was prepared by the staff of Information Ventures, Inc., under a subcontract from S. Cohen & Associates, Inc., for submission to the U.S. Environmental Protection Agency (EPA), Office of Air and Radiation, under Work Assignment 2-97, Contract Number 68D90170. The project was the result of an interagency agreement between the EPA and the U.S. Department of Transportation (DOT), the Federal Railroad Agency (FRA), and the Volpe National Transportation Systems Center (Volpe Center). The scope of the project was suggested by Dr. James T. Walker, the EPA Work Assignment Manager. The report is designed to provide a broad overview of the potential biological effects of electromagnetic fields in order to anticipate possible health consequences of introducing new high-speed rail technology.

This review surveys reports on all biological effects of maglev-type electromagnetic fields, both positive and negative, and discusses data characterizing exposure parameters of maglev, conventional electric rail transportation, and other common environmental and occupational sources of EM fields. It concludes with a discussion of possible mechanisms for electromagnetic field bioeffects. Dr. Walker and other EPA staff provided helpful recommendations based on an earlier draft of this report, but the literature review and its conclusions have not been formally reviewed or approved by the EPA.

Primary authors of this report are Doctors Robert B. Goldberg and William A. Creasey, Information Ventures, Inc., and Kenneth R. Foster from the Department of Bioengineering at the University of Pennsylvania. Analysis of measurement data from the TR07 system and of the field characteristics of the other electric transportation systems evaluated by Electric Research and Management, Inc. (ERM), and preparation of the figures comparing the maglev envelope to field characteristics reported in the literature to produce bioeffects was performed by Dr. Foster. Analysis of *in vitro* methodology was performed by Dr. Joseph Tumilowicz, and technical assistance in preliminary analysis of the literature was provided by Mr. James W. Elliott. The authors would like to thank Dr. James Walker (EPA Work Assignment Manager) and Lynne Gillette for their help and suggestions, Dr. Aviva Brecher (DOT/Volpe Center), the Technical Monitor, for her many helpful comments on the draft report, and Dr. Donald Goellner (S. Cohen & Associates Task Leader) for his assistance in managing the project. Mr. Arne Bang, Senior Manager of Special Programs at the FRA and sponsor for this research effort; and Mr. Robert Dorer, the Volpe Center Manager of the High Speed Guided Ground Transportation Safety Program, are also thanked for their guidance.

SYSTÈME INTERNATIONAL (SI) UNIT DEFINITIONS AND CONVERSIONS USED IN THIS REPORT

DISTANCE (ENGLISH-TO-SI CONVERSION):

1 inch (in)	= 2.54 centimeters (cm)	= 0.025 meters (m)
1 foot (ft)	= 30.5 centimeters (cm)	= 0.305 meters (m)
1 yard (yd)	= 91.4 centimeters (cm)	= 0.914 meters (m)
1 mile (mi)	= 1.61 kilometers (km)	= 1,610 meters (m)

ELECTRICAL QUANTITIES:

Electric Fields

1 Volt/meter (V/m)	= 0.01 Volts/centimeter (V/cm)
1 kiloVolt/meter (kV/m)	= 1000 Volts/meter (V/m)
1 kiloVolt/meter (kV/m)	= 10 Volts/centimeter (V/cm)

Magnetic Flux Densities (English-to-SI Conversion)

10,000 Gauss (G)	= 1 Tesla (T)
10 milliGauss (mG)	= 1 microTesla (μT)
1 milliGauss (mG)	= .1 microTesla (μT)
0.01 milliGauss (mG)	= 1 nanoTesla (nT)

Electromagnetic Frequency Bands

1 cycle per second	= 1 Hertz (Hz)
1,000 cycles per second	= 1 kiloHertz (kHz)

Ultra Low Frequency (ULF) Band	= 0 Hz to 3 Hz
Extreme Low Frequency (ELF) Band	= 3 Hz to 3 kHz
Very Low Frequency (VLF) Band	= 3 kHz to 30 kHz
Low Frequency (LF) Band	= 30 kHz to 300 kHz

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF TABLES

1. EXECUTIVE SUMMARY

There is growing public and scientific concern over potential human health risks associated with exposure to extremely low frequency (ELF) electromagnetic fields (EMF) from a variety of sources, primarily engendered by epidemiologic studies conducted in the past two decades which have linked residential and workplace exposures to 50 or 60 Hertz (Hz) fields to increased incidence of cancer. However, limited knowledge and understanding of the mechanisms of interaction between ELF EMF fields and biological systems; negative results in some epidemiologic studies; and a lack of conclusive proof of a causal relationship between EMF exposures and health effects have resulted in uncertainty about the potential health consequences. New technology which generates high levels of ELF EMF or produces ELF EMF with unusual frequency or time-varying characteristics has been the focus of more intense public scrutiny, based on environmental impact and health concerns. However, such issues are hard to address given the lack of definitive information on the safety of ELF EMF in general.

The U.S. has implemented a National Maglev (magnetic levitation) Initiative (NMI) to design and develop high-speed transportation systems. Maglev systems use strong magnetic fields for suspension and propulsion, and there is concern over possible health effects that may result from exposing humans to these fields. Because of this concern, the Federal Railroad Administration (FRA) asked the Environmental Protection Agency's (EPA) Office of Radiation Programs (ORP) to assist in determining the potential health implications of maglev and high-speed rail systems. This review was conducted as part of that cooperative effort. The primary purpose of this review was to assess all potential biological and health effects associated with human exposure to ELF EMF of the type generated by maglev, high speed rail transportation, similar automated mass transit systems, and other commonly-encountered sources, based on a comprehensive review of the published research literature.

Magnetic field exposure assessment measurements of the TR07 German maglev system performed by Electric Research and Management, Inc. (ERM), under contract with FRA (Contract No. DTFR53-91-C-00047), represented the only complete set of measurements of maglev available at the time of writing. Analysis of the specific TR07 maglev ELF field characteristics and their potential to produce biological effects therefore provided the focus for this aspect of the review. In addition, there was a generalized consideration of the anticipated field characteristics of other systems, such as the Japanese maglev design which uses a DC superconducting magnetic repulsion system to suspend the train. Recent publication of similar measurements of electrified Amtrak and MBTA rail systems in the North-East Corridor, the Washington DC metro system, and the French TGV high speed rail system were evaluated for comparison with the maglev system.

ERM measurements indicated that the TR07 system produces DC magnetic fields within 40 microtesla (μT; 100 μT = 1 gauss (G)) of geomagnetic field levels at the maglev train floor, and within about 10 μT of ambient levels at head height. AC magnetic field emissions from the TR07 maglev system occur primarily in the ELF range (0-300 Hz), with more intermittent (transient) and intense exposures in the 2-45 Hz range than is typical of power system (50 or 60 Hz) exposures. Total ELF EMF field intensities are usually less than 100 mG at floor level and under 20 mG at head level for passengers, with occupational exposures slightly higher in intensity and richer in higher frequency components. The fields are highly time-varying (changing with the speed of the train and other conditions) so that any "typical" exposure estimates have a variability in the flux density at any given frequency of about 100%. Actual operating conditions, with multiple trains in the system or with changes to the system design to increase the clearance distance between the train and guideway, could increase flux density substantially above those measured by ERM, perhaps by an order of magnitude or more.

ERM has also reported measured magnetic field characteristics of conventional electric rail systems (AMTRAK, Metro Northeast Corridor, New Jersey Transit, MBTA DC subway and trolley systems) and high-speed rail systems (the French TGV-A system). These systems generally produce exposures which are somewhat higher in intensity than the maglev system but have a different frequency distribution. Conventional electric rail systems, like the TR07 maglev, show high spatial and temporal variability (depending on train speed, etc.) and expose passengers and crew to "spikes" of fairly high exposure. Unlike the maglev however, the frequency range is generally more narrow, centering on the power frequency (e.g., 25 or 60 Hz for AC systems) and its odd harmonics. DC-powered systems have a large DC component with some extremely low frequency components resulting mainly from ripples in the rectification electronics.

The flux densities measured by ERM in the maglev and other electric rail systems generally fall well within existing international exposure guidelines in terms of time-averaged field intensity, the basis for current exposure standards. However, the particular pattern of frequencies and the variation of intensity with time appear to be unique to maglev systems, and consequently, particular attention was given to evaluating the bioeffects literature for effects in the 0-45 Hz extremely low frequency range. For each of the bioeffects discussed, the frequencies and intensities reported to produce the response were analyzed in relation to a typical averaged TR07 maglev exposure estimate.

Alterations in calcium flux in isolated chicken brain tissue, other tissues, and cells of neural and non-excitable tissue origin provide the largest body of evidence for the existence of bioeffects which are only produced by ELF EMF having specific frequency/intensity characteristics (a "window" effect). In general, these effects involve a statistically significant, but highly variable increase or decrease in the flux of calcium through cellular membranes, when the system is exposed to low-intensity ELF EMF at a particular combination of frequency and intensity. In contrast to the typical dose-response relationship seen with chemical or other physical agents, higher intensity signals do not necessarily produce a greater response and may actually generate a diminished response. Some of the variability in experimental results can be attributed to uncontrolled differences in the biological status of the system, or to experimental parameters such as the pattern of temperature change. Calcium flux effects have been reported in fields which fall within TR07 exposure characteristics, but these reports remain controversial because they have not been adequately replicated. The physiological significance of these effects are also unclear because they have been demonstrated only in isolated cells and tissues, but only rarely and indirectly in whole animals. Therefore, these results do not directly imply a health hazard or even necessarily a biological effect in the intact organism, but do support the possibility of a complex dose-response pattern in other biological responses to ELF EMF. If "window" responses were to apply to hazardous effects, exposure limits could not be established solely on the basis of time-averaged flux density levels.

There is a limited and somewhat contradictory body of research which indicates that certain time-varying magnetic fields can produce developmental abnormalities in rodents and chickens. A systematic effort made in Project Henhouse, a multi-laboratory attempt to replicate experiments in which abnormalities were induced in chicken embryos by pulsed magnetic fields, indicated that a combination of biological and field-specific factors may underlie the inability to obtain consistent effects with ELF EMF exposure. Production of abnormalities by the action of certain EMF fields on chicken embryos is supported by statistical analysis of the studies taken as a whole. However, the high degree of variability and inability to consistently replicate the response, points to interaction with uncontrolled biological factors. A more problematic body of epidemiologic studies suggest that human exposure to ELF EMF from electric cable heat, electric blankets, or video display terminals (VDTs) may increase the incidence of spontaneous abortion early in pregnancy. The available studies do not strongly support a human risk, but it is not clear if any of them have adequately addressed the possibility of effects on the earliest stages of pregnancy, as has been suggested on hypothetical grounds.

A variety of effects of ELF EMF have been reported on immune system responsiveness expressed as effects on antibodies in the blood (the " humoral" responses), activation of killer cells (the "cytotoxic" immune responses), or the biochemical characteristics of isolated white blood cells (lymphocytes) in culture. While there are still many inconsistencies in results among experiments, it seems likely that lymphocytes which have been stimulated by exposure to highly active plant antigens (lectins) are more responsive to EMF than unstimulated cells, and that immune system cells show indications of responding to EMF only in specific frequency and/or intensity "windows." Methodological defects or ambiguities in many of the cell culture experiments make much of this data difficult to interpret. Most of these effects are produced at field levels substantially higher than the TR07 maglev fields, but experiments were performed at field intensities chosen for their convenience, without an effort being made to determine threshold conditions for the effects. No coherent pattern can be described for the more variable measures of immune response in cultured lymphocytes following ELF EMF exposure.

Melatonin is a neuroendocrine regulatory hormone produced by the pineal gland. Among other effects, disruption of circadian melatonin release has been linked in clinical studies to behavioral disorders such as seasonal affective disorder, suppression of immune system function, and, in experimental studies, to more rapid growth of transplanted tumors. Pineal responses to ELF EMF represent one of the most active areas of current research, and one which provides a possible mechanistic link between ELF EMF and postulated adverse human health effects such as cancer and depression. In animal experiments, the sensitivity of the pineal response to ELF EMF varies, possibly depending on species/strain differences and other physiological characteristics, such as previous recent nocturnal light exposures or stress. Decreased nocturnal pineal melatonin production has been demonstrated in humans exposed to ELF EMF from polymer wire electric blankets, but the health consequences of this effect were not determined. As with immunologic effects, the magnetic field intensity levels at which melatonin effects were observed were at least an order of magnitude greater than those measured in the TR07 maglev, with the exception of some experiments which reported inhibition of nocturnal melatonin secretion in response to rapidly inverted DC fields of geomagnetic intensity.

Physiological effects of ELF EMF have been reported in humans, notably a small but significant decrease in heart rate and a modest decrease in task performance. These responses are within the physiologically normal range and are not considered a health hazard: they would only be of concern if they result in hazardous negative effects on human performance (e.g., increase the accident rate), or are indicative of more extreme ELF EMF effects in particularly susceptible individuals. Various effects on animal behavior have been reported under EMF exposure conditions which fall within or close to the TR07 maglev frequency/intensity exposure range, but these effects are hard to interpret in terms of human performance. More severe effects such as cardiac arrhythmia and electroencephalographic abnormalities have been reported in workers exposed to high levels of ELF EMF, but these reports mainly appeared in the Soviet and Eastern European literature and have generally not been confirmed by Western studies.

A number of experiments have reported signal-specific effects of ELF EMF on gene expression (primarily at the level of transcription) in a variety of normal cells and tumor cell lines. Effects on cell proliferation have also been observed in a number of cell lines, and clinical results indicate a statistically significant effect of pulsed EMF signals in promoting healing in bone non-unions and soft tissue injury. Much of the research literature in this area describes responses which are indicative of normal differentiated tissue responses rather than malignant growth, but there are a few reports of stimulated oncogene expression and increased contact-independent cell proliferation. There is considerable interest in finding an *in vitro* correlate for the EMF-cancer link suggested by epidemiological studies, but such results have appeared in the literature inconsistently. At the present time it is not clear to what extent enhanced gene expression depends on specific features of the EMF stimulus, and to what extent the nature of the response is a function of the target tissue.

A number of hypothetical mechanisms can be proposed to explain ELF EMF bioeffects, including some which are operative at the whole animal level (effects on pineal melatonin or other hormones), at the tissue level (cellular movement and morphogenesis guided by electric fields induced in tissues), or at the cell level (stimulation of membrane receptors, ion flux, or cytoplasmic components). These mechanisms, although each is plausible and supported by limited experimental evidence, remain primarily speculative.

In summary, there are a variety of bioeffects that have been reported to occur at ELF EMF frequencies such as those produced by the TR07 maglev system, but usually at intensities substantially greater than those characteristic of anticipated maglev exposures. To date, no significant bioeffects have been reported in maglev fields or in experimental systems designed to simulate maglev fields, but very little experimental work has been done under these specific ELF EMF exposure conditions.

In terms of possible negative health effects of chronic exposure to maglev magnetic fields, the mechanism of action of ELF magnetic fields is a critical issue. If only the time-averaged intensity of magnetic fields is important for producing bioeffects, the TR07 maglev does not seem to present potential health risks exceeding those associated with transmission line and common electrical appliance exposures. However, if biological effects are produced by ELF fields in a frequency/intensity-specific manner or by rapidly changing fields (termed the dB/dt effect), it becomes necessary to evaluate the specific profile of maglev emissions in terms of these features, since it is not otherwise possible to predict the bioeffects and possible health consequences.

2. INTRODUCTION AND BACKGROUND OF REVIEW

There is growing public and scientific concern over potential human risks associated with exposure to electromagnetic fields (EMFs). Recent epidemiologic studies have linked residential and workplace exposures to 60 Hertz (Hz) fields with increased incidence of cancer. However, limited knowledge and understanding of the mechanisms of interaction between these fields and biological systems, and lack of conclusive proof of a causal relationship between exposures and health effects, have resulted in uncertainty about the health consequences.

The U.S. has implemented a federal maglev (magnetic levitation) initiative to design and develop high-speed transportation systems. This initiative is under the direction of the Federal Railroad Administration (FRA), Department of Transportation (DOT), and the U.S. Army Corps of Engineers. Maglev systems use relatively strong magnetic fields for suspension and propulsion, and there is concern that possible health effects may result from exposing humans to these fields. Because of this concern, the FRA has asked EPA to assist them in determining the potential health implications of maglev and high-speed rail systems. EPA's Office of Radiation Programs (ORP), FRA, and DOT have entered into an Interagency Agreement through which ORP will provide this assistance. The primary purpose of this project is to assist the FRA by assessing the potential biological and health effects associated with human exposure to the EMFs generated by maglev, high speed rail, similar automated mass transit systems, and other commonly encountered sources.

This report, prepared under work assignment 2-97, provides a summary of research information and discussion about the potential biological and health effects associated with human exposures to EMFs generated by maglev and other high-speed rail systems. Information Ventures, Inc., has completed a preliminary survey of the EMF bioeffects literature relevant to maglev for DOT under VNTSC Order No. DTRS57-91-P-81743. Magnetic field exposure assessment measurements of the TR07 German maglev system have been performed by Electric Research and Management, Inc. (ERM), under contract with FRA (Contract No. DTFR53-91-C-00047). The ERM report is summarized in section 3 of this report. This report extends our preliminary evaluation of the literature, providing a more rigorous basis for analysis of the specific TR07 maglev fields and their potential to produce biological effects. In addition, this report reviews a broader range of potential biological effects including all reported effects, beneficial as well as potentially hazardous, which may be associated with EMF characteristic of maglev-type systems.

ERM measurements indicate that the TR07 system produces DC magnetic fields within 40 microtesla (μT; 100 μT = 1 gauss (G)) of geomagnetic field levels at the maglev train floor, and within about 10 μT of ambient levels at head height. AC magnetic field emissions from the TR07 maglev system occur primarily in the extremely low frequency (ELF) range (0-300 Hz) with more intermittent (transient) and intense exposures in the 2-45 Hz range than is typical of power frequency (50 or 60 Hz) exposures. Total ELF EMF field intensities are usually less than 10 μT at floor level and under 2 μT at head level for passengers, with occupational exposures slightly higher in intensity and richer in higher frequency components. These levels generally fall within existing international exposure guidelines in terms of time-averaged field intensity, the basis for current exposure standards. However, the particular pattern of frequencies and the intensity variation with time appear to be unique to maglev systems. Extremely low frequency EMF has been reported to produce a range of frequency- and intensity-specific bioeffects, but for various reasons these field characteristics have not been addressed by present exposure standards. In terms of possible negative health effects of chronic exposure to maglev magnetic fields, the mechanism of action of ELF magnetic fields is a critical issue. If only the time-averaged intensity of magnetic fields is important for producing bioeffects, the TR07 maglev does not seem to present potential health risks which exceed those of transmission line and common electrical appliance exposures. On the other hand, if biological effects are produced by ELF fields in a frequency/intensity-specific manner or on the basis of rapidly changing fields (termed the dB/dt effect), it becomes necessary to evaluate the specific profile of maglev emissions in terms of these features, since it is not otherwise possible to predict the bioeffects and possible health consequences.

2.1 BASIS FOR CONCERN OVER POSSIBLE HEALTH EFFECTS OF ELF EMF

There has been very little research conducted with ELF EMF with characteristics exactly like the fields produced by maglev vehicles, but those fields are similar in intensity to fields produced by electric power transmission. Concern has been raised about potential human health effects of EMF from power systems (50/60 Hz, its harmonics and subharmonics) based on indirect evidence of human health effects from residential and occupational epidemiologic studies. These studies, and a limited number of reports of acute effects, are discussed in detail in Sections 12 and 13 of this report. Laboratory studies have suggested biological effects (not necessarily hazardous) with low intensity ELF EMF, and the evidence for these bioeffects is reviewed in Sections 4-11 of this report. At the present time, there is no clear laboratory data to provide an unequivocal explanation of the epidemiologic results, but there are hypothetical mechanisms which might link the two which are discussed in the final section of this report (Section 14).

Based on epidemiologic studies, current thinking about potential health risks of ELF EMF has focused on magnetic rather than electric fields produced by alternating current (AC) lines. Power transmission lines and household wiring produce both electric and magnetic fields, but field exposure levels are generally low except in close proximity to appliances, in certain industrial applications, and for individuals directly under transmission lines. Magnetic field levels range from 0.03-30 μT in the home, up to 35 μT near transmission lines, and as high as 50 mT in a few industrial processes. The background geomagnetic field is a static field with a maximum vertical component (at the magnetic poles) of 67 μT, and a maximum horizontal component (at the magnetic equator) of 33 μT (World Health Organization (WHO) 1987). Static magnetic fields from the few high-voltage direct current (DC) transmission lines in current use are no greater in intensity, and are usually somewhat smaller than, the earth's field. Acute exposure studies have been conducted with such fields with no significant indication of health hazards, but there is no epidemiologic data. While at low frequencies electric and magnetic fields are largely independent, many experimental and epidemiologic studies have not discriminated between the electric and magnetic field components producing bioeffects. It is generally assumed, however, that time-varying (AC) magnetic fields are more likely to be responsible for any bioeffects because they represent the greatest man-made deviation from ambient conditions, and because time-varying magnetic fields induce electric fields in body tissues. Much recent work has attempted to define, in more detail, the applied fields used, including their relationship to the geomagnetic field, and to include complete dosimetry in the experimental design. The impact of these attempts is limited, however, by a lack of information on exactly which field parameters are most important in affecting biological systems.

This report therefore considers effects produced by a range of waveforms at frequencies under about 1000 Hz, and only occasionally considers reports of bioeffects produced by microwave frequency and radiofrequency EMF which are amplitude modulated in the ELF range. In the latter reports, bioeffects are attributed by the experimenters to the ELF amplitude modulation frequency, since they are not seen with unmodulated carrier waves, but this interpretation is open to question. There are not enough data at present to allow generalization across the spectrum of EMF bioeffects seen at one frequency, and there is suggestive evidence that EMF effects may, at least in some cases, be quite frequency-specific. For this reason, this report attempts to specify frequency and waveform characteristics in reviewing the bioeffects literature with the caveat that a reported effect might be closely dependent on the specific experimental conditions.

2.2 OVERVIEW OF APPROACH TO THE REVIEW

The ELF EMF bioeffects literature has been divided by topic, based on the primary biological system (endpoint) involved. In some cases, categorization of work may be almost arbitrary. For example, calcium flux effects in lymphocytes may be considered as a general ion flux effect (Section 4) or an effect on the immune system (Section 6), since calcium ion flux is associated with lymphocyte proliferation and consequently, with immunoreactivity. In surveying such a broad range of biological effects, it is impossible to avoid some inadvertent scattering of related work, but we have made an effort to point out the interrelatedness of topics wherever possible.

We have also introduced an element of critical analysis of the results in an attempt to provide some feel for the reliability of various reports. The EMF research literature is a very diverse and multidisciplinary body of work, and it is not always easy to resolve reports of conflicting results based on published information. Many experiments reported in the EMF bioeffects literature are in the nature of exploratory studies, and have not been independently replicated. Two areas in particular, reports of effects of ELF EMF on calcium ion flux in cells and tissues, and reports of teratologic effects of ELF EMF in chicken embryos, constitute a substantial body of controversial experimental results which is in need of further investigation. Many of the individual reports do not provide sufficient methodological detail to allow critical evaluation (many are meeting abstracts), but we have attempted to point out generalized strengths and weaknesses of the research methods and approach whenever possible.

As is pointed out at several points in the discussion of various areas of research, there is no unequivocal indication of human health risk from electric rail transport fields or any other low-level ELF EMF source. Rather, the research points to certain areas of potential concern, among them results indicating nonlinear dosimetry, which clearly applies to certain bioeffects and therefore potentially to biological hazards. Biological effects are produced at frequencies and intensity levels comparable, in some cases, to maglev and other electric rail system exposures. At the same time, research results are frequently ambiguous and results indicate areas where further research is needed.

Throughout this report, quantitative information on magnetic field strength (induction) is given in International System (SI) units (tesla, T) with occasional cross references to field levels in centimeter-gram-second (CGS) units (gauss, G) for the convenience of those more accustomed to these units. A tesla unit is equal to 10,000 gauss (1 G = 10^{-4} T; 1 mT = 10 G, 1 μT = 10 mG), and is approximately equivalent to 0.8 A/m in air.

3. MAGLEV FIELD CHARACTERISTICS

The EMFs associated with a maglev vehicle arise from both the suspension and propulsion systems, which may differ greatly depending on the maglev design. For example, the National Japanese Railways maglev utilizes for suspension, DC fields generated by superconducting magnets at points along the sides and underneath of the car to center it in a semicircular runway. On the other hand, the German TR07 design uses AC magnetic fields generated below the train to pull the car up towards magnets on the guideway's lower surface, and others to stabilize it against sideways displacement from the guideway.

These different technologies will profoundly affect the types of EMFs associated with the vehicles. No fields have been measured or calculated for the proposed American maglev designs. Fields generated by a small maglev system operating over a short distance near Birmingham, U.K., have been measured, but the data have not yet been released officially. The Japanese maglev was destroyed in an accident before complete dosimetry had been carried out. It was calculated, however, that maximum DC fields would be 1-20 mT (10-200 G) and AC fields in the car no more than 10-100 μT, the latter chiefly when passing another train (Nakagawa 1992).

Thus, the only prototype system for which actual data are available is the German TR07 Maglev System at the Emsland Transrapid Test Facility; these data will be described in the next section. A later section will briefly compare the magnetic field exposure from this vehicle with that from several conventional rail systems.

3.1 EMF CHARACTERISTICS OF THE TR07 (GERMAN) MAGLEV SYSTEM

An extensive set of measurements on the TR07 was reported recently by Electric Research and Management, Inc. (ERM) (Dietrich, Feero *et al.* 1992). The dosimetry focussed on DC and time-varying magnetic fields, in part because of public health concerns about such fields, and in part because electric fields within the metal passenger compartment are quite small.

Measurements were conducted on the vehicle itself, in the vicinity of the vehicle, guideway, passenger station, control center, and power supply facilities at the Transrapid Test Facility in Emsland, Germany. The data consisted of magnetic field waveform recording collected using a computer-based digital magnetic field monitoring system (*Multiwave*™). Multiple scans, using ten sensors, were performed in rapid succession to document the temporal fluctuations in magnetic fields throughout the vehicle. The data were analyzed by Fourier transform techniques to determine the spectra of the magnetic fields between 2.5 and 2562.5 Hz, in 5 Hz frequency bands.

According to this report, measurable fields exist in four different frequency ranges: DC and very low frequency fields (<1 Hz), sub-power frequency fields (2.5-47.5 Hz), power frequencies (47.5-62.5 Hz), low supra-power frequencies (62.5-302.5 Hz), and high supra-power frequencies

(302.5-2562.5 Hz). The magnetic fields at all of the measured sites had a complex spectra that was highly variable in time.

In the passenger compartment, the average time-varying magnetic flux density between 2.5 Hz - 2 kHz ranged from approximately 10 μT near floor level to 2 μT at standing head level (see Figure 3-1). The fields were highly variable, both in strength and frequency content, with the maximum values at any height above the floor often 2.5 times higher than the average value.

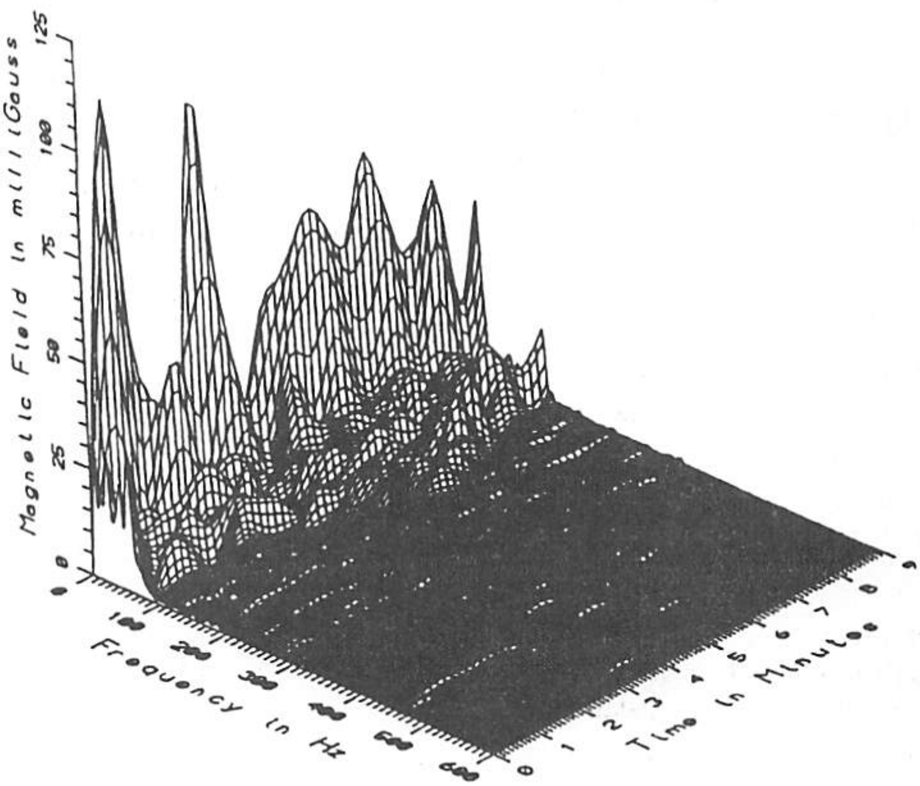

FIGURE 3-1. MAGNETIC FIELDS AT FLOOR LEVEL -- WINDOW SEAT IN REAR OF PASSENGER SECTION, CAR #2 (FROM DIETRICH *ET AL.*, 1992).

The DC magnetic field within the passenger compartment was dominated by the geomagnetic field (50 μT) at head level of a standing person, with higher fields (83.5 μT) at floor level, including measurable contributions from vehicle-generated fields. Dietrich *et al.* concluded that the static magnetic fields onboard the TR07 vehicle were comparable to those commonly experienced in the environment from the earth's magnetic field and other static sources.

Outside of the vehicle, within 3 m of the centerline of the guideway, the maximum magnetic fields were comparable to average field levels within the vehicle, but they existed only for the brief periods when the guideway was energized and the vehicle was passing.

At the passenger station, magnetic field levels were similar to those near the guideway; in the waiting area, the maximum time-varying flux density produced by a passing vehicle was approximately 2 μT.

Near power equipment, the time-varying fields had a complex frequency dependence. However, the average flux densities were low (<0.2 μT). Fields associated with the feeder cables were small (<0.2 μT) and difficult to measure accurately in this study.

In summary, the exposure characteristics of the TR07 maglev vehicle are complex. At **low-frequencies** (power-frequencies and below) the magnetic flux densities within the passenger compartment are comparable to those from other common sources of exposure. However, their frequency characteristics are more complex, and are (as quoted from the ERM report) "unique from those that have been measured to date anywhere else in the environment" (Dietrich *et al.* 1992).

At **higher frequencies**, above 50-60 Hz, the magnetic fields associated with the TR07 are comparable to environmental fields produced by harmonic distortion of power frequency electric currents in appliances and wiring. At DC (or frequencies below 1 Hz) the fields are comparable to the geomagnetic field.

Figure 3-2 compares the spectra of the time-varying magnetic fields within the passenger compartment of the TR07 maglev at several distances above floor level. These data were taken sequentially over a single ten-minute run, in which the velocity of the vehicle accelerated from 150 to 400 km/hr and back to 150 km/hr. Each envelope represents the average of the spectra of 20 sequential sets of data. This provides a representative indication of the frequency spectra of the magnetic fields at various distances above the floor of the passenger compartment averaged over the 10 minute run.

In examining the data, two things need to be kept in mind.

(a) First, the fields are highly variable in time. In the data set analyzed, the standard deviation of the amplitude of any frequency component was approximately equal to the average value of the component at that frequency. The variability in the flux density at any frequency range is thus about 100%;

(b) Second, the "average" exposure indicated in the figure pertains to the single 10 minute run. Under actual operating conditions, the flux density in the passenger compartment will depend on the vehicle's velocity and other operating conditions and its spectra over extended periods of time might vary considerably.

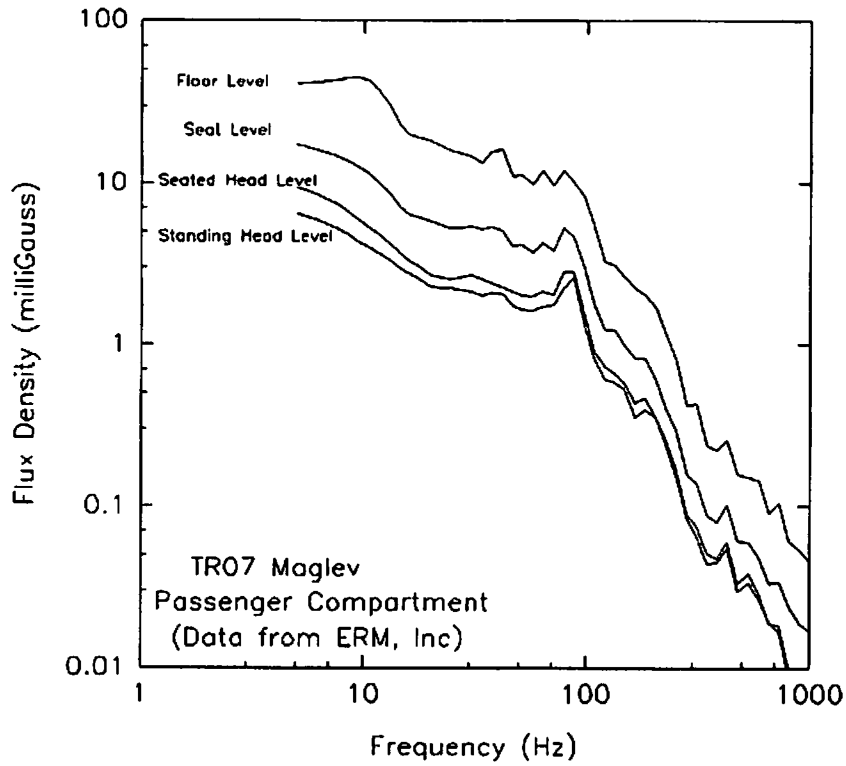

FIGURE 3-2. SPECTRAL DENSITY OF MAGNETIC FIELDS IN PASSENGER COMPARTMENT OF THE TR07 MAGLEV. THE DISTANCES ABOVE THE FLOOR ARE 12.7, 47, 112, AND 175 CM. DATA FROM ERM (ELECTRIC RESEARCH AND MANAGEMENT, INC).

3.2 MAGNETIC FIELD EXPOSURES FROM OTHER RAIL SYSTEMS

ERM recently (1993) extended these studies to include measurements from other rail systems as well: the AMTRAK and Metro North Northeast Corridor (NEC) rail systems, the French train, a Grande Vitesse-Atlantique (TGV-A); and the Massachusetts Bay Transit Authority (MBTA) mass transit system in the Boston area (Dietrich, Feero *et al.* 1993; Dietrich, Papas *et al.* 1993; Dietrich 1993). The trains are powered with either DC current (the MBTA system, part of the TGV-A system) or with 25, 50, or 60 Hz (parts of the AMTRAK and TGV-A systems).

Each of these trains produces characteristic magnetic field patterns which, like the TR07, vary with power use (train speed). Typical profiles for one of the systems is shown in Figure 3-3, which illustrates the frequency/intensity characteristics of the NEC011 train in a graphic which is comparable to the information shown for the TR07 maglev in Figure 3-1.

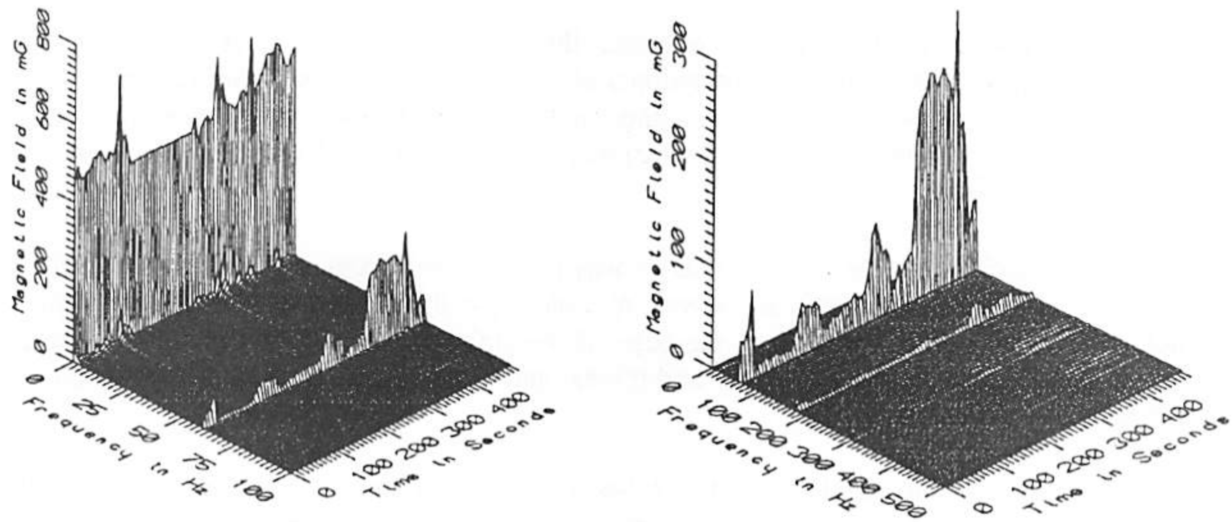

FIGURE 3-3. MAGNETIC FIELDS IN A COACH OF THE NEC011 TRAIN. MEASUREMENTS MADE 60 CM ABOVE THE FLOOR AT THE EDGE OF THE AISLE. DC AND LOWER FREQUENCY DETAIL SHOWN AT LEFT, FULL SPECTRUM AT RIGHT (FROM DIETRICH, FEERO *et al.* 1993).

The strong DC component in the NEC011 measurements comes primarily from the geomagnetic field. Dominant time-varying components are from power-frequency fields (in this system, at 60 Hz) and their harmonics. In contrast to the magnetic fields produced by the TR07, the time-varying fields produced by conventional rail systems occur in discrete frequency bands with little or no intensity between them (compare Figures 3-1 and 3-3). This constitutes one of the major differences between the magnetic field characteristics of the TR07 and conventional electric rail systems.

The results of the ERM studies may be summarized as follows:

a. The alternating electric fields onboard all trains are generally low, a few volts per meter.

b. The time-dependent magnetic fields onboard the trains are complex and vary greatly with time. For trains powered by alternating current, the strongest frequency components are at the frequency of the power supply, or odd harmonics of that frequency. However, measurable components exist at many other frequencies as well. The strongest magnetic fields in conventional electric rail systems are at power-line frequencies (eg. 25, 50, or 60 Hz, depending on the system) and their intensity is on the order of several tens of microtesla. In general, the magnetic fields measured onboard the AC-powered trains resemble those present near electrical motors.

c. For DC powered systems, the strongest fields (up to several tens of millitesla; up to several gauss) are static fields, and are associated with the propulsion systems. However, measurable frequency components are found over a broad range of frequencies as well.

d. The magnetic field intensities onboard the TR07 maglev are, roughly speaking, comparable to those found onboard conventional electric-powered trains. The frequency range and average intensity levels are similar, although there is a different frequency distribution (greater intensity at frequencies from 2-45 Hz) and a higher degree of variability in frequency and intensity with time.

e. Magnetic fields near the trains' rights-of-way mostly result from currents in the catenaries and tracks. Their maximum levels are several microtesla, at the frequency of the power supply, but fall off rapidly with distance from the edge of the right of way. Significant contributions are present from currents in the catenary and tracks, and from power transmission lines that are located in or near the right of way.

Figure 3-4 summarizes magnetic field data (also from ERM) obtained from the TR07 maglev and these other electric rail transportation systems. The "envelopes" represent the spectra of magnetic fields measured onboard the trains at seat level, and attempt to condense a great deal of measurement information into a single profile of magnetic field frequency/intensity. The limitations and assumptions involved in using these "envelopes" should be recognized. The "envelope" gives some indication of the exposure to a passenger or train employee onboard the train, and facilitates comparison of this exposure with those used in laboratory studies. However:

a. The actual exposure onboard the maglev (and other trains) is highly variable. The actual magnetic fields present onboard a train (either conventional or maglev) are extraordinarily complex, both in amplitude and time dependence. The frequency spectrum is broad, with maximum recorded values at most frequencies that were approximately 3 times the average, and deviations of as much as 2-fold. This spatial and time-variability cannot be described adequately in a figure.

b. The envelope provides an indication of typical field strengths measured at seat level by ERM. At other locations within the train, the measured field levels varied considerably. For example, at floor level the fields were typically several times stronger than those measured at seat level. Therefore, the "envelopes" are only representative field levels onboard the trains, and there is perhaps a 3-fold uncertainty in exposure resulting from the factors discussed above.

This variability creates obvious problems in relating the exposure onboard the rail systems to the laboratory studies. Most experimental work is conducted using sinusoidal fields of constant frequency and constant amplitude, amplitude-modulated sine waves that contain only a few harmonics, or pulsed magnetic fields of relatively simple time dependence. By comparison, the exposures onboard the trains are intermittent, generally of shorter duration than in most experimental studies, and much more variable in frequency and amplitude. The significance, if any, of these differences depends on the particular effect and its biophysical mechanisms.

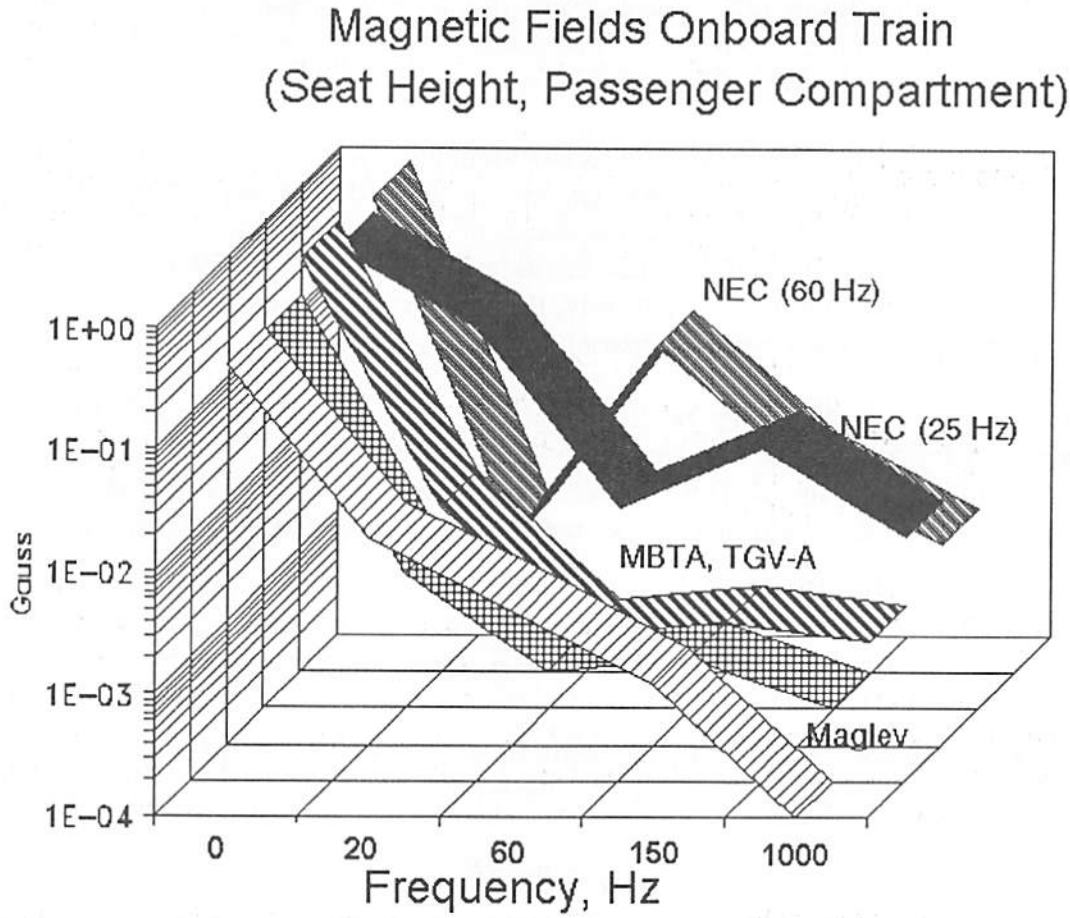

FIGURE 3-4. ENVELOPE OF TYPICAL MAGNETIC FLUX DENSITIES MEASURED AT SEAT LEVEL. NOTE THAT THE HORIZONTAL (FREQUENCY) AXIS IS NOT A LINEAR SCALE. (FROM DATA IN DIETRICH 1993A, 1993B, 1993C).

3.3 EXTERNAL VERSUS INTERNAL FIELDS

An important consideration is the difference between fields <u>external</u> to the body, and <u>internal</u> fields induced within the body. Quite different situations exist for electric and magnetic fields.

a. **Magnetic field** - Since tissues are nonmagnetic, the internal magnetic field is the same as the external field. Therefore, for magnetic field-induced effects, the external magnetic field is likely to be a useful exposure index for comparing laboratory studies with human exposures.

b. **Electric field** - Tissues are conductive, and the internal electric field is quite different from the external field, in a way that depends on the body size, frequency of the field, and other factors. Therefore, great care is needed when comparing the results of laboratory studies involving the exposure of biological preparations to external electric fields, with human exposures. This report considers few, if any, biological effects from external electric fields, however.

c. **Induced electric fields from external magnetic fields** - Time-dependent magnetic fields will induce time-dependent electric fields within the body. Some investigators have suggested that these are the cause of reported biological effects from time-dependent magnetic fields. Since the induced electric fields within the body vary as a function of position within the body, the body size and orientation, and other factors, great care is needed when comparing laboratory studies with human exposures.

A simple analysis suggests scaling relations that can help facilitate such a comparison. The **maximum** electric field induced by a vertically-oriented magnetic field occurs near the periphery of the body. It can be estimated from the formula that applies to a cylindrical object of radius R, where E is the induced field in volts per meter, and B is the flux density in tesla:

$$E_{induced} = \frac{R}{2}\frac{dB}{dT}$$ **Equation 1.**

From this result, we see that the induced electric field has **zero DC component**. For sinusoidal fields of frequency f and peak amplitude B_{peak} (tesla) this becomes:

$$E_{induced} = \pi * f * R * B_{peak}$$ **Equation 2.**

Thus, for time-dependent magnetic fields, the frequency spectrum of the induced electric fields can be found from the frequency spectrum of the magnetic field simply by multiplying by a constant proportional to the frequency.

Figure 3-5 compares the envelope of the magnetic fields at seat level onboard the TR07 maglev with the induced electric fields in the body using equation 2, assuming R = 0.15 meters. Smaller fields would be induced within the head and limbs (because of the smaller radius of these parts of the body).

Several caveats are needed about these results. The first is that the actual induced electric fields within the body will vary, because of (a) the geometric dependence of the induced field, as indicated in Equation 1; and (b) the geometric complexity of the body; and (c) the complex spatial and time-dependence of the actual magnetic fields onboard the trains. Nevertheless, these estimates are useful when comparing human exposure from rail systems with laboratory studies.

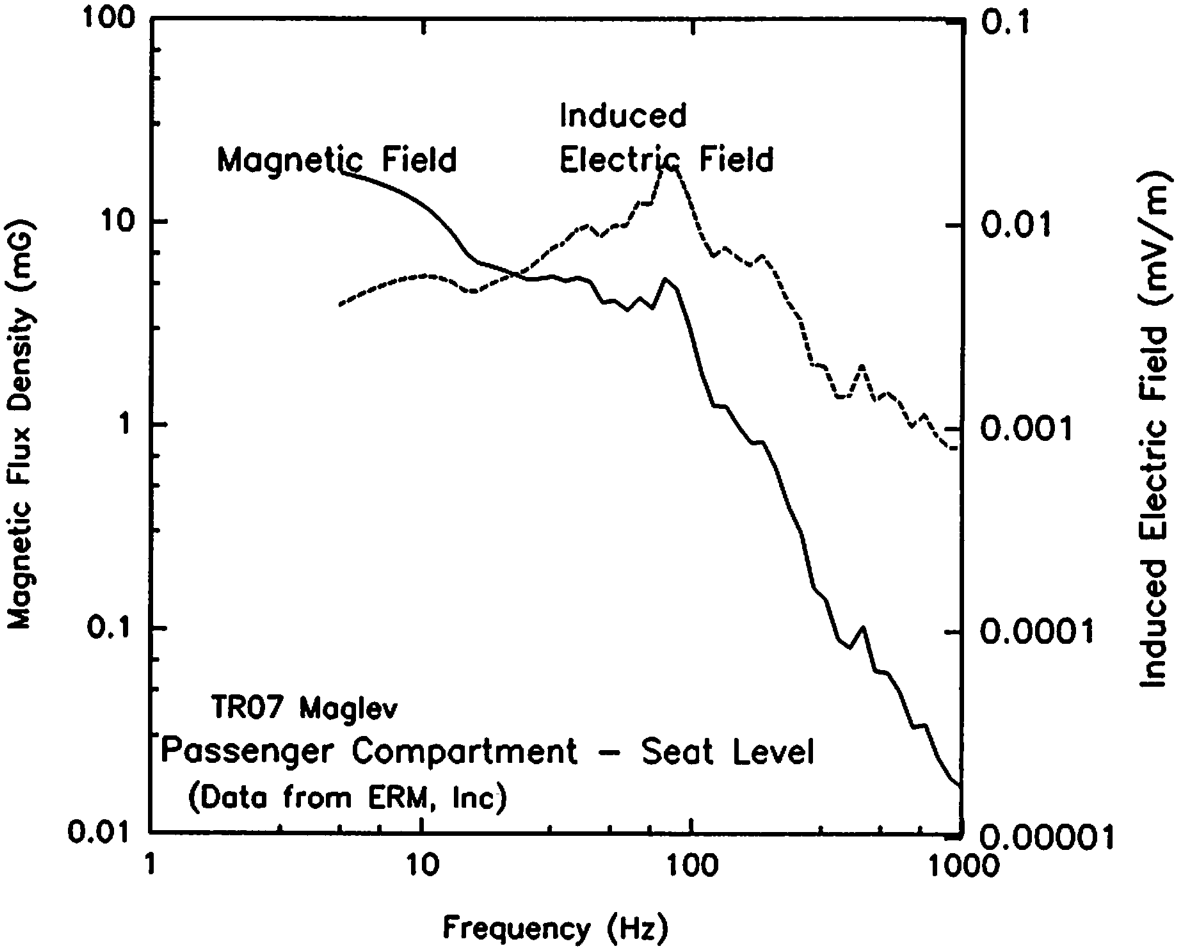

FIGURE 3-5. ESTIMATED MAXIMUM MAGNITUDE OF INDUCED ELECTRIC FIELDS IN THE TORSO OF A PERSON EXPOSED TO ALTERNATING MAGNETIC FIELDS. ELECTRIC FIELDS IN mV/m.

We note that the induced electric fields within the body, calculated above, are quite small compared to fields that are naturally present. These endogenous electric fields associated with nerve action potentials and other natural bioelectric sources are about 10,000 times stronger (0.1-1 V/m) and occur in a comparable frequency range (Carstensen 1987).

Finally, the comparisons facilitated by the envelopes in the above figures pertain to the amplitude of frequency components of the magnetic fields, measured over rather short time periods (seconds) onboard the train. They include no information about the time-dependence of the fields over longer periods of time, the direction of orientation of the fields, transient characteristics, field coherence, or other factors. Some investigators (*vide infra*) believe that such factors are important in effects reported in *in vivo* or *in vitro* studies. Given the poor understanding of such effects, and the lack of clear dose-response relations for many of them, the relevance of such factors to human health hazards is presently unclear.

In spite of these caveats, the scaling considerations presented above have an important implication. If an effect from a time-dependent magnetic field in a biological system arises from induced electric fields, any comparison between human exposure and laboratory studies must take into account the effect of body size. Therefore, it would make sense under those circumstances to scale the field strength of human exposure by the ratio human body size: test animal size. This will result in many more reported effects of magnetic fields falling within or near the envelope for maglev exposure.

3.4 COMMON OCCUPATIONAL AND RESIDENTIAL EXPOSURES AT POWER FREQUENCIES

In a report based on EMDEX meter measurements, Kaune, *et al.* (1989) gave summary exposure statistics for workers in various job-title categories. Parametric tests indicated that there were substantial differences in work-day exposure among the six job categories, with workers in power plants on standby status having the lowest (geometric means of the median and 90th percentile being 0.1 μT (1.0 mG) and 0.31 μT (3.1 mG), respectively), and transmission linemen the highest (geometric means of median and 90th percentile, 0.54 μT (5.4 mG) and 6.17 μT (61.7 mG), respectively) exposure. Bowman *et al.* (1988) have taken the approach of measuring ELF (below 100 Hz) electric and magnetic field intensities at 114 work sites of classes of workers identified in epidemiologic studies as being at higher risk for leukemia, presumably due to EMF exposure. In all work environments, the geometric means of the magnetic field exposures were higher than mean residential exposures, with the exception of radio dispatchers and a few specific environments of electronics engineers, technicians, and assemblers. Measurements ranged from 0.3 x 10^{-7} T for radio operators to 103.1 x 10^{-7} T for electricians -- a 343-fold difference. The geometric means within the electronic assembler category varied 810-fold among the different environments. Forklift operators, an occupational group not previously identified as an "electrical" occupation in epidemiologic studies, were exposed to the highest magnetic fields, ranging from 54 to 109 x 10^{-7} T while in steady motion, with surges up to 1,250 x 10^{-7} T when accelerating.

Estimates of residential exposure have been attempted, and generally they also indicate substantial variation in individual exposures within groups of individuals identified as "exposed." Power-frequency (50 Hz) magnetic field assessments of the residential environment, using small light-weight personal exposure monitors developed by the Institut de Recherche d'Hydro-Quebec (IREQ), were being conducted by National Grid Research and Development Centre, Central Electricity Generating Board, UK, and were reported by Renew, Male, and Maddock (1990). Typical daily variations in field intensity were significant: mean exposure for mobile "at home" readings (108 mA/m) exceeded mean static readings by 65 mA/m, indicating a significant contribution from appliances and localized fields; the range of variation in average magnetic fields in different homes was considerable (5-97 mA/m), and was significantly greater when a high voltage line was located within 100 m (16-327 mA/m). The range of average "at work" exposures was even larger (45-7073 mA/m).

3.5 IDENTIFICATION OF BIOLOGICAL HAZARDS DUE TO MAGLEV

The exposure characteristics of the TR07 maglev vehicle on the whole are similar to fields from appliances and other common sources of 60 Hz magnetic fields. They differ from the latter in that (a) the frequency spectrum is more complex and variable, extending up to several hundred Hz, and (b) the fields are highly variable with time, reflecting motion of the vehicle.

Any discussion of the health significance of these fields must refer to specific hazards. Well known hazards of electromagnetic fields below the kilohertz frequency range include shock and burn. However, these require very strong fields (many times greater than those associated with the maglev) or direct contact of a subject with a charged conductor. The fields associated with the maglev (as well as other common sources of ELF magnetic fields) are well below the threshold for such recognized hazards. They are also well below limits set by several international and national exposure standards for low-frequency magnetic fields.

There is, however, considerable public and regulatory concern about possible hazards from comparatively low level ELF magnetic fields, mostly those associated with the electrical distribution system and appliances. This concern will undoubtedly extend to magnetic fields associated with maglev systems. Most of this concern results from epidemiologic studies that reported increases in cancer risk associated with exposure to electromagnetic fields, whether measured or deduced from surrogate measures of field exposure, typically at levels commonly found in the home or workplace. However, as has often been pointed out by regulatory agencies and scientists, there is no scientific consensus that the epidemiologic evidence reflects a cause-effect relation between health and field exposure; or if it does, what parameters of exposure are important.

Given this situation, there is no basis to predict any adverse health consequences of the electric or magnetic fields associated with the maglev. Certainly, the fields associated with the maglev are far smaller than those that are clearly identified as hazardous with respect to shock, burns, etc. There is some uncertainty with regard to effects of low-level, long-term exposure and possible hazards such as increased risk of cancer. Based on the limited experimental information available, there is no basis to conclude that the complex frequency and time dependence of the maglev fields necessarily represents a particular hazard.

On the other hand, few standardized screening studies have been performed using the particular exposure characteristics of the maglev (or of other rail systems, for that matter). To the extent that the magnetic field exposure from a maglev vehicle (or other trains) is comparable to that from power lines and appliances, this issue is clearly linked with that of possible health effects of power line fields.

In the following sections that describe reported bioeffects of EMFs, we will compare the anticipated magnetic-field exposure in the passenger compartment of the TR07 Maglev to field conditions at which the biological effects of time-varying magnetic fields have been reported. This might yield insight into potential interactions of maglev fields with biological systems.

4. CALCIUM FLUX IN ELF EMF FIELDS

Much of the initial research indicating that ELF EMF and ELF-modulated high frequency EMF could modulate the movement of ions in cells and tissues is based on concepts developed in the field of electrophysiology. This is not surprising since two of the key laboratory directors in this area, W. R. Adey and J. M. R. Delgado, spent substantial segments of their careers doing research in neurophysiology. Adey's expectation that brain tissue would be sensitive to certain weak oscillating electromagnetic fields was based on an understanding of endogenous electrical signals in the brain (as expressed in the electroencephalogram) and the intuitive feeling that the response to exogenous fields must reflect normal physiological processes (Adey 1978). The initial results of calcium flux experiments in isolated cat and chicken brain preparations by Bawin and Adey were interpreted in terms of coherent responses to specific signals (which may mimic to some degree endogenous signals), and therefore EMF was seen to act by providing a "triggering event" rather than acting as a motive force for the reported ion flux changes (Bawin, Sheppard, and Adey 1977). Arguments against the possibility that weak oscillating fields can have any biological effects based on assessing the total energy involved in relation to random thermal energy (Adair 1991) or endogenous electrical signals from muscle and nerve, (Wachtel 1988) do not address a basic premise that underlies much of the work discussed in the following sections of this report: the expectation that signal specificity (information content) rather than the total energy input may be the critical factor in the interaction with cells and tissues. Calcium flux experiments constitute the largest body of experimental evidence in the literature for the existence of frequency/intensity specific effects of ELF EMF in biological systems.

4.1 CALCIUM FLUX IN ISOLATED CHICKEN BRAIN TISSUE

Since the maintenance and modulation of electric potentials across membranes are essential for cell viability and function, anything that perturbs membrane charges has the potential for profoundly altering cellular metabolism. Oscillating low frequency EMFs might be thought capable of such action. Ionic gradients are major elements in maintaining membrane potentials, and calcium flux across membranes has been a particularly active field of study, in view of the importance of this ion.

A series of experiments by Bawin and Adey demonstrated that weak sinusoidal electric fields can selectively inhibit calcium efflux from freshly isolated chicken brain hemispheres and cat cerebral cortex tissue samples from the visual, auditory, somato-sensory, and suprasylvian areas. These studies were based on the observation that there are weak oscillating electric gradients, on the order of 1-20 mV/cm, which occur spontaneously in brain tissue (as revealed in the electroencephalogram), and the expectation that the application of similar external weak fields might produce biological responses in this system (Bawin and Adey 1976; Adey and Bawin 1982). Most of the work was done with ELF-modulated radiofrequency EMF rather than ELF EMF directly, because a low frequency electric field does not penetrate very deeply into tissue (Bawin and Adey 1977; Sheppard, Bawin, and Adey 1979). Other experiments, however, obtained similar results with 50-1000 mV/cm ELF electric fields at 175 Hz applied using a pair of 1-m^2 parallel metal plate electrodes (Bawin and Adey 1975; Bawin and Adey 1976). More recently, Blackman has also demonstrated that results which are essentially identical to those obtained with ELF-modulated radiofrequency fields can be obtained in the chicken brain system with ELF fields (Blackman 1989).

In a typical experiment, freshly prepared neonatal chicken brain hemispheres were incubated at 36°C for 30 min in physiological glucose-saline containing $^{45}Ca^{++}$, washed repeatedly to remove unbound radioactive calcium, then exposed or sham-exposed for 20 min to an electric field. At the conclusion of the exposure period, samples of the bathing solution and brain tissue were assayed for radioactivity, and exposed samples compared with the corresponding sham-exposed controls. Results repeatedly indicated maximal efflux to be associated with specific frequency/intensity "windows." Calcium efflux from isolated chick brain exposed for 20 min to 16 Hz sinusoidally-modulated radiofrequency radiation (450 MHz) was increased by about 10% at power levels of 0.1 and 1.0 mW/cm^2. However, calcium efflux was not significantly changed at a lower power level of 0.05 mW/cm^2 or at higher power levels of 2.0 and 5.0 mW/cm^2 (Sheppard, Bawin, and Adey 1979). The response was characteristic of nervous tissue: no changes in calcium efflux were seen in chick muscles exposed to 6 and 16-Hz modulation frequencies. Experiments with chicken and cat brain tissues demonstrated enhanced calcium efflux following exposures to fields of 10 and 56 V/m at frequencies of 6 and 16 Hz, while other frequencies tested (1, 32, and 75 Hz) and other field amplitudes (1, 5, and 100 V/m) did not induce changes in calcium efflux (Bawin and Adey 1977).

The results of these studies have been supported by an extensive series of experiments conducted on chicken brain preparations by Blackman *et al.* which further refined the concept of "window effects:" biological effects produced only at specific and fairly narrow combinations of frequency and intensity, a type of action spectrum suggestive of a resonance mechanism (Blackman *et al.* 1977, 1982; Blackman, Benane, and House 1985; Blackman *et al.* 1985, 1989b; Blackman 1989; Adey 1988b).

Blackman and coworkers confirmed a window response for a narrower range of modulation frequencies, indicated that only a narrow range of intensities were effective, and suggested that effective frequency/intensity combinations were related to the dielectric constant of the tissues at each carrier frequency (Blackman 1988). In one series of experiments, intensity window results were reported for 50 MHz radiofrequency (RF) irradiation amplitude modulated at 16 Hz. A power density series was tested with results as follows: Ca^{++} efflux was enhanced at 1.75, 3.85, 5.57, 6.82, 7.65, 7.77, and 8.82 mW/cm^2; no change in efflux was noted at 0.75, 2.37, 4.50, 5.85, 7.08, 8.19, 8.66, 10.6, and 14.7 mW/cm^2. Repeat trials were conducted several months later at 3.85, 5.57, 6.82, and 7.77 mW/cm^2: these trials replicated the initial results (Blackman *et al.* 1989b).

Blackman's group has also reported a multiple frequency-windowed response to extremely low levels of ELF EMF in patterns that are consistent with his modulated radiofrequency results obtained at much higher field energy levels. In one series of experiments, Blackman reported a peak of calcium efflux in a 16 Hz field with a peak to peak voltage (Vpp) of 5 Vpp/m, corresponding to a magnetic field intensity of 8 nT (0.08 mG) (Blackman 1989). In other experiments, Blackman reported peaks in the efflux of radioactive calcium from brain tissues exposed for 20 min at 37°C in a custom-built transmission line to sinusoidal fields between 1 and

60 Hz, at electric field intensities between 8 and 32 uV(rms)/m (in air) and magnetic-field flux densities between 36.0 and 144.2 fT (i.e., 0.360 and 1.44 x 10(-9) G) (Blackman *et al.* 1989a). It should be noted that these results are obtained using extremely tight control of the experimental variables (for factors which have been identified in over a decade of experimental work), and each exposed brain hemisphere is paired with its contralateral half as the sham-exposed control. The magnitude of the calcium efflux changes are very small and variation between brains often exceeds the variation between experimental and controls. These extremely low level field results have not, to our knowledge, been replicated in other laboratories.

In his more recent reports, Blackman has described a complex series of frequency and intensity windows in two groupings with ELF EMF exposures. In experiments performed with 16 Hz ELF fields (peak to peak voltage (Vpp) varied from 1 to 70 V, corresponding to a magnetic component of 1.6 nT/Vpp/m), peaks of calcium efflux centered around 5, 6.5, and 7.5 Vpp/m, with null results at 1, 2, and 10-30 Vpp/m (Blackman 1989). Extending the dose rate further revealed a second intensity "window" of activity: a peak extending through 35, 40, 45, and 50 Vpp/m, followed by null results at 55, 60, and 70 Vpp/m. When the experiment was extended over a range of frequencies, in addition to the peak to peak voltage variation, distinct frequency peaks were seen at harmonics of 15 Hz, until at n=8 (120 Hz) the effect disappeared. The experiment was carried on to 510 Hz using a 15 V/m (42.5 Vpp/m) field, and significant effects were seen at all odd multiples of 15 Hz, with a notable exception at 165 Hz; the response at 135 Hz was strongly positive (Blackman 1989).

An apparent inconsistency between the results of Bawin and Adey, and Blackman has caused some to question the validity of the reported window responses. Blackman has reported that similar specific frequency and intensity combinations were effective in altering calcium flux, interspersed with combinations that were not. However, the direction of the change Blackman reported was opposite that reported by Bawin and Adey (Bawin reported decreased flux from chicken brain preparations whereas Blackman reported enhanced flux). Blackman *et al.* have published results which established a series of experimental conditions to explain, at least in part, some of the anomalous results. Using an extremely weak ELF EMF exposure (16 Hz sinusoidal field, at 14.1 V rms/m (in air), and 0.064 μT rms), Blackman reported that it is possible to obtain inhibition or enhancement of the release of calcium ions, or a null result, depending upon the thermal condition of the samples prior to and during exposure. When the sample temperature was ascending during the 20 min exposure to a final temperature of either 35, 36, or 37, but not 38 or 39°C, enhanced release was observed; when stable during exposure (i.e., within $\pm$ 0.3 C) to a final temperature of 36 or 37, but not 35 or 38°C, the release was reduced; and when descending to any final temperature between 35 to 38°C, a null result occurred (Blackman, Benane, and House 1990).

The influence of the local geomagnetic field (LGF) on the response was also evaluated as a factor in calcium efflux experiments. By using Helmholtz coils to reduce or increase the DC field to fractions or multiples of the measured LGF orthogonal to the horizontal plane containing the oscillating electric and magnetic fields (38 μT), it was possible to block a positive result at 15 Hz or cause a null value at 30 Hz to be positive. When a frequency of 315 Hz was used, complete nullification of the LGF eliminated the positive calcium efflux seen earlier. Calcium efflux was found to be dependent on the ambient DC field: increased efflux was only obtained when the DC magnetic field was in the normal orientation or perpendicular to the oscillating magnetic field. Because the LGF is the earth's field distorted by ferromagnetic components of the building, the LGF may vary considerably in different locations in a room as well as between different laboratories. This may be a significant factor in the failure to replicate these and other experiments dependent on calcium efflux (Blackman 1989; 1990).

Other variables are likely to be of biological origin. Most important of these is the existence of several types of calcium channels, associated with different biological functions, and very importantly, affected by different sets of inhibitors; EMF also would not be expected necessarily to affect each type of channel in the same way. A very subtle difference that has been reported is that tissue from the right and left sides of the chicken brain appear to differ in their endogenous rates of calcium efflux (Brand *et al.* 1986); this too may stem from differences in calcium channel distribution.

Blackman has also reported experiments which indicate that the history of EMF exposure during development can have an effect on responsiveness of the chicken brain tissue to imposed ELF EMF. Eggs were incubated in incubators with applied 10 V/m fields at 50 Hz or 60 Hz. Brain tissue from chickens hatched from eggs incubated at 60 Hz gave a null result at 60 Hz when tested 1.5 days after hatching, but a statistically significant efflux for 50 Hz fields. In contrast, when eggs were incubated at 50 Hz, a null result was obtained for both 50 and 60 Hz exposures (Blackman *et al.* 1986; 1988).

The existence of frequency/intensity specific responses to low levels of EMF is controversial, as some efforts to replicate chicken brain experiments have been unsuccessful (Brand *et al.* 1986; Bellossi 1986). The chicken brain system is recognized by Blackman as a model system which presents some formidable technical difficulties. The responses of isolated (and dying) brain tissue are also of questionable physiological significance, although Adey's group has obtained some parallel results with cat brain preparations *in situ*. Many of the experiments were performed using amplitude-modulated radiofrequency fields (Adey 1988a), and it is unclear whether these effects reflect the biological action of the high frequency carrier wave or of the low frequency amplitude-modulated wave. Results favor the interpretation that it is the modulation frequency which is important since unmodulated carrier frequencies had no effect in this experimental system, and sinusoidal electric fields at low frequency produced effects similar to ELF-modulated radiofrequency fields in experiments by both Bawin and Blackman (Blackman 1988). Results with the chicken brain are supported to some extent by evidence of similar frequency and intensity windows in the calcium flux response of other tissues and isolated cells, discussed in the following sections of this report.

4.2 CALCIUM FLUX IN OTHER TISSUES

There are a number of other studies in the literature reporting effects of EMF on ion flux in tissues other than brain. Studies have been carried out in the frog heart, another electrically-responsive tissue (Schwartz, House, and Mealing 1990). In this study it was reported that 240 kHz continuous wave, or the same wave modulated at an ELF of 0.5 Hz, in synchrony with electric stimulation of the heart had no effect on calcium efflux. However, modulation at 16 Hz led to a significant increase in efflux, and there were distinct intensity windows at 0.15 and 0.3 mW/kg, with the latter window being extremely narrow (0.06 mW/kg on either side).

In a report from the Soviet literature, exposure of intact male Wistar rats to industrial frequency EMF (50 kV/m, 50 Hz, for 5 hr) was claimed to significantly reduce tissue calcium levels in the liver and thymus; and significantly increase levels in the kidneys, brain, hip muscle, tongue, testicles, and prostate. Magnesium levels were also reported to be significantly reduced in the liver and thymus; and significantly increased in the brain, hip muscles, tongue, testicles, and prostate (Dishlovoi *et al.* 1980). Albert *et al.* found that 147 MHz electromagnetic radiation, sinusoidally modulated at 16 Hz, caused an 11% increase in $^{45}Ca^{++}$ efflux from pancreatic tissue slices when compared to sham exposed samples; although there was no coincident effect on calcium-dependent release of pulse-labeled protein from either control or carbamylcholine stimulated tissue slices (Albert, Blackman, and Slaby 1980). This result underscores the difficulty in interpreting calcium flux responses to ELF EMF as an effect on normal physiologically integrated systems.

To assess the possibility that specific ionic resonances can influence bone development, organ cultures of 8-day chick femoral rudiments were exposed to combined 16 or 80 Hz, 20 μT peak sinusoidal and various static magnetic fields tuned to calcium, magnesium, potassium (16 Hz), and combined Ca/Mg (80 Hz) ion cyclotron resonances (ICR) for 30 min/day for 7 days. Tuning for Ca, Mg, or Ca/Mg was reported to increase rudiment length and mid-shaft diameter, diaphyseal collar length and mid-shaft thickness, and reduce the gross L/D and diaphyseal L/T ratios, indicating greater robustness. Tuning for K^+ produced exactly opposite results (Smith, McLeod, and Liboff 1991). Finally there is a negative report in the literature for a tissue expected to be particularly responsive to resonance conditions found to be effective in other systems. No response to resonant frequency/intensity combinations for a variety of ions (H^+, Li^+, Na^+, K^+, Mg^{++}, Ca^{++}, Cl^-) was obtained with turtle colon, a sodium-absorbing epithelium in which Na enters the cell across the apical membrane via Na-channels and exits the cell through an electrogenic pump which exchanges Na for K (Liboff, Parkinson, and Dawson 1988).

4.3 CALCIUM FLUX IN SINGLE CELL SYSTEMS

Over the past few years, a substantial number of reports have appeared in the literature reporting similar frequency/intensity specific effects of ELF EMF on calcium flux in single cell systems. These reports include work by Blackman and coworkers who exposed cells to the same fields found to produce calcium efflux in chicken brain preparations, obtaining similar results. In one such study, human neuroblastoma cells (IMR-32) were exposed to a 147-MHz carrier frequency 80% sinusoidally amplitude modulated at 16 Hz at specific absorption rates (SARs) of 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 W/kg. Enhancement of $^{45}Ca^{++}$ efflux occurred only at unique intensity windows, with statistically significant changes of 37% at 0.05 W/kg and 27% at 0.005 W/kg. Additional modulation frequencies were tested in the IMR-32 cells and modulation frequencies centered on 16 Hz, and at around 60 Hz, they caused enhanced efflux with 147-MHz carrier waves (as was true in a previous report with 915-MHz carrier waves). The authors concluded that the cell lines derived from tumors of the central nervous system respond to modulated RF fields in a manner identical to the normal forebrain-tissue preparations from newborn chickens (Dutta, Gosh, and Blackman 1989). In comparing results of chicken brain and cell experiments, Blackman concluded that frequency-specific effects on calcium flux show two general patterns: nonexcitable tissues like lymphocytes appear to respond to a broad range of frequencies from 16 to 100 Hz; while excitable tissue like brain hemispheres appear to respond to a broad band of frequencies ranging from 60 to 90 Hz, and also to narrower bands occurring at multiples of 15 Hz. These patterns are further modified by age, physiological and biochemical state, and species differences (Blackman 1988).

Liboff and coworkers have produced an extensive series of reports of calcium flux effects related to specific frequency/intensity combinations meeting ion cyclotron resonance (ICR) conditions for calcium and other ions (Liboff, McLeod, and Smith 1989). In this theoretical model, the rate of ion transport is increased by an external EMF when the field provides a resonant energy input to the helical movement of an ion through its membrane transport channel. Positive effects are linked to the ICR condition of $f_0 = (q/2\pi m)B_0$, where f_0 is the oscillating field frequency, q = ionic charge, m = ionic mass and B_0 = static magnetic strength. This model was developed as a unifying hypothetical mechanism to explain results of earlier studies in which the imposition of a weak DC magnetic field (below the intensity of the earth's magnetic field) was found to influence the effect of a 60-Hz magnetic field on operant behavior in rats (Thomas, Schrot, and Liboff 1986) and on ^{3}H-thymidine incorporation in fibroblasts (Liboff *et al.* 1984). For the past few years, ICR theory has been a dominant model in many attempts to find frequency/intensity specific responses and explain the effect of the magnitude and direction of a DC or geomagnetic field in different biological systems.

The motility of the marine diatom *Amphora coffeaeformis* depends on the calcium ion concentration, [Ca^{++}], in the surrounding medium, and this system has been employed as a biological assay to test for EMF conditions that enhance Ca^{++} influx. Liboff, McLeod and coworkers examined diatom mobility as they varied static field conditions around a set of ICR conditions for Ca^{++} (a magnetostatic field of 0 vertical and 20.9 μT horizontal flux density, in an AC frequency of 16 Hz at a peak flux density of 20.9 μT). They reported that at resonance there was a sharp reduction in the [Ca^{++}] threshold so that diatom mobility was enhanced 5-fold in 0.25 mM Ca^{++} medium. At 20.9 μT, the resonance peak around 16 Hz was quite sharp, dropping off significantly at 14 and 18 Hz. When the frequency and magnetic field flux density were doubled, keeping the same ratio, a second peak appeared at 41.8 μT, 32 Hz as predicted by ICR theory. Response to harmonics of the fundamental resonance frequency at [Ca^{++}] of 0.25 and 0.5 mM showed significant stimulation of mobility at odd harmonics (16 Hz and 48 Hz), while even harmonics (32 Hz and 64 Hz) did not differ from control levels (Smith *et al.* 1987; McLeod *et al.* 1987).

Although the diatom system has been advanced as strong evidence for ICR, reports in the literature have not been uniformly positive. A group at Battelle Laboratories attempted to replicate the diatom experiments and found that the mobility of diatoms in low calcium medium was extremely variable (at 0.25 mM, 27.0 $\pm$ 15.4% for exposed; 13.2 $\pm$ 15.4% for sham-exposed cultures). They concluded that the effect was real (a conclusion supported by a consistent trend in the results and some statistically significant differences between exposed and control cultures), but that high variability between preparations in the number of cells that move, makes the culture method insufficiently reproducible for examination of frequency-dependent responses to extremely low frequency electromagnetic fields (Reese *et al.* 1991). Another group, attempting to replicate the diatom mobility effect, exposed over 400 cultures to various EMF conditions around cyclotron resonance conditions, and reported no statistically significant difference between exposed and control plates for any of the field conditions or for any calcium concentration (Parkinson and Sulik 1990; 1992).

In experiments with lymphocytes, Liboff *et al.* reported that $^{45}Ca^{++}$ incorporation varied in a resonant manner; and with the maximum occurring precisely at a specific frequency (14.3 Hz) that is downshifted from the frequency maximum in the diatom experiments (16.0 Hz) by the inverse ratio of the $^{45}Ca^{++}$ ion to the ordinary Ca^{++}-ion mass (44.95:40.08). They interpreted this close agreement between observed and calculated resonance frequencies as very strong evidence for an ICR mechanism. They also reported amplitude window results which showed a linear response up to a few tens of microtesla, then falling off sharply at higher field intensities (Liboff *et al.* 1987a; 1987b).

Grande *et al.* investigated the effects of ICR field combinations on bovine articular chondrocytes *in vitro*. Labelled calcium uptake increased significantly ($p<0.001$) after 30 min of exposure to a Ca^{++}-tuned signal, but declined to levels not significantly different from controls thereafter. By 48 h after exposure, thymidine and sulfate incorporation increased and then declined at 72 h to levels not significantly different from controls. In the presence of a Mg^{++}-tuned signal, thymidine incorporation was significantly increased in exposed cells at all time intervals, but sulfate incorporation was significantly increased only at the 48 h and 72 h timepoints. Tuning of exposures to stimulate K^{+} transport was not accompanied by significant increases in thymidine or sulfate incorporation at any of the time intervals examined (Grande *et al.* 1991). Somewhat different biological responses to K^{+}-tuned fields were reported by Liboff *et al.* in experiments with the human lymphoma line HSB-2 (ATCC CC1 120.1). There was an increase in proliferation relative to controls (mean ratio 1.36) when cells were exposed to ICR conditions corresponding to the third harmonic for the K^{+} ion at 60 Hz; and when the exposure frequency was changed from 60 Hz to 16 Hz, the DC field for maximum proliferation shifted from 51.1 μT to 40.8 μT, corresponding to the predicted first harmonic at 16 Hz (Liboff, Jenrow, and McLeod 1992).

In an independent test of the ICR hypothesis, calcium uptake was monitored in normal and malignant murine lymphocytes exposed to a combined weak ELF magnetic field and DC magnetic field. Calcium-ion uptake by normal and leukemia lymphocytes was significantly increased by a 30 min exposure to a 13.6 Hz, 20 μT peak sinusoidal magnetic field. In contrast, there was no effect when the same field was applied for 30 min immediately before -- as opposed to during -- introduction of calcium ions (Lyle *et al.* 1991).

Calcium flux responses to 60-Hz EMF in lymphocytes have also been independently reported by Liburdy and Walleczek, who also identified a biological factor influencing their responsiveness (Walleczek and Liburdy 1990b). Concanavalin-A (con-A) activated rat thymocytes exposed to an ELF magnetic field (60 Hz, 1.0 mV/cm peak induced electric field) showed a 2.7-fold ($p<0.05$) increase in $^{45}Ca^{++}$ net transport across the cell membrane, while $^{45}Ca^{++}$ flux remained unaltered during field exposure of quiescent, non-activated thymocytes (Walleczek and Liburdy 1990a). Lyle *et al.* also reported a 3-fold increase in $^{45}Ca^{++}$ transport in normal rat spleen lymphocytes activated for 24 h with con-A. They were also able to obtain a similar increased response by pretreating cells with 0.01 mM to 1.0 mM ouabain, which suggests that the field might not be affecting calcium channels directly, but may be increasing calcium by indirect mechanisms (Lyle *et al.* 1990). Later work confirmed EMF enhancement of calcium transport in con-A simulated lymphocytes, and showed that an opposite effect could be obtained when the DC field component was removed. A co-linear AC/DC magnetic field was established with nulled orthogonal static magnetic field components. Con-A triggered a 175% increase in calcium influx in the absence of the AC/DC field combination. When AC/DC fields were present with con-A, no increase in calcium influx was detected, indicating that signal transduction was inhibited (Liburdy, Yost, and de Manincor 1991).

Other sources of biological variability may also complicate results reported in the literature. In a study involving exposure of unstimulated human peripheral mononuclear cells to a 50 Hz, 0.1 mT sinusoidal field, an increase in cytosolic free calcium occurred in only 10% of the total cells: the remainder were unaffected (Lindstrom *et al.* 1992). This subpopulation effect has also been discussed by Weaver, Barnett, and Astumian (1990). Culture methodology can also have a profound effect on the response of cells, especially lymphocytes. These considerations are discussed in some detail in the context of immunological responses of cells (Section 6.4 of this report). It is clear from reported differences in the response of activated lymphocytes to ELF EMF, as discussed above, that any culture procedure which alters immunoreactivity will also have a strong effect on ion flux responses.

Lyle and coworkers have begun to address the nature of the ^{45}Ca which is associated with cells; this includes both intracellular isotope and that which is bound to the membrane (Lyle *et al.* 1992), so that, in this context, calcium "uptake" represents two distinct processes. Using flow cytometric procedures to follow the uptake of a fluorescent dye (fluo-3) as a marker of intracellular calcium in Jurkat E6-1 human leukemic T-cells, these workers found no significant changes as a result of exposure to 60 Hz, 1 mV/cm electric field; suggesting that transmembrane calcium signalling was unaffected by this field. However, some caution is needed in interpreting these findings, since Liburdy (1992) has reported data which suggest that 60 Hz fields affect primarily the plateau phase of calcium signalling; whereas the early rise in intracellular calcium represents mainly the release from internal stores, a process which is not as sensitive. It should be noted in this context that changes in calcium flux also have been reported for intracellular structures such as isolated sarcoplasmic reticulum (Surgalla 1988), or microsomal membranes (Stagg *et al.* 1992) exposed to EMFs.

In contrast to the series of reports of resonance effects in various cell types, there are a number of negative reports in the literature. Parkinson and Hanks (1989) monitored intracellular calcium ion concentrations with a fluorescent dye (fura2) in BALB/c3T3 (clone A31), L929 (NCTC clone 929), ROS (rat osteosarcoma, clone 17/2.8), and V-79 (Chinese hamster lung fibroblast) cells exposed to ICR and nonresonant conditions, and found no indication of an ICR effect. Another group, attempting to replicate the results reported for lymphocytes by Liboff *et al.*, found no evidence of a resonance effect (Prasad *et al.* 1991). Hojevik *et al.* (1991) measured total current of Ca^{++} ions through patch-clamped cell membranes while exposing clonal insulin-producing b-cells (RINm5F) in an ICR field and found no effect on the transport of Ca^{++} ions through the protein channels of the plasma membrane.

4.4 EVIDENCE FOR FREQUENCY/INTENSITY SPECIFIC EFFECTS

The results of experiments with tissues and cells discussed in the previous two sections of this report provide a substantial, but not totally consistent, body of evidence supporting the existence of nonlinear responses to ELF EMF in a variety of biological systems. Theoretical unifying constructs, such as the ICR hypothesis, have motivated much of the experimental work, but the validity of observations of nonlinear responses are not necessarily tied to the validity of any theoretical framework. At the present time, the ICR mechanism seems unlikely for reasons which will be discussed in the final section of this report. The reasons for contradictory experimental observations and failures to replicate reports of extremely low level time-varying and DC magnetic field effects still need to be explained.

Stimulation of both efflux and influx by ELF EMF in similar experimental situations may not be contradictory results, but could reflect a common mechanism of enhanced ion movement. A number of investigators have described models that may explain how ELF EMF may alter calcium fluxes (Caratozzolo *et al.* 1984; Blank 1987; Findl 1987; Liboff *et al.* 1987a; McLeod and Liboff 1987; Adey 1988b). While a model such as ICR may present fundamental theoretical problems (Sandweiss 1990), there are special factors involved in the organization of cells into living tissue (McLeod *et al.* 1992), and the presence of magnetite in some tissues (Kirschvink and Kobayashi-Kirschvink 1992), which might alter the physical attributes underlying theoretical calculations, and thus, the sensitivity to EMFs, by orders of magnitude. Apart from potential changes in signal transduction, other reports tie responses to weak intensity ELF EMF to biochemical processes, such as the increased glycosylation of plasma membrane protein that has been described in human fibroblasts and rat osteosarcoma cell lines (Fisher, Dulling, and Smith 1986), or altered extracellular glycosaminoglycans in chicken embryo tissues (Trillo *et al.* 1990).

In summary, there appear to be three basic problems in accepting the calcium flux data as "real:" (1) effects are reported in similar systems which are opposite in direction (e.g., increased efflux is reported by one group, decreased efflux by another), (2) effects are not readily replicated by independent laboratories, and (3) effects have been reported at extremely low field intensities, not obviously different from ambient field levels or other forms of noise. There are experimental results to explain the first two points, at least for certain specific cases, in terms of biological or uncontrolled experimental variables. As noted here and in other sections of this report, many of the experimental systems used for EMF research are nonstandard and difficult to work with, and in most cases not enough careful work has been done by independent laboratories to clearly establish a body of work as replicated or not. The third point has been argued in theoretical terms both ways. There are a series of theoretical arguments to explain why field intensity calculations underestimate true field levels at the cell membrane level when cells are arranged in tissues, and how cells may respond to extremely weak signals as informational stimuli rather than energy inputs. Similarly, there are physical arguments which explain why cells should be unable to distinguish weak ELF EMF signals reported to produce bioeffects, from thermal noise. With such a large body of reports of nonlinear responses to ELF EMF in the literature, it seems likely that some real biological response(s) underlie these observations, but their physiological significance is not obvious. At this point it does not seem possible to reach a more definite conclusion based on the information in the literature: a substantial and systematic effort is needed to replicate and define the effective parameters of at least one biological response in order to resolve this question.

4.5 GRAPHIC ANALYSIS OF CALCIUM-RELATED EFFECTS IN TERMS OF TR07 FIELDS

Figure 4-1 summarizes a variety of reported effects of time-varying magnetic fields on calcium transport.

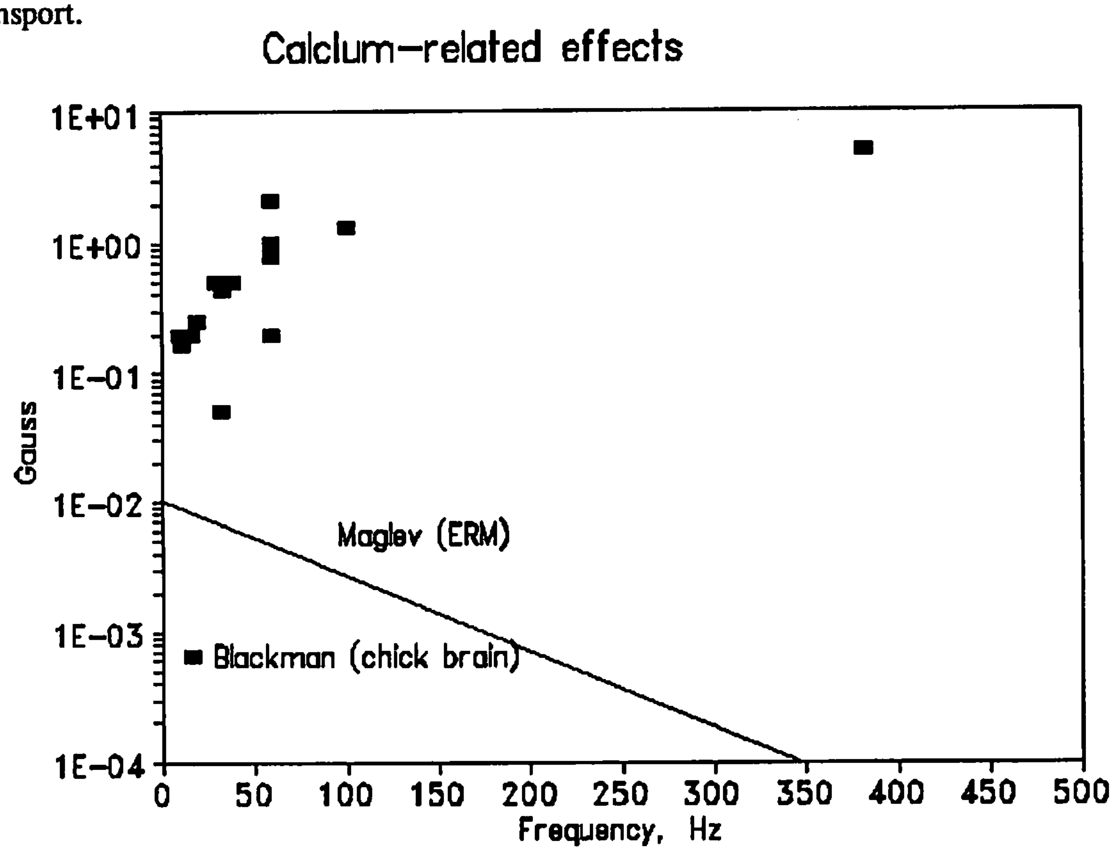

FIGURE 4-1. SUMMARY OF REPORTED EFFECTS OF ALTERNATING MAGNETIC FIELDS ON CALCIUM TRANSPORT IN BIOLOGICAL SYSTEMS, IN COMPARISON TO THE "ENVELOPE" OF EXPOSURE FOR THE TR07 MAGLEV.

Most of these studies fall into one of two groups: studies of "calcium efflux" from excised brain tissue (Blackman), and studies related to Liboff's "cyclotron resonance" hypothesis. Several of the reported effects by Blackman's group fall well within the envelope corresponding to TR07 magnetic field exposures.

These studies are problematic in their interpretation for several reasons. Both Liboff *et al.* and Blackman *et al.* consider the effects as resulting from magnetic fields (rather than induced electric fields). However, these reported effects occurred under diverse combinations of amplitude and frequency conditions, and their dose-response functions are both complex and poorly understood. Many of the studies cited in the figure employed limited ranges of magnetic field intensity and only one or a few frequencies.

As discussed in earlier sections, Liboff's "cyclotron resonance" hypothesis has been criticized on technical grounds (Halle 1988), and no longer enjoys widespread acceptance, although other theoretical models invoking alternate resonance mechanisms have been proposed. Moreover, attempts by other investigators to independently confirm some of Liboff's results have been unsuccessful (Prasad *et al.* 1991).

5. TERATOLOGICAL EFFECTS

A number of studies have investigated the teratogenic potential of ELF EMF, primarily using chicken embryos or mammalian fetuses exposed *in utero*. The results of these studies are strikingly variable: many report little or no effect of field exposure while apparently replicate experiments report a significant teratogenic effect. This inconsistency has been interpreted either as random biological variation (noise), or as a valid teratogenic effect which is strongly dependent on specific field or biological characteristics that are not adequately understood or controlled. In the following sections, this literature will be reviewed and evaluated to reach some tentative conclusions regarding the teratogenic potential of maglev type fields.

5.1 MAMMALIAN STUDIES

A limited number of chronic exposure studies have been done with mammals using morphology and early postnatal behavior to study first or second generation offspring raised in high electric fields. The results of these studies, which mostly employed kilovolt strength electric fields with no magnetic field component, have been predominantly negative. Relatively large scale studies with rats demonstrated no significant effects on reproductive or developmental parameters (Cerretelli *et al.* 1979; Seto *et al.* 1984; Sikov *et al.* 1984; Rommereim *et al.* 1987; 1988; 1990), while another study conducted with Hanford miniature swine showed a marginally significant increase in the number of musculoskeletal malformations in the offspring of exposed sows (Sikov *et al.* 1987). Tests of neuromuscular function in the apparently normal exposed F2 progeny from an earlier replicate of this experiment failed to demonstrate any functional differences (Lovely, Creim, and Phillips 1982). A small study of rats exposed prenatally and postnatally to 60-Hz electric and magnetic fields found no significant differences in cerebellar and cerebral development associated with exposure (Gona and Yu 1987). Sienkiewicz, Robbins, and Saunders (1991) exposed pregnant CD1 mice to a power frequency magnetic field (20 mT, 50 Hz) and observed some statistically significant but minor behavioral differences in exposed animals in their air righting reflex and Rota-rod performance (measures of balance and coordination). These studies were generally on too small a scale and populations were not followed long enough to evaluate more subtle effects like a small increase in cancer rate.

A number of studies have reported slightly decreased growth rates in animals exposed prenatally or perinatally to EMF. A study of young rhesus monkeys from 1 to 54 months of age, chronically exposed to ELF electric and magnetic fields, demonstrated a significantly lower body weight and higher testosterone levels in exposed males compared with the control group. However, no difference in weight or steroid hormone concentrations in the females was apparent (Lotz and Saxton 1984). Small but significant decreases in maternal body weight and lower rates of weight increase in male (but not female) pups were reported for exposed rats, and were interpreted as a response to chronic stress (Sikov *et al.* 1988). Offspring of gravid laboratory rabbits, chronically exposed to a strong 50 Hz electric field during gestation and/or during the first six weeks postpartum, were found to have small endocrine differences associated with a mild stress response (Portet and Cabanes 1988). Mild stress effects, discussed more fully in a later section of this report, may be a confounding factor in teratogenesis studies.

In contrast to the largely negative results with sinusoidal AC electric fields, two studies conducted in Sweden have suggested a teratogenic effect associated with pulsed magnetic fields of the type produced by video display terminals (VDTs). Interest in this area was stimulated by reports of clusters of abnormal pregnancies and miscarriages among VDT operators (discussed in the following section), and field characteristics were chosen to simulate this exposure. A group from the Swedish Agricultural University reported a significant increase in the frequency of placental resorptions in C3H mice exposed to a 20-kHz sawtooth pulsed magnetic field with a peak field strength of 15 μT, suggesting an effect early in development (Frolen *et al.* 1987); while investigators from the Karolinska Institute, using the same strain of mice and apparently identical exposure conditions, found a significant increase in external malformations with no increase in resorptions or mortality (Tribukait, Cekan, and Paulsson 1987). Frolen's results have been confirmed recently by one group (Svedenstal and Paulsson 1992), but others have been unable to replicate the results or have obtained results which suggest significant differences in the reported patterns of abnormalities.

A recent report indicated that sawtooth waveform pulsed EMF with a peak-to-peak flux density of 40 μT enhanced the incidence of cleft palate and/or cleft lip induced in mice by cytosine arabinoside (Chiang *et al.* 1992a). In Tribukait's experiments, mice exposed to 1- or 15-μT, 100 Hz rectangular pulsed magnetic fields (rather than the sawtooth waveform) showed no change in the frequency of malformations (Tribukait, Cekan, and Paulsson 1987). However, the importance of pulse shape in producing teratologic effects is not clear. A teratological assessment of rats exposed to similar sawtooth pulsed magnetic fields, performed in Canada, indicated no significant change in resorptions or the frequency of abnormalities (Stuchly *et al.* 1988). Similar lack of teratologic effects were seen in studies which involved exposing pregnant rats to a homogeneous 50 Hz field with a 15 mT magnetic flux density (Brinkmann *et al.* 1988); or pregnant mice to a 50 Hz, 20 mT sinusoidal magnetic field (Kowalczuk, Robbins, and Saunders 1991); or to exposures which attempted to more or less replicate the teratologic effects reported by Frolen *et al.* (Juutilainen, Huuskonen *et al.* 1992; Wiley, Corey *et al.* 1992). Because there are (as yet unpublished) experiments confirming the initial reports from the Swedish groups, Juutilainen and others are still trying to independently replicate the results and/or determine the reasons for failure to replicate the initial observations.

5.2 ELECTRIC BLANKET STUDIES

Electric blankets represent a relatively common source of high-level magnetic field exposure at power frequencies because of the close proximity of the heating wires to the body when the blanket is in use (Florig and Hoburg 1990; Casamento, Crowl, and Bassen 1992). A number of studies, which are discussed in other sections of this report, have been conducted to assess the effect of this exposure on circadian rhythms and pineal function, and on the incidence of certain forms of cancer based on theoretical considerations developed by Stevens and Davis (1987). Discussion in this section concerns studies which suggest that exposure to electric blankets and similar power-frequency EMFs may contribute to increased rates of fetal abnormalities or an increase in abortion incidence.

Wertheimer and Leeper reported results of two small-scale retrospective epidemiologic analyses that suggested a correlation between increased fetal loss and conception at a season when there is increasing use of electrically heated beds (Wertheimer and Leeper 1986) or ceiling cable electric heating (Wertheimer and Leeper 1989). They suggested that the results reflect an increased incidence of spontaneous abortion resulting from increased ELF magnetic field exposure at the earliest stages of development (first trimester).

Wertheimer and Leeper's hypothesis remained essentially untested by larger studies until recently. Dlugosz *et al.* (1990; 1992) analyzed electric blanket and heated waterbed use in relation to the risk of cleft palate, cleft lip, anencephalus, and spina bifida using data from the NY Congenital Malformations Registry. A total of 542 mothers of case infants were compared to mothers (1:1) selected at random from birth registrations that individually matched the case mothers on race, age, home country, month of last menses and child's sex. Relative to nonusers, conditional logistic relative risk estimates for women who used electric blankets or heated waterbeds were nearly identical and below unity (0.7-0.9). Adjustment for potential confounders did not materially alter these findings. It should be noted, however, that these malformations are not lethal, and may or may not occur in parallel with abnormalities which lead to spontaneous abortion.

Different results were obtained by Yong *et al.* (1992) who conducted a case-control study of pregnancy complications and electric blanket use with 986 cases (68% spontaneous abortions) and 975 controls from the Zhejiang Gynaecology and Obstetrics Hospital in China. The details of electric blanket usage, drug usage, occupational exposure to chemicals and noise, and other factors (a total of 45) during pregnancy were obtained from doctors or questionnaires in the mail. After statistical analysis, there was a significant increase in the odds ratios for spontaneous abortion and total abnormal pregnancy with electric blanket usage during early pregnancy: 1.95 (1.31-2.90 95% confidence interval (CI)) and 1.61 (1.10-2.34 CI), respectively. The odds ratios for electric blanket usage during middle or later pregnancy were not elevated.

Chiang *et al.* (1992a; 1992b) recently reported results of a small scale study in which pregnant mice were exposed to the 50 Hz field from an electric blanket, and showed significant decreases in the catecholamine and succinic dehydrogenase levels in specific regions of the developing brains of their offspring.

5.3 VDT STUDIES

Particular concern has been raised, especially in the popular press, by studies of workers using computer video display terminals (VDTs) and by animal studies involving analogous fields. VDTs produce a complex mixture of low-level EMF. A typical pattern of EMF emitted by a VDT unit includes components from the control circuitry (30 Hz to 120 MHz), the 60 Hz magnetic field used to power the electron beam (with harmonics and subharmonics at 15, 30, 120, 180 Hz), a pulsed magnetic field produced by the fly-back transformer (emissions in the

15-18 kHz frequency range and its harmonics, extending to the MHz range, generated as a pulse 8 μs in duration, once every 55-70 μs), and the two pairs of deflection coils for vertical line-by-line scanning (57 MHz with upper harmonics of up to 60 MHz) (Sharma 1987). Nonionizing radiation levels are generally comparable in intensity to many appliance and weak industrial exposures, and are well below guidelines for occupational exposure (Frankenhaeuser 1987).

Concern over possible health effects from VDTs was initiated in the early 1980s by a series of clusters of cases of reproductive problems reported in several groups of women who worked with VDTs (Foster 1986). These reports and subsequent epidemiologic and physical studies have been reviewed by Bergqvist (Bergqvist and Knave 1988; Bergqvist 1989), who attempted to define the VDT radiation risk with emphasis on adverse effects on pregnancy outcome. Although VDTs produce small amounts of ionizing radiation (soft X-ray and UV), the levels reaching the user are considered far too low to produce any mutagenic effect. Nonionizing radiation, primarily ELF magnetic fields, have been the primary focus of concern, although physical and laboratory studies have generally not indicated that there are mutagenic effects associated with such fields (with the exception of mouse studies noted above) (Frankenhaeuser 1987).

Most of the larger epidemiologic studies of populations exposed to VDTs have not demonstrated a statistically significant increased risk for adverse reproductive outcome with VDT exposure (Bergqvist and Knave 1988); with one notable exception. Goldhaber, Polen, and Hiatt (1988) used data from the Kaiser Permanente Medical Care Program (KPMCP) in Northern California to conduct a case-control study of early and late miscarriages among clerical and other workers using VDTs. A total of 9173 pregnancies were followed with pregnancy outcomes and estimated VDT exposures determined from medical records and questionnaire responses. The odds ratio (OR) for risk of miscarriage was significantly elevated for women working with a VDT for more than 20 h/wk during the first trimester (OR = 1.8, 1.2-2.8 95% confidence interval (CI)), but not for women working 5-20 h/wk (OR = 1.0, 0.6-1.6 CI), or for women working less than 5 h/wk (OR = 0.9, 0.6-1.4 CI). No significantly elevated risk of birth defects was found among women working with VDTs, although the OR was 1.4 for both high and moderate exposure (CI= 0.7-2.9 and 0.7-2.7, respectively). When VDT use was examined within occupational groups, a dose-response trend for miscarriage was observed among administrative support/clerical workers, but not among managers/professionals, technical/sales, and service/blue collar groups. As an indicator of adverse effects from VDT radiation, this study has two major weaknesses: (1) recall bias in the estimated exposure to VDTs, as women who had an adverse reproductive outcome and subsequently read about possible VDT effects may have tended to overestimate VDT use, while women with uneventful pregnancies may have underestimated VDT use, and (2) effects of other occupational factors, particularly stress, which can be strongly associated with VDT work, especially at the technical/support level.

More recently, Lindbohm and coworkers reported results of a study of Finnish clerical workers which indicated a significantly elevated risk of spontaneous abortion for women using VDTs with relatively high EMF emissions (Lindbohm, Hietanen et al. 1992). For women using VDTs with high ELF magnetic field measurements (> 0.9 uT or > 9 mG), the OR was 3.4 (1.4-8.6 CI) relative to workers using a VDT with a low ELF magnetic field level (OR = 0.4 (0.2-0.8 CI)). To factor both duration and intensity of exposure, a variable was constructed by multiplying field strength by number of hours per week of VDT use. Based on categories derived from this variable, the OR was increased for the highest exposure category for both the ELF frequency range (OR = 3.8, 1.6-8.8 CI) and the VLF frequency range (OR = 2.7, 1.2-6.1 CI). In the highest exposure category, the adjusted OR was higher for late abortions, defined as 12 weeks or after, (OR = 9.5, 1.8-51.5 CI, 8 cases) than for earlier abortions (OR = 1.9, 0.6-6.3 CI, 11 cases).

The National Institute for Occupational Safety and Health (NIOSH) conducted a major study of groups of full-time female directory assistance and general telephone operators with similar work situations in an attempt to resolve some of these issues (Schnorr *et al.* 1991). Although both groups had similar occupational demands and presumed stress levels, the directory assistance operators used VDT units manufactured by, Computer Controls, Inc. (CCI) or International Business Machines (IBM) to provide telephone numbers to customers; while general operators used units which employed a light-emitting diode (LED) or neon glow tube (NGT) to display the numbers rather than a VDT. EMF emissions in the various occupational settings involved in the study were measured directly, and an estimate of exposure was calculated from those measurements and a work history, rather than by subjective recall. A multiple logistic-regression analysis showed no association between VDT use in the first trimester and spontaneous abortion (OR = 0.93, 0.63-1.38 CI). However, analysis of the EMF emissions in the two occupational settings indicated differences in the mean emissions in the very-low-frequency range (15 kHz); but in the ELF range, magnetic field exposures were similar for all groups of operators: 62.3, 57.7, 62.4, and 32.4 mA/m for CCI VDT, IBM VDT, LED, and NGT units, respectively. These measurements are in the range of exposure levels found in the average home (40-200 mA/m). The authors concluded that their results indicate no increase in the risk of spontaneous abortion associated with the occupational use of VDTs. However, elements of the study design did not allow assessment of effects on early abortion (subclinical fetal loss), and measurements indicate that, although there were differences in levels of 15 kHz exposures, both groups were exposed to essentially similar levels of ELF magnetic fields, the EMF exposure of greatest concern.

Therefore, while many human studies completed to date suggest little reproductive effect from exposure to ELF EMF from VDTs, the issue is unresolved and is still a matter of significant public concern. For example, Wertheimer and Leeper suggested a correlation between increased fetal loss and conception at a season when there is increasing use of electrically heated beds (Wertheimer and Leeper 1986) or ceiling cable electric heating (Wertheimer and Leeper 1989) (discussed in the previous section). Their hypothesis of an increased incidence of spontaneous early abortion resulting from increased ELF magnetic field exposure at the initial stages of development means that fetal loss would occur when it is least likely to be reported as clinical miscarriage or to require medical attention. Epidemiologic designs such as that of the NIOSH study, used to avoid errors of recall bias, are therefore also less able to address questions of early fetal loss raised by Wertheimer and Leeper.

5.4 PROJECT HENHOUSE

The EMF research literature contains a relatively large body of experimental work reporting teratogenic effects of extremely low frequency magnetic fields on chicken embryos. Current interest in this system can be traced to the work of a Spanish research group headed by J. M. R. Delgado. Using a pulsed field generated in a coil by a Grass S88 stimulator (nominally, rectangular waves of 0.5 ms duration), these investigators initially reported up to a 78% incidence of abnormalities in the morphogenesis of chicken embryos exposed during the first 48 h of incubation to a 1.2 μT (12 mG) 100 Hz magnetic field (Delgado *et al.* 1982). The effect was described as a "window effect," in keeping with the concept of Bawin and Adey (discussed previously), because the number of embryos with developmental retardation was greatest with a 1.2 μT/100 Hz field, and less and/or more variable at the other frequencies (10 and 1000 Hz) and intensities (0.12 and 12 μT) tested. Different organ systems appeared to react with different sensitivity to various field conditions. The cephalic nervous system appeared the most sensitive, while the vascular system was less sensitive. Somites were unaffected at 1.2 μT/100 Hz; while at 1000 Hz, embryos exposed to 1.2 or 12 μT (120 mG) exhibited widespread developmental delays and defects, especially in organs of mesodermal origin, with weaker effects at 0.12 μT (Delgado 1985). Developmental effects were ascribed to a biochemical mechanism which involved changes in glycosaminoglycans so that alteration of these cell surface components resulted in altered cell contacts and disrupted morphogenic cell associations.

Although the reported effects and hypothetical mechanism proposed to explain them initially seemed to present a coherent picture, additional work by Delgado's group and others indicated that the effect, if real, was much more complex than it appeared initially. Subsequent work suggested additional field-specific effects. For example, at 100 Hz with a pulse risetime of 100 μs, teratogenic effects were reported to be greatest at intensities of 1.0-13.9 μT, but not at lower or higher intensities; but with pulse risetimes of 2 and 42 μs, intensities of 0.4 and 1.0 μT were reported to cause the greatest teratogenic effect (Delgado 1985).

Over the next decade, a number of attempts made to replicate these results met with varying degrees of success. Work which continued in Delgado's laboratory after his retirement was directed by J. Leal (Leal *et al.* 1988). A number of other laboratories attempted to replicate Delgado's results in Sweden (Mild 1986; Sandstrom, Mild, and Lovtrup 1987), Finland (Juutilainen and Liimatainen 1986), and the U.S. (Sisken *et al.* 1986; Maffeo *et al.* 1988; Litovitz, Montrose, and Litovitz 1991). These groups reported no teratologic effects; effects only under certain field conditions; effects only with certain strains of chicken; or claimed results which suggested that a previously uncontrolled variable, such as the orientation of the egg in the geomagnetic field, could account for discrepancies in results between research groups. Those groups which did report significant teratogenic responses observed a much lower abnormality rate than initially reported by Delgado *et al.* (Juutilainen and Saali 1986; Juutilainen, Laara, and Saali 1987). In each case, replication of Delgado's experiment was only approximate, and many details of the field exposure conditions (especially waveforms) and biological system (strain and source of embryos) varied among the laboratories.

As preliminary results from various groups accumulated, no consistent pattern of results emerged: not only did different groups confirm or fail to observe teratogenic results, but attempts to define systematically those experimental variables which could account for inconsistent results between experiments were unsuccessful.

In 1985 and 1986, the Office of Naval Research sponsored "Project Henhouse," an international effort to determine if specific low-level pulsed magnetic fields produced teratological effects in the chicken embryo system, and to resolve the discrepancy in experimental results obtained in different laboratories (summarized by Berman *et al.* 1990). Scientists at the US Environmental Protection Agency labs in Las Vegas designed and built six pairs of identical incubation-exposure systems at the individual participating laboratories and calibrated field and vibration characteristics in them. The intent was to use identical protocols, methods, exposures, and evaluation criteria for experiments at six different locations around the world with the expectation of achieving agreement in results between laboratories. Each exposure system consisted of two modified VWR Model-6000 water-jacketed incubators with the door heaters disconnected to eliminate them as a source of a 50/60 Hz magnetic field, and the magnetic latch removed to eliminate it as a source of static magnetic field. In each lab incubators were oriented with the door facing magnetic East, and the incubators were placed at least two meters apart so that the field exposure from the active incubator was minimal in the sham-exposure incubator. The magnetic field was produced using two vertical 44.2 cm Helmholtz coils connected in series, and fed by a pulsed signal from a Wavetek Model-801 pulse generator. The input waveform was a unipolar pulse with a repetition rate of 100 pulses per second, 500 μs duration, nominal 1 μT peak magnetic field. This signal was similar to signals which had been reported in the literature to produce a teratogenic effect, but was well enough defined to allow reasonable dosimetry and replication of the exposure system.

The overall results of the henhouse project indicated a statistically significant teratologic effect: exposed embryos had an abnormality rate of approximately 25%, while controls had an abnormality rate of approximately 19%, a difference which was statistically significant ($p<0.001$). More remarkable, however, was the fact that results only served to underline the inconsistency between laboratories, in spite of the precautions taken. Two laboratories had highly significant increases in the proportion of abnormal embryos in the exposed groups with a four-fold increase in one lab ($p<0.001$) and a two-fold increase in the other ($p=0.03$); one had a marginally significant 9% increase in abnormalities in the exposed group ($p=0.08$); and the remaining three labs, including one lab which ended up using a different strain of eggs, showed no significant difference between exposed and sham-exposed embryos ($p=0.606$, 0.617, and 0.402)(Berman *et al.* 1990). A careful independent statistical reanalysis of the Henhouse results confirmed this overall significance, but also indicated significant intralaboratory differences which included uncontrolled differences between experimental replicates in the same lab and between controls in different labs (Handcock and Kolassa 1992).

A number of investigators have suggested reasons why chicken embryo teratology results might vary between experiments. Because these teratology experiments are among the most replicated of ELF EMF studies, it is worthwhile reviewing possible reasons for variable results; not only because of interest in teratologic results themselves, but as a model for factors which might contribute to the general variability in the results of other EMF experiments.

5.4.1. EMF (Physical) Factors

Investigators have noted in their discussion that the experimental and sham-exposure field conditions used can differ in small but significant details from those reported by other investigators (Sandstrom, Mild, and Lovtrup 1987). Those investigators reporting negative results are careful to note that the lack of effect applies only to the specific set of exposure and biological conditions examined, and may not necessarily generalize to other similar (but not identical) experimental conditions (Maffeo *et al.* 1988).

Although applied field exposures may be well controlled, ambient field conditions for experimental and control embryos are a complex function of power-frequency sources in the laboratory environment, the local geomagnetic field, and masses of ferromagnetic material in the building and incubator which modify the orientation and intensity (flux density) of time-varying field waveforms and the DC magnetic field. The true ambient field is rarely measured in detail and reported in experimental work. After shipping identical incubator/exposure chambers to the six laboratories involved in Project Henhouse, Mantiply and Wagner performed a series of measurements at each site and noted differences in the local static and 50/60 Hz ambient magnetic fields. Their measurements found 50 Hz fields ranging from 0.53 to 0.67 μT, 60 Hz fields of 1.1 to 1.2 μT RMS with incubation heater on, and static fields between 12 and 44 μT at angles of 19 degrees to 74 degrees from vertical (Wagner and Mantiply 1988). Analysis of these differences according to ion resonance theory indicated that even these small differences could be significant if resonance effects are involved in the teratogenic response (Mantiply and Wagner 1988).

Leal and coworkers have systematically explored subtle variations in physical parameters, attempting to identify factors which could account for variable results. They have shown that several different types of ELF EMF conditions can produce increases in the number of abnormal embryos. For example, a series of 192 exposed and 191 sham-exposed embryos demonstrated a three-fold, statistically significant ($p=0.003$) increase in abnormalities produced by exposure to a 50 Hz sinusoidal magnetic field with a 200 μT (2 G) rms flux density (Ubeda *et al.* 1990). Previously, the same research group reported that a horizontal 100 Hz 100 μT pulsed field produced a higher incidence of abnormal embryos when they were oriented South-West in the geomagnetic (static) field, and a decreased number of abnormal embryos when embryos were oriented in a geomagnetic South position (Leal *et al.* 1984). More recently, Leal and coworkers have attempted to explain variability of results as a function of natural variations in the intensity of the geomagnetic field with time (over a range of approximately 100 nT (1 mG)), and found a correlation of embryo responsiveness with recorded geomagnetic field intensity or change in intensity during the initial 24 h of embryonic development (Leal *et al.* 1987; 1989b). Subsequent experiments and analyses were performed to test the teratogenic effect of field patterns established according to ion cyclotron resonance (ICR) theory (specific DC and sinusoidal AC magnetic fields establishing ion-resonance conditions for calcium and sodium). Calcium ICR conditions were produced by cancelling the geomagnetic DC field and imposing defined DC and sinusoidal magnetic fields using 3 pairs of orthogonal Helmholtz coils. These field conditions produced significant increases in specific abnormalities, although the magnitude of the effect was small in comparison with the effects of the pulsed fields used previously (Leal *et al.* 1988; Chacon *et al.* 1989). In one series of reports, the group noted an effect related to the position of embryos in the magnetic field coil. While the coil produces a uniform magnetic field, the induced electric field vector varies along the diameter of the coil, and the magnitude of the induced electric field was found to be inversely correlated with the frequency of abnormal embryos (greatest when the electric field was lowest) (Leal *et al.* 1988).

Berman has conducted a detailed overview analysis of chicken embryo and other vertebrate teratology experiments in an attempt to determine if there are trends or similarities in the characteristics of fields reported to produce teratologic effects (Berman 1990). Analyzing the experiments in terms of the type of signal used, he noted no statistically significant pattern, but a greater ratio of positive reports of teratologic effects when the repetition rate was in the 60-100 Hz range and the change in field intensity per unit time (dB/dt) was over 0.1 T/s. Martin compared several similar 3 μT, 60 Hz sinusoidal signals which differed primarily in switching, having the effect of altering the rapidity of the rise and fall time of the pulse. A split phase signal produced by a light dimmer switch was expected to be most teratogenic because of the rapid rise and fall time, but produced no significant increase in the malformation rate (Martin 1989). Therefore, although there are some suggestions that certain field characteristics define an "effective" teratogenic EMF signal in the chicken embryo system, it is evident that results do not (yet) support a clear conclusion as to what these characteristics are.

Recently, the importance of a field characteristic described as coherence has been advocated by Litovitz *et al.* (Farrell *et al.* 1992; Litovitz, Montrose, and Doinov 1992). The need for field coherence is based on a theoretical mechanism which suggests that membrane components are the primary sensors of biologically active EMF signals, and that a critical minimal number of adjacent membrane components on a single cell must be stimulated in a coordinated manner in order to initiate a biological response. Random EMF signals and thermal noise lack the needed coherence in space and time to produce a coordinated effect, which explains why effective signals can exist at energy levels at or below thermal noise energy levels (discussed by others as "the kT problem"). Litovitz and coworkers reported that a significant increase in the abnormality rate of chicken embryos exposed to a 100 Hz pulsed field (the Henhouse signal) could be abolished if simultaneously a "noise field" consisting of a semi-random 30 Hz to 1 kHz signal was superimposed on the pulsed signal with a signal-to-noise ratio of 1 (Litovitz, Montrose, and Doinov 1992). If these results can be replicated, they suggest that ambient EMF signals, which certainly may vary between laboratories and over time, are capable of cancelling out the effects of otherwise biologically active EMF signals.

Developmental effects may be supported by a plausible biological mechanism, which is discussed in section 14 of this report in the context of hypothetical mechanisms. There are a substantial number of reports in the developmental biology literature indicating that electrochemical field gradients (DC electric fields or ionic currents) play a major role in morphogenesis. Imposition of a time-varying magnetic field, which induces a corresponding time-varying electric field in the embryonic tissue, may disrupt or redirect these endogenous DC electrical fields in the developing embryo. A recent report by Hotary and Robinson (1992) confirms the central role of such DC fields in the chicken embryo system. These investigators used conductive implants to shunt endogenous electric currents out of the embryo at the mid-trunk level of stage 11-15 embryos, and observed a range of abnormalities in 92% of the embryos with conductive implants, but in only 11% of embryos with nonconductive implants. The abnormalities included defects in neural tube and other axial structures similar to those reported to be produced by imposed pulsed magnetic fields. Vibrating probe measurements of the axial electric field density indicated that fields in the posterior intestinal portal of normal embryo were on the order of 108 $\mu A/cm^2$, and the conductive shunt (which produced abnormalities) decreased this current by 30 ± 6%. The developmental abnormalities were morphologically similar to the pattern of abnormalities produced by the "rumpless" genetic mutation, and these embryos also had a reduction in the current measured at the posterior intestinal portal (44 $\mu A/cm^2$). Many of the reported biological effects appear to be frequency-specific however, and it is not clear if ELF electric fields induced in tissue could have morphogenic activities similar to endogenous DC fields.

5.4.2. Biological Factors

Genetic differences between strains have been explored as factors in the teratologic response of chicken embryos by Koch and Koch (1991). Using strains which included those that showed both significant and insignificant differences between exposed and sham-exposed embryos in the Henhouse project experiments, they exposed groups of embryos from 4 different strains to the 100 Hz, 1 μT Henhouse signal. They found no significant increases in abnormalities with any of the 4 strains. This result appears to contradict an earlier report by Martin. He found that Arbor Acre strain embryos, the strain used by one of the laboratories in the Henhouse study which observed no teratogenic effect, was genetically unresponsive to the Henhouse pulse, while the same signal produced significant abnormalities in White Leghorn strain embryos (Martin 1988). However, Koch and Koch reported an abnormality rate in their sham-exposed controls which was as high as the rate in exposed groups in the Henhouse experiments, while Martin had a control embryo abnormality rate which was similar to the rate reported for the controls in the Henhouse experiment. The intrinsic abnormality rate has been cited as a factor in these experiments by Litovitz, Montrose, and Litovitz (1991), Leal *et al.* (1989a), and Juutilainen (1991). All of these investigators maintain that a low rate of abnormalities under control conditions is a necessary prerequisite for any significant differential effect of EMF exposure. Leal has suggested that the effect of the EMF exposure is to amplify the expression of genetic abnormalities already present in the population but not expressed in unexposed (control) embryos. Perhaps a high abnormality rate in the controls indicates that some other factor has already increased the incidence of abnormalities to the point where ELF EMF can have no further effect.

Although early experiments involved exposing chicken embryos to EMF at various periods of development, recent results indicate that there is a specific developmental stage or limited series of stages which are sensitive to teratogenic effects of EMF. Martin showed that exposure to the Henhouse pulsed field only during the initial 24 h of incubation produced rates and patterns of abnormality identical to those produced by 48 h or 7 day exposures (Martin 1988). Recently, Martin further subdivided the critical time period by showing that exposure to EMF during the first 12 h of incubation produced no apparent increase in abnormalities, while exposure from 12-24 h of incubation produced a statistically significant increase in abnormalities (Martin 1991). This period of development corresponds to a time of rapid cell proliferation; establishment of the three basic body layers by cell movement (gastrulation); and formation of the neural tube and brain structures (neurulation).

Biological variability in chicken embryos is well known to chicken embryologists but is not generally discussed in the literature. Fertile chicken eggs for laboratory work are usually obtained from commercial suppliers who select hens for productivity rather than genetic continuity, and change their flocks annually to keep productivity high. These practices cause possible genetic drift year to year even in a laboratory which uses the same source of supply, and results obtained at certain times of the year (usually late winter or spring) when the egg supply may change abruptly due to turnover of the flock. Chicken strains are not inbred lines, and the population contains many recessive lethal mutations which make the production of inbred lines problematic. Fertile chicken eggs are usually collected at the convenience of the supplier over a period of time (commonly 24 h) and are shipped to the laboratory by commercial transport. Little or no effort is made to avoid exposing the eggs to moderate temperature changes or electromagnetic fields during this time period, in which some superficial cleavage of the blastomere and early morphogenesis occurs. Quantitative work with chicken embryos therefore occurs against a much more variable genetic and environmental background than is common in laboratory animal experiments, and this must be considered in interpreting variable results from such experiments.

5.4.3. Other Factors

Overinterpretation of chance observations has been suggested as a possible factor in reports of "phantom" EMF biological effects (Foster 1992). Foster notes that many of the experiments comprising the EMF research literature are small-scale studies which remain unreplicated by other laboratories. There is a substantial amount of hypothesis building and speculation in the EMF bioeffects literature, in keeping with the largely exploratory nature of much of the work, and many observations reported in meeting abstracts are never published in peer-reviewed journal articles. Research in this area is not well funded, and it is often not clear whether work was not completed because of funding constraints or through lack of reproducibility.

Unintentional investigator bias (or a desire to see a meaningful pattern of results in random experimental noise) has also been suggested as a factor in reports of EMF bioeffects which cannot be repeated by others. Bias has been explicitly controlled in the design of many EMF experiments, including Project Henhouse. While the observation of embryo abnormalities is inherently subjective, abnormal embryos in the Project Henhouse series were scored according to established criteria by observers who were blinded as to exposure conditions, and results of individual laboratories were coded for statistical analysis. In fact, one of the laboratories reporting no significant difference between sham-exposed and embryos exposed to the Henhouse pulsed magnetic field, was Leal's group (Berman *et al.* 1990).

Many of the chicken embryo experiments are difficult to interpret because of small sample size and the statistical weakness of small-scale experiments. Because of high variability in background rates of chicken embryo abnormalities and the cost of running large scale experiments, many of the results of teratology experiments suggest trends but lack statistical significance. Berman has made a case for reinterpreting previous negative (insignificant) results based on new information: he suggests, for example, that the lack of a statistically significant teratologic effect reported by Sisken *et al.* (1986) for two groups of embryos exposed to pulsed EMF for either 24 h or 7 days, should be reinterpreted in light of Martin's observation of a critical exposure period in the initial 24 h of development. If Sisken's two exposed groups are combined into a single exposed group, the combined abnormality rate is significantly greater than the abnormality rate in the combined sham-exposed groups (Berman 1990). The statistical validity of this approach may be questionable, but it does emphasize the role of small sample size in lending uncertainty to the interpretation of these results.

In summary, an evaluation of the current published reports of teratological effects in chicken embryos in response to pulsed magnetic fields suggests that the effects are real, but not well enough understood to be easily replicated. The observation of chicken embryo abnormalities under certain pulsed magnetic field conditions seems to be well supported by independent replication of the observations in independent laboratories (Berman 1990). It is still not clear, however, what biological and electromagnetic field conditions are critical variables in obtaining a reproducible teratologic response.

5.5 OTHER TERATOGENETIC REPORTS

Relatively few studies have been performed with other systems or field conditions. The effects of strong magnetic field gradients were evaluated in frog embryos (*Xenopus laevis*) by Ueno, Harada, and Shiokawa (1984). They exposed fertilized eggs to homogeneous 1.0 T fields and fields with gradients ranging from 1 to 10,000 T/m, observing only insignificant changes in the rate of abnormalities. In later work, the same group subjected *Xenopus* embryos to 10-15 mT alternating magnetic fields (20 Hz, 2.0 kHz, or 20 kHz) with no harmful effects (Ueno *et al.* 1985).

Generally, exposure of embryos to strong static (DC) magnetic fields has not been found to produce developmental abnormalities. Kay, Herfkens, and Kay (1988) exposed *Xenopus* embryos to the field of a magnetic resonance imaging device (1.5 T, 64 MHz) and found no significant effects on the rate of development, morphology, behavior, or the pattern of extracted DNA, RNA, or protein. Buntenkotter *et al.* (1991) found no effect on fetal development after exposing pregnant rats from the morning after mating to 30 mT (300 G) static fields for 24 h/day. Murakami, Torii, and Masuda (1992) exposed 84 pregnant CD-1 ICR mice to a 6.3 T static magnetic field for 1 h/day from day 7-14 of gestation and found no difference in the incidence of morphological abnormalities or growth patterns when they examined the fetuses at day 18 of gestation. There is an isolated report of developmental retardation and neural tube defects in chicken embryos exposed to an extremely high intensity magnetic field (5000 Oe or approximately 500 mT), but sample numbers were small and temperature control uncertain (Joshi, Khan, and Damle 1978).

At the other extreme, there is one interesting report of developmental defects resulting from a decrease in normal static magnetic field exposure. Asashima, Shimada, and Pfeiffer (1991) used a facility at the Japanese Institute of Space and Astronautical Science at Sagamihara, which was designed to simulate magnetic field conditions in interplanetary space, in order to expose developing newt embryos to a static magnetic field of 5 nT, about 10,000 times weaker than the geomagnetic norm (which ranges between 30 and 60 μT at the earth's surface). Significantly higher rates of abnormalities were observed in the shielded embryos in all groups exposed early in development (before stage 16). Bi-handedness and intestinal protrusion were observed in magnetically shielded larvae but not in controls. Other abnormalities more frequently observed in shielded larvae were spinal curvature, malformed eyes, and retarded or blocked development. These results imply that normal morphogenesis depends to some extent on the presence of the geomagnetic field in the environment, and suggest that unusual magnetic field conditions could interact with an endogenous magnetoreceptor system to produce biological effects. Alternatively, the need for a geomagnetic field could reflect the same process reported with exogenous ELF EMF when the orientation and strength of the DC magnetic field (or geomagnetic field) is critical for the bioeffect. Developmental experiments are now being conducted on space flights, but these are complicated by the additional factor of zero gravity effects.

5.6 GRAPHIC ANALYSIS OF TERATOLOGIC EFFECTS IN RELATION TO TR07 FIELDS

Figure 5-1 summarizes several reported teratological effects of alternating magnetic fields on biological systems, in most cases the chick embryo. Most of the reported effects of EMF on embryological development involved pulsed magnetic fields, and are not included in this figure. Three authors have reported teratological effects of alternating magnetic fields on chick embryos. One (Delgado) reported effects under exposure conditions that are well within the range of expected field levels in the TR07 maglev passenger compartment (although the duration of exposure was quite long); the other two reports involved exposures at the upper end of the range of expected maglev exposures. For reasons discussed above, the induced electric fields in the human body would be considerably higher than those in the chick eggs in these experiments.

The literature on the teratological effects of pulsed magnetic fields on chick embryos and other systems is large and inconsistent (see the review by Berman 1990). Berman concluded:

> "... we cannot clearly relate an increase in the incidence of abnormal embryos resulting from exposure to pulsed magnetic fields to any patterns of pulse frequency, field intensity, pulse shape, or rate of change in the intensity.... Until the important variables in pulsed magnetic fields are determined and the mechanism of effects is identified, it may not be possible to extrapolate such effects to humans."

Berman suggests, nevertheless, that the exposure conditions that most often lead to observed developmental anomalies are pulse repetition rate of 60-100 Hz, and a maximum time rate of change of the magnetic field (dB/dt) above 0.1 T/s. By contrast, the maximum value of dB/dt in the passenger compartment of the TR07 maglev (Figure 3-5) is about a hundred times smaller.

Using Equation 1, we estimate that the field conditions which Berman has identified as associated with teratogenesis (0.1 T/s, pulse repetition rate of 60 Hz) results in an induced peak electric field of 500 microvolts per meter in a 1 cm radius subject, corresponding to an induced current of about 0.05 microamperes per cm^2. The frequency spectrum of this stimulus is largely above 60 Hz. This falls at the upper end of the envelope of induced electric fields in a hypothetical exposed passenger of the TR07 maglev, as shown in Figure 3-5.

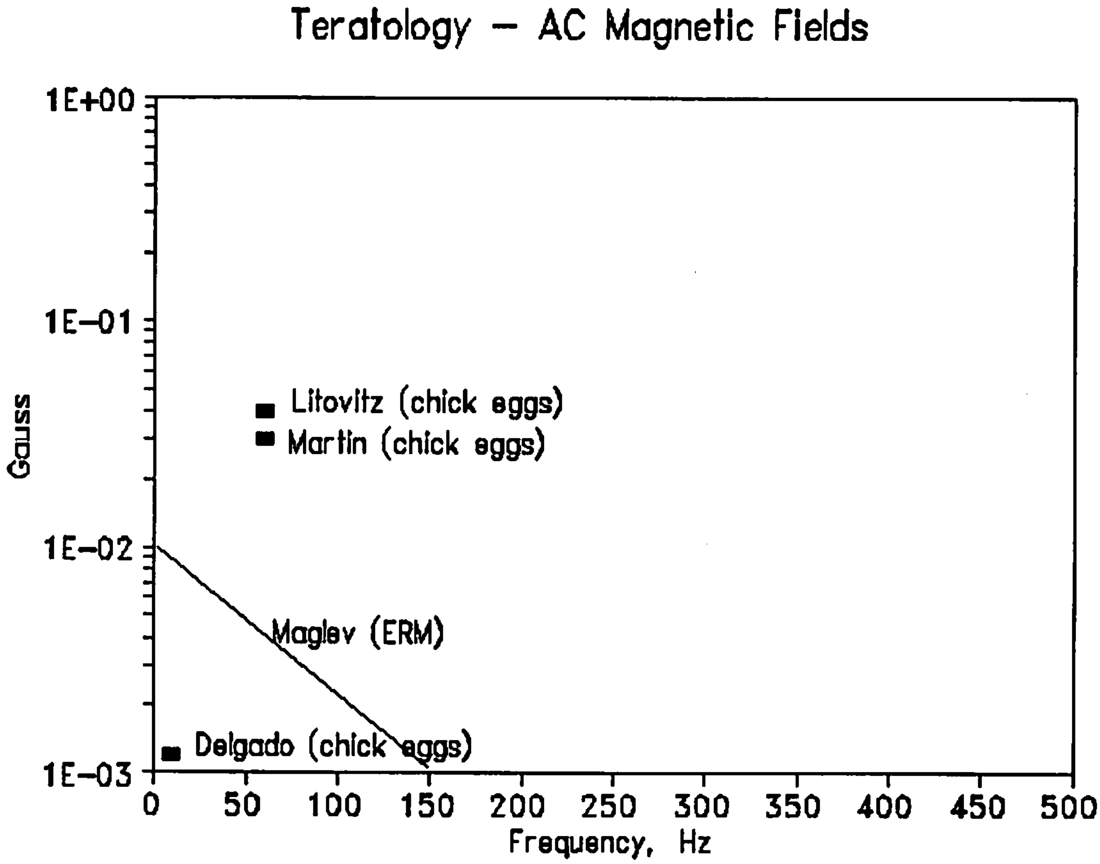

FIGURE 5-1. REPORTED TERATOLOGICAL EFFECTS OF ALTERNATING MAGNETIC FIELDS RELATIVE TO ENVELOPE OF EXPOSURE ONBOARD THE TR07 MAGLEV.

The time rate of change dB/dt is unlikely to be, by itself, an adequate measure of exposure. It provides a useful measure of the amplitude of the instantaneous induced electric field in the subject. However, one would expect that the biological effectiveness of an electric or magnetic field would also depend on its time or frequency dependence, as well as the duration of exposure. Most teratological studies involved chronic exposure to the pulsed magnetic fields, for hours, days, or weeks during the development of the embryo; although it would appear that there is a biological window of efficacy of relatively short duration corresponding to gastrulation.

6. IMMUNOLOGICAL EFFECTS

6.1 HUMORAL AND CYTOTOXIC RESPONSES IN VIVO

It has been suggested that EMFs acting at the cell membrane may modulate the function of the immune system (see review by Budd and Czerski 1985). The literature on the interaction of EMFs with the immune system is highly conflicting. Only data from exposure of whole animals to 50 or 60 Hz electric or electromagnetic fields can be said to show any consistency, and even here the picture is one of relatively small or null effects.

Russian investigators (Liubchenko 1982) reported that chronic exposure of rats to 50 Hz fields (1-15 kV/m) caused only a transient increase in blood complement, while human volunteers exposed to 12 or 15 kV/m exhibited transient elevations in complement titer. Another Russian group showed that exposure of mongrel white mice to a 2,000 V/cm electrostatic field for 24 h produced an immunodepressive effect: a reduction in the number of antibody-forming cells in the spleen, a decrease in the hemolysin titer in the peripheral blood, and a reduction in the immune rosette-forming cell population (Tovmasian, Shekoian, and Artsruni 1982).

In a series of experiments in which mice were exposed to 60 Hz fields at 100 kV/m for 30 to 150 days, Morris and his colleagues found no significant perturbation of the immune system in terms of primary antibody response or mitogen stimulation of spleen cells. An increased mitotic index for phytohemagglutinin-stimulated spleen cells from exposed, as compared with sham-exposed, mice was not statistically significant (Morris and Phillips 1982; Morris 1985). This study was extended to rats, where no major changes in cell-mediated immune responses were observed (Frazier, Reese, and Morris 1988; Morris *et al.* 1989). There were, however, small statistically-insignificant changes in natural immunity, as reflected in increased release of ^{51}Cr from labeled target cells *in vitro*, when rats were exposed to either continuous or intermittent (1 min on/1min off) 10 G, 60 Hz magnetic fields (Morris *et al.* 1990). In contrast, when female sheep were penned beneath a 60 Hz power line for 2 or 5 months, there was a marginally-significant reduction ($p=0.049$-0.060 in 3 experiments) in mitogen-stimulated IL-1 production by their peripheral leukocytes, as compared with cells from age-matched sheep penned in an ambient field. Furthermore, 6 weeks after vaccination with Lepto-5, there was reduced antibody response to the Lepto-5 antigen in the exposed sheep; while after 6.5 months, 13 of the 14 exposed animals developed dermatological lesions associated with fungal soil organisms (*Scopulariopsis sp.*) not found in any controls, which is suggestive of impaired immune function (McCoy *et al.* 1992).

6.2 EFFECTS ON ISOLATED LYMPHOCYTES

Data obtained by exposure of isolated components of the immune system to ELF EMFs *in vitro* have been conflicting. A series of reports have claimed that exposure of human lymphocytes to pulsed EMFs enhances their response to mitogens such as phytohemagglutinin, as measured by DNA synthesis (Hellman *et al.* 1985; Emilia *et al.* 1985; Cantini *et al.* 1986; Franceschi, Bersani, and Mario 1986; Cadossi *et al.* 1986). On the other hand, Conti *et al.* (1983), using square wave pulses at 1 to 200 Hz, reported that lectin-stimulated mitogenesis of human lymphocytes was inhibited. Mooney *et al.* evaluated the effect of ELF pulsed magnetic fields on the response of human peripheral blood mononuclear cells to mitogenic stimulation and also reported an inhibitory effect (Mooney, Smith, and Watson 1986). Cossarizza *et al.* (1989) could find neither stimulatory nor inhibitory effects of either a 0.1-mT, 60 Hz cyclic rotating field or a 2.5 mT, 50 Hz pulsing (2 ms pulse) field on the activity of human natural killer (NK) or IL-2-induced lymphokine activated killer (LAK) cells. Vecchia *et al.* (1992) reported inhibition of cell proliferation and cytotoxic activity of both NK and LAK cells incubated in a homogeneous 50 Hz, 2.5 mT sinusoidal magnetic field for 3 days.

A report in the literature indicates that differences in field conditions used by different investigators may explain this apparent conflict. Bersani *et al.* showed that the effects obtained in lymphocyte experiments are extremely dependent on the physical characteristics of the field. Whereas a square wave 3 Hz, 5 mT-pulsed field inhibited mitogen-stimulated increase in thymidine incorporation by human lymphocytes, a 50 Hz triangular wave pulsed field enhanced the uptake, and the findings were reproducible between two research groups (Bersani *et al.* 1992).

Studies with sinusoidal ELF EMFs have generally shown modest depression of various components of the immune process. Phillips (1986) found that 60 Hz magnetic fields alone, or combined electric and magnetic fields, inhibited natural killer-cell-induced cytolysis of irradiated Colo 205 cells *in vitro*. Lyle and his group have also reported inhibition of the allogeneic cytotoxicity of a normal murine T-lymphocyte cell line (CTLL-1) by 60 Hz sinusoidal electric fields (Lyle *et al.* 1988a). In human lymphocytes, a 15 Hz, 0.1 mT field stimulated activated B- and T- cell proliferation, and reduced the activity of LAK, but not of natural killer cells (Mehta *et al.* 1991). A recent study using the same field conditions and activated human lymphocytes, produced similar proliferative effects and enhanced release of IL-2 by T-cells (Blackinton *et al.* 1992); whereas Cossarizza *et al.* (1992) reported that lymphocyte proliferation was inhibited by exposure to a 50 Hz electric field (5 kV/m) without any effect on cytotoxicity. Finally, Winters (1986) summarized a project involving human and canine lymphocytes by concluding that 60 Hz EMFs had no effects on ligand receptors, immunoglobulins, mitogen response, or the synthesis of DNA, RNA, and protein. He did find, however, that cultured human colon cancer cells showed a mitogenic response, increased transferrin receptor contents, and resistance to natural killer cells after exposure.

This variability in response may arise from several sources apart from the difference between pulsed and simple sinusoidal waveforms mentioned above. Distinct "window effects" for frequency and intensity somewhat analogous to action spectra, have been identified. Thus, Cadossi *et al.* (1986) reported that inhibition of the lectin response occurred in 10 mV fields, in contrast to the stimulation seen at other intensities; and, Conti *et al.* (1985) found that inhibition did not take place at various combinations of frequencies for particular lectins. Similarly, Franceschi, Bersani, and Mario (1986) obtained a bimodal response at low phytohemagglutinin concentrations in which the effect of EMF was inhibitory rather than stimulatory. Even the components of the EMF complicate the data: Morris and McClanahan (1986) reached the conclusion that a 60 Hz electric field with a 5 μT magnetic contaminant was needed to enhance the mitogenic response of lymphocytes when they were unable to find an effect with 60 Hz pure electric or magnetic fields alone. Given that mitogenesis is a calcium-dependent process, and that mitogen-stimulated increase in calcium uptake appears to be modulated significantly by a specific EMF frequency (12.5 Hz) (Lyle *et al.* 1990), the immunologic data obtained *in vitro* is compatible with an ion cyclotron resonance effect of EMF.

The effects of ELF EMF on calcium flux in lymphocytes, discussed in Section 4.3 of this report, have important implications for immune function. Lymphocyte mitogenesis requires the influx of calcium, and it was first reported in 1985 that pulsed EMF stimulates calcium influx and lymphocyte mitogenesis in tandem (Emilia *et al.* 1985). Subsequently, Liburdy, Miller, and Durney (1988) described modulation of intracellular calcium movements within lymphocytes. This group has explored the effects of varied types of field and the relationship with the biological state of the lymphocytes. Resting rat thymocytes did not show any effect of a 60 Hz sinusoidal field, but when the cells were first stimulated with a mitogen (con-A) to initiate signal transduction, there was a further increase in calcium uptake on exposure to field values significantly greater than that seen with mitogen alone (Walleczek and Liburdy 1990b). In contrast, a pulsed magnetic field (3 Hz, quasi-rectangular, B peak 6.5 mT) tended to inhibit calcium uptake in mitogen-stimulated cells (Walleczek and Budinger 1991). The possible role played by the interaction between a static and a time-varying field was addressed in another study (Liburdy, Yost, and de Manincor 1991; Liburdy and Yost 1992) comparing the effects of the simple sinusoidal field with combined AC/DC field conditions that satisfy the requirements for ion resonance (Liboff and McLeod 1988; Lednev 1991). The results obtained in the 60 Hz field reproduced the earlier findings (Walleczek and Liburdy 1990b), but when the AC/DC field was applied together with con-A there was no increase in calcium uptake, indicating that signal transduction induced by con-A was inhibited by the field. Lyle *et al.* (1989; 1990) found that weak oscillating magnetic fields (12.5 to 15.3 Hz) increased the association of ^{45}Ca with CTLL-1 cells (a murine normal cytotoxic T-lymphocyte cell type maintained in continuous growth with interleukin-2), mouse and rat spleen lymphocytes, and EL-4 mouse lymphoma cells. Other cell types studied included rat thymocytes, murine B-lymphocytic line MPC, and human myeloid leukemia K-562. Rat thymocytes differed from spleen lymphocytes in that exposure to the field did not increase association with ^{45}Ca in resting cells, and inhibited the increase in uptake seen on activation with con-A. This suggests that the response depends upon the degree of maturation of these immune cells. Proliferation did not necessarily correlate with changes in ^{45}Ca uptake, and the authors concluded that changes in the latter caused by these weak fields either do not enhance, or may inhibit lymphocyte function; whereas in leukemic cells, there may be a variety of responses, including increased proliferation which is potentially important for cancer promotion.

In spite of the large amount of experimental data obtained, it is difficult to arrive at any firm conclusions regarding the action of EMFs on the immune system. This is due to the use of multiple exposure parameters, the unexpected dose/response relationships explained as window effects, inconsistent results, and the many different measures of immunity that were used.

6.3 METHODOLOGICAL FACTORS IN LYMPHOCYTE EXPERIMENTS

In vitro experiments with lymphocytes constitute a substantial body of results reporting, or contradicting reports of, significant biological effects of weak ELF EMF. The reliability of these *in vitro* results depends to a large extent on the care used in working with cultured cells. Although methodology has been critically analyzed as an issue in interpreting epidemiologic studies, we are not aware of any similar analysis of cellular studies.

Dr. Joseph Tumilowicz, a recently retired scientist who worked in tissue culture and virology research at Baylor College of Medicine (Houston, TX), has analyzed a series of representative EMF studies involving lymphocytes and other cultured cells. This section of the report, based on his analysis, outlines those factors which may contribute anomalous results in EMF experiments performed with isolated lymphocytes. A similar discussion of concerns applicable to adherent cells *in vitro* appears in Section 10 of this report.

The reproducibility and reliability of experiments with lymphoid cells can be affected by seemingly insignificant variations in methods and materials. In experiments with human lymphocytes where autologous human sera are used to cultivate the lymphocytes, the type of tube used for collection of blood may alter the activated status of the cells. Ulreich and Chvapil (1982) reported stimulation of proliferation of fibroblasts by human serum collected in a particular tube-stopper unit.

In many experiments, antibiotics are routinely used in the medium to support sterile technique. The addition of antibiotics does not assure eradication of contaminating microorganisms, because those present may be resistant or only partially suppressed by the types and concentrations of antibiotic included in the medium. Thus, absence or ineffective combinations and concentrations of antibiotics may result in high levels of microbial contamination even in typically short term experiments with lymphocytes. Few investigators report testing cultures for low-level microbial contamination, especially for mycoplasma which can produce a variety of subtle effects in a culture (from increased ^{3}H-thymidine incorporation in the culture due to incorporation by the mycoplasma, to suboptimal cell growth and viability), without producing noticeable changes in the appearance of the culture. The use of antibiotics introduces ionophores, and, depending on the kinds and concentrations of antibiotic, may be toxic or inhibit metabolic function at levels near minimal inhibitory doses. For example, gentamicin, a commonly used antibiotic, inhibits entry into S phase and retards DNA replication of mouse granulopoietic and erythropoietic cells (Benes 1982).

Experiments carried out with crude isolates of white cell populations in the buffy coat layer, after centrifugation of blood, may yield different results than experiments with populations enriched in lymphocytes by isolation, e.g., in Ficoll-Hypaque density gradients. The latter enriched populations are more uniform and reproducible.

The incompletely defined part of media used to cultivate cells and serum, varies greatly in composition and the presence of contaminating, adventitious microbial agents (according to lot, season obtained, supplier, age and species of donor animal, processing, etc.). While most of these aspects are beyond the control of the investigator, lots with the lowest endotoxin and hemoglobin (prooxidant) levels should be selected. Many suppliers in the U.S. can provide sera pretested for low capacity to stimulate lymphoid cell proliferation in the absence of mitogens. Some workers heat serum (56°C, 30 min) before use to destroy or reduce the number of viable adventitious agents. Heating may also reduce the capacity of serum to promote growth. Most careful researchers test serum batches, then use the same lot of serum, retrieved from a properly frozen state as needed, for an experimental series. This limits a series of experiments in time, since frozen serum gradually degrades over a period of six months to a year.

The use of zwitterionic HEPES buffer in medium RPMI 1640, the most commonly employed medium for culture of lymphoid cells, plays a role in phototoxicity of RPMI 1640 exposed to fluorescent lighting (Spierenburg 1984). In their experiments with murine thymocytes, Zigler *et al.* (1985) found that the principal cytotoxic product in HEPES-containing RPMI 1640 exposed to visible light for 3 h, as measured by a virtually total loss of ^{3}H-thymidine uptake and the protective effect of catalase, was hydrogen peroxide. All media, regardless of the presence of HEPES, should be exposed to fluorescent light for as short a time as necessary or shielded during such exposure, because of the accumulation of phototoxic products (Wang 1976).

Suppliers of liquid media assure that the osmolality of media conforms to expected values. However, researchers usually prepare the medium from powdered form without ascertaining the osmolality of the reconstituted medium. Additional measurements of osmolality should be made after addition of organic buffers like HEPES, which can affect osmolality. Changes in osmolality should not be overlooked after the medium is added to cells, particularly when using 24-well and 96-well culture plates, commonly employed in experiments with lymphoid cells. These plates are designed for use in humidified incubators. Without humidification, the osmolality of media can change drastically in a short time as a result of evaporation. Furthermore, evaporation may not be uniform from inner and peripheral wells within the covered plate or from plate to plate, depending on location within the incubator. Investigators who decline to humidify an incubator because of the increased probability of mold contamination may, if feasible, change media frequently, seal plates, or consider preparing media with a slightly decreased osmolality. Shifts in osmolality can cause obvious effects, such as changes in cell size, cell shape, nutrient transport, and cellular synthesis. Sodium and hydrogen ion exchange in thymic lymphocytes is stimulated by changes in osmotic pressure (Grinstein 1985). Presumably, shifts in fluxes of magnesium, calcium, and potassium, which contribute significantly to maintaining osmotic pressure, also may occur.

Use of ^{3}H-thymidine in measurement of proliferation should be confined to short exposures to prevent cellular radiation injury as evidenced by a block in G2 + M in phytohemagglutinin-stimulated lymphocytes exposed for 18 h (Pollack 1979). These authors found no perturbation of the cell cycle when cells were exposed to a relatively high concentration of ^{3}H-thymidine for only 20 min.

Some of the uncertainty in replicating experiments with established cell lines results from genetic drift or uncertain identification. Lymphoid cell lines should be fully and accurately identified, and obtained from recognized cell repositories such as the American Type Culture Collection. Cell lines from such a source are likely at the outset to be free of exogenous microbial contaminants compared with cell lines received from other laboratories. According to Dr. G. McGarrity, former director of the Coriell Institute of Medical Research which houses the largest number of mammalian cell cultures of any repository, approximately 12% of the cultures received by the Institute from the U.S. and about 25% of those received from Europe and Japan were contaminated with mycoplasmas. All cultures, regardless of source, should be monitored for presence of microbial contaminants.

Conclusions about the nature of a lymphoid sub-type under investigation should be based on fully developed characterization, which is rarely reported. Two examples from the EMF research literature may be used to illustrate the impact of this uncertainty. Phillips refers, with unacceptable certainty, to lymphoid cells as natural killer (NK) cells, based on an admittedly unclear connection between transferrin receptors and target structures for NK-induced lysis (Phillips 1986). Characterization of the cytotoxic cells with monoclonal, anti-NK would have been required to support the claim for NK identity of these cells. In a different vein, Lyle *et al.* (1988a) did not state the provider or source of the T-lymphocyte line, CTLL-1, used in their work. Furthermore, they describe CTLL-1 as normal, based apparently only on its dependence on interleukin (IL-2). No karyotypic data were provided, although the use of CTLL-1 in 1987 by Lyle *et al.* follows its development by Gillis and Smith by approximately ten years. The cause of reproducibility might have been served better if Lyle *et al.* had used culture CTLL-2, apparently an IL-2-dependent clone of CTLL-1, submitted by S. Gillis to the Tumor Immunology Bank of the American Type Culture Collection. Thus, all investigators attempting to reproduce the work of Lyle *et al.* could begin their experiments with the same preserved stock of partially characterized cells. This stock has been tested and found negative for bacteria, fungi, and mycoplasma, and the species has been verified.

These concerns are raised not as criticism of specific studies, but to indicate the range of purely technical problems which may lead to a failure to replicate results or produce artifactual positive results. The practices outlined above are not unique to EMF research but characterize much of the *in vitro* research literature. These factors underscore the uncertainty we have indicated in interpreting the body of positive and null results reported for ELF EMF effects on lymphocytes.

6.4 GRAPHIC ANALYSIS OF EFFECTS ON CELLS OF THE IMMUNE SYSTEM IN RELATION TO TR07 FIELDS

Figure 6-1 shows reported effects of time-varying magnetic fields on the immune system or on immune-system cells *in vitro*. These effects were reported only with magnetic fields that were considerably stronger than the envelope corresponding to the TR07 maglev. However, most of these experiments were done using convenient laboratory fields which were not intended to define threshold levels.

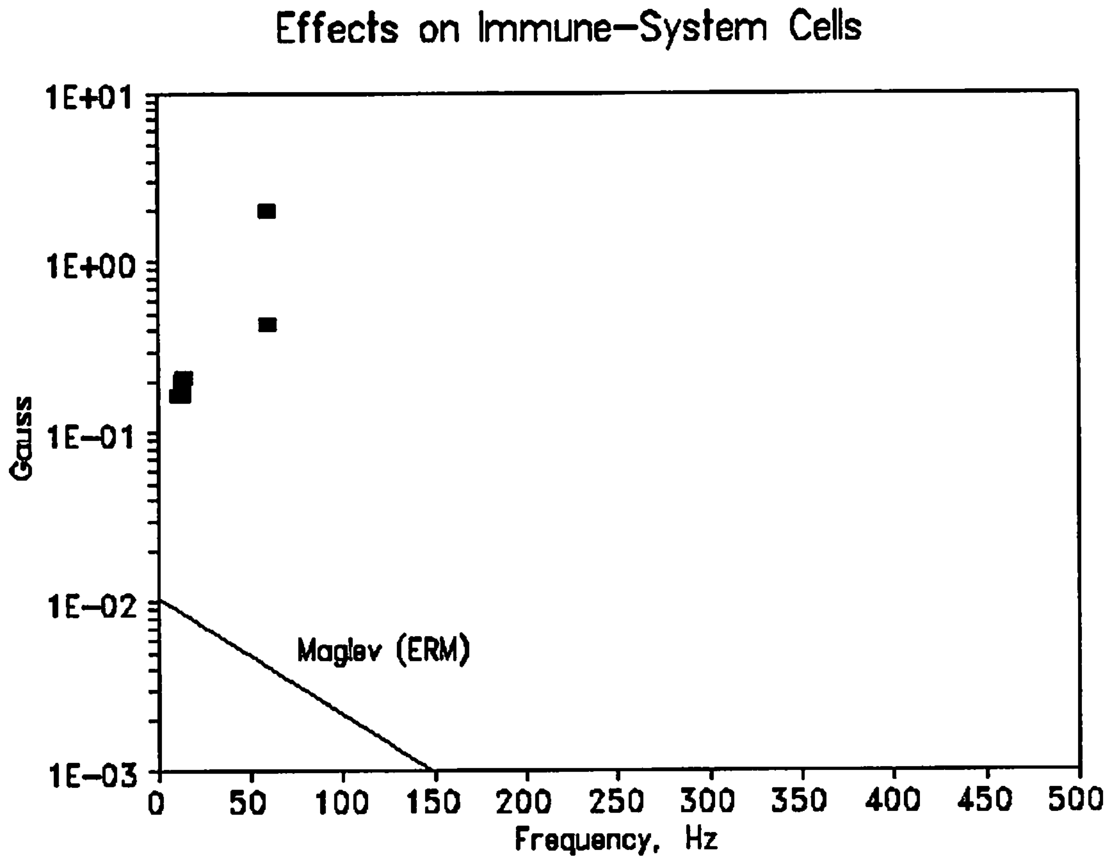

FIGURE 6-1. REPORTED IMMUNE-SYSTEM EFFECTS OF ALTERNATING MAGNETIC FIELDS RELATIVE TO ENVELOPE OF EXPOSURE ONBOARD THE TR07 MAGLEV.

7. PINEAL AND OTHER HORMONAL EFFECTS

7.1 PINEAL MELATONIN

The most interesting and potentially significant endocrinologic action of ELF EMF, and the one that is receiving much current attention, is the interaction with pineal function. The particular relevance of this effect to maglev type fields has been pointed out by Wilson *et al.* (1992). These workers are currently preparing a major literature review for the Environmental Protection Agency on possible bioeffects of maglev type fields, with an emphasis on pineal-based effects and mechanisms (*Broadband Magnetic Fields and Their Possible Role in EMF-Associated Bioeffects*, prepared under Contract No. 68D20185).

Exposure of rodents to 60 Hz EMF fields can abolish the pineal circadian rhythms for the synthesis and secretion of melatonin (Wilson and Anderson 1989; Wilson, Stevens, and Anderson 1989; Wilson, Chess, and Anderson 1985; 1986; Groh, Ehret, and Readey 1988); 60 Hz electric fields also reduce the nocturnal peak serum levels of this hormone (Leung *et al.* 1988a; Grota *et al.* 1991). This effect may be related to alterations in circadian rhythms of activity and metabolism in mice and rats exposed to 60 Hz electric fields (Groh and Readey 1990). Similar findings have been reported in Djungarian hamsters exposed to a 60 Hz, 0.1 mT sinusoidal magnetic field (Yellon 1991). Djungarian hamsters were exposed to the 60 Hz magnetic field for 15 min, beginning 2 hr before lights off, and that single exposure significantly ($p<0.05$) reduced the duration of increased melatonin in the pineal at night and blunted the nocturnal rise in serum melatonin. This result is remarkable because exposure occurred during the daylight period, producing an effect which lasted at least six hours into the dark period (while pineal effects reported with rats occur with EMF exposure during the dark period). Experiments are currently underway to characterize this effect using gene probes in order to determine which specific genes participate in biological clock mechanisms controlling pineal melatonin synthesis (Haggren *et al.* 1992).

A group at Battelle Laboratories initially reported suppression of nighttime 6-hydroxymelatonin excretion with continuous 60 Hz, 65 kV/m electric fields (Sasser, Morris, and Anderson 1990), or lower pineal melatonin (significant at $p = 0.054$) levels specifically with exposure to intermittent 60 Hz, 8 mT (80 G) magnetic fields (Wilson *et al.* 1990). These electric field effects were confirmed, at least for their effect on serum melatonin levels, by Grota *et al.* (1991). However, continued attempts to replicate the experiments at Battelle Laboratories with a 60 Hz electric field (65 kV/m) or 60 Hz, 0.5 mT magnetic field (Sasser *et al.* 1991a; 1991b), or with a DC magnetic field, $\pm$ 40 mT (reversed at 1 min intervals with a time constant of 2.7 ms), a 60 Hz sinusoidal 8 mT magnetic field, or a 3.8 Hz asymmetric sawtooth magnetic field (Sasser *et al.* 1992) have yielded results which were variable and often inconsistent. These authors attribute their failure to document a reproducible reduction in pineal melatonin levels in rats exposed to several different types of EMF exposure to experimental factors, such as phase shifts of the effect during the dark period, or to preexperimental factors, such as selective breeding practices by the animal supplier (Charles River Laboratory, Raleigh, NC) or stress during shipping. Further work is necessary to see if rodents which are genetically closer to the wild condition, like the Djungarian hamster, have a pineal system which is more strongly affected by ELF EMF than laboratory animals, which have been selected for many generations for rapid and dependable reproduction in all seasons. The human health implications of these studies rests on determining which animal model (the laboratory rat or the Djungarian hamster) is more similar to human physiology with respect to circadian physiology.

These studies have been extended to other species, but not all investigators have been able to reproduce EMF effects consistently in non-rodents. To date, a study carried out under the aegis of the Bonneville Power Administration (Chartier, Dickson, and Stearns 1991; Lee *et al.* 1992b) has failed to demonstrate any effects on endocrine systems and their circadian rhythms in Suffolk sheep penned for up to 10 months beneath a 500 kV transmission line. The mean 60 Hz electric field ranged from 4.1 to 7.5 kV/m, and the magnetic field from 1.5 to 5 μT. Control ewes were penned in ambient fields (<0.01 kV/m; <0.04 μT). A study carried out in baboons (*Papio cynocephalus*) used three different exposure conditions (Rogers *et al.* 1991; Rogers, Reiter, and Orr 1992). The first group was exposed to a variably-scheduled, intermittent, 30 kV/m (perceptible) and 0.1 mT (below the limit of perception) field with rapid onset. The magnetic field was produced first, followed in about 2 minutes by the electric field brought by relay closure to 14 kV/m, and then increased to 30 kV/m at 1.5 kV/m/s. Daily exposure was day-time only through the tenth day, and lengthened to include night-time by day 20. Significant reductions occurred in the nocturnal serum melatonin concentrations of exposed animals. On the other hand, six weeks of regularly scheduled, day-time exposure to 6 kV/m (not perceptible) and 50 mT fields, introduced over a 4 s period without rapid onset characteristics, was not associated with altered melatonin levels; although the latter did fall progressively in both exposed and control animals over the study period; similar results were seen with 30 kV/m and 0.1 mT fields.

Some efforts have been made to isolate the pineal response *in vitro* in order to characterize the response to EMF independent of neuroendocrine physiology. Some *in vitro* data with rat pineal glands (Lerchl *et al.* 1991b) was reported, indicating suppression of melatonin synthesis in whole rat pineal glands exposed to a 33.7 Hz, 44 μT (rms) sinusoidal magnetic field producing ICR conditions for the calcium ion. Leung *et al.* have been attempting to establish an *in vitro* exposure system for organ cultures of chicken pineal glands, and have established assay and culture conditions to the point where they can demonstrate that chicken pineal glands do maintain a strong rhythm of melatonin cyclicity *in vitro* for 3-7 days. Experiments are proceeding to examine the possible direct effects of magnetic fields on melatonin metabolism in the pineal using this system, but no effect of EMF on pineal melatonin production *in vitro* has been reported to date (Leung *et al.* 1989; Leung, Miller, and Anderson 1992). Luben *et al.* compared the short-term effects of exposure to low energy EMF, of the type used for clinical fracture healing (EMF pulsed at 72 Hz), on mouse bone cells, fibroblasts, and explanted mouse pineal glands. They reported that this EMF inhibited adenyl cyclase activation by the β-adrenergic agonist isoproterenol, apparently through lowering of receptor affinity (Luben, Huynh, and Morgan 1991). Luben has suggested that this may reflect a generalized effect on G protein-linked receptors common to all these tissues (Luben 1992).

One of the possible reasons for a variation in pineal responses to different types of EMF exposure lies in the characteristics of the field onset. In humans, a study of pineal function in relation to the use of different types of electric blankets (Wilson *et al.* 1988), concluded that continuous polymer wire blankets, which switch on and off twice as frequently as conventional blankets and subject the user to a 50% greater magnetic field, are associated with significant but transient increases in 6-hydroxymelatonin excretion for several nights after onset and cessation of blanket use (Wilson *et al.* 1989; 1990). This dependence on field characteristics for an effect on melatonin is also found in rodent experiments. Lerchl *et al.* (1990; 1991a) and Reiter *et al.* (1990) described a fall in pineal serotonin N-acetyltransferase activity; increases in the melatonin precursor, serotonin, and a methylated metabolite 5-hydroxyindole acetic acid, indicative of altered melatonin metabolism; and, lower melatonin levels in rats exposed to weak static magnetic fields (8×10^{-5} T) that underwent rapid automated (5 ms) reversal at 1 min intervals. No effect on pineal melatonin patterns was observed when manual reversal (1 s) was used. Further study of this system indicated that both induced eddy currents and inverted EMF were needed to affect the pineal; and also, that the latter is more sensitive during the plateau and declining phase of melatonin production (Reiter *et al.* 1991; 1992).

7.2 STRESS HORMONES

A concept has developed that seeks to explain the biological interactions of EMF on the basis that such fields act as generalized stressors, setting up changes in the neuroendocrine axis that lead to a range of biological consequences. Stress is known, for example, to produce increased plasma levels of adrenocorticosteroids, which, in turn, create changes in physiological functions such as heart rate, and have also been associated with decreased immune response, one of the secondary effects of which could be an enhanced rate of tumor growth (Riley 1981).

Studies aimed at demonstrating an activation of the hypothalamo-hypophyseal-adrenal axis by EMF have produced equivocal results. A report from the Soviet Union (Udintsev, Ivanov, and Moroz 1986) indicated that in rats exposed to an alternating magnetic field (50 Hz, 20 mT) the hypothalamo-hypophyseal-adrenal system was activated. Plasma concentrations of 11-hydroxyketosteroids and ACTH were elevated, as were plasma and tissue levels of free fatty acids and phospholipids, but after prolonged, repeated exposures the activity of the endocrine axis was inhibited. Similar activation of the adrenals and thyroid was shown in mice (Kartashev and Ivanova 1988). In contrast, Quinlan *et al.* (1985) failed to show generalized activation of the hypothalamo-hypophyseal-adrenal axis, although the level of growth hormone was elevated in exposed rats.

There was evidence of increased lipid peroxidation after chronic exposure to EMF. Such an activation does indeed resemble a type of stress reaction. Another piece of evidence suggestive of a stress reaction in rats exposed to a 60 Hz, 100 kV/m electric field is chromodacryorrhea, the secretion of porphyrin by the Harderian glands (Leung *et al.* 1988a). This response was observed with high level electric field exposure, however, not lower-level magnetic field exposure. Ragan *et al.* (1983) and Michaelson and Lu (1988) were unable to detect a stress reaction in rats exposed to 80-100 kV/m 60 Hz electric fields. Another group reported that prolonged exposure of rats to strong 60 Hz electric fields slightly lowered the plasma levels of corticosterone, together with those of testosterone and prolactin, but effects on thyroid activity and follicle-stimulating hormone were equivocal (Free *et al.* 1981). Studies carried out with rat adrenal tissue *in vitro* indicated that 60 Hz fields, while not directly altering steroidogenic activity, greatly stimulated the response to ACTH (Lymangrover *et al.* 1987).

Other endocrinologic effects that have been reported include evidence based on tissue iodine contents of suppressed thyroid activity in rats exposed to a weak field (50 Hz, 0.11 mT) for 20 or 30 days (Marsakova 1982), and a reduction in insulin release by isolated rabbit islets of Langerhans (Jolley *et al.* 1983).

7.3 THE ROLE OF ENDOCRINE EFFECTS IN THE POTENTIAL HEALTH IMPACT OF ELF EMF

These reported endocrine effects are of strong interest because of endocrine interaction in the function of the immune system, behavior, and resistance to many diseases including cancer. Estrogens and androgens have long been known to play major roles in the etiology and growth of such tumors as breast and prostate cancers, and roles for other endocrine factors have been defined more recently. Elevated levels of prolactin have been associated with increased risk of breast cancer (Wang *et al.* 1987). Melatonin plays the role of an immunomodulator by stimulating immune responses (Maestroni, Conti, and Pierpaoli 1987), and has also been implicated in the direct control of cell proliferation and as a suppressor of the growth of transplanted tumors (Reiter 1988; Blask 1989a). Melatonin may, therefore, play a major role in the incidence of human breast cancer (Stevens 1987; Stevens and Davis 1987; Reiter 1988; Blask 1989b; Stevens *et al.* 1992). In a recent study, the oncostatic action of melatonin on the growth of estrogen positive MCF-7 breast cancer cells, which appears to involve a G1-S transition delay, was partially blocked by exposure to a sinusoidal 60-Hz, 1.05 μT magnetic field (Liburdy *et al.* 1992). These results suggest a possible mechanism for tumor promotion by ELF EMF, in which EMF field exposure upsets the nocturnal circadian peaks of melatonin, which normally suppresses the growth of environmentally-induced preneoplastic cells. At the present time, evidence for several links in this hypothesis is still tentative, and a great deal of experimental work remains to be done (Wilson and Anderson 1989; Goldberg and Creasey 1991).

In summary, the most consistent and potentially significant of the hormonal responses to ELF EMF exposure appears to be the reported abolition of the pineal circadian rhythm for melatonin synthesis and secretion. Although recent failures to replicate rat experiments at Battelle have raised questions about the significance of this effect, it is a major direction of current research. Interference with circadian rhythms could potentially have wide ranging human health consequences, including effects on human work performance; therefore, research to establish whether or not this effect occurs, and its impact in humans, is vital.

7.4 GRAPHIC ANALYSIS OF NEUROENDOCRINE EFFECTS IN RELATION TO TR07 FIELDS

Figure 7-1 shows reported effects of time-varying magnetic fields on an aspect of the neuroendocrine system that has received particular attention, namely melatonin metabolism. Two of the reported effects involved 50 or 60 Hz magnetic fields at strengths that are within the envelope for the TR07.

Several of the reports of effects of magnetic fields on melatonin or related neuroendocrine products involved exposures that consisted of sudden inversion of the earth's magnetic field, typically within several tens of milliseconds. So far, these effects have not been explained in terms of a biological mechanism, and the reports themselves have not been independently confirmed. The frequency spectrum of the time-varying magnetic and induced electric fields corresponding to this exposure is complex, but presumably concentrated at higher frequencies than relevant to TR07 maglev exposure.

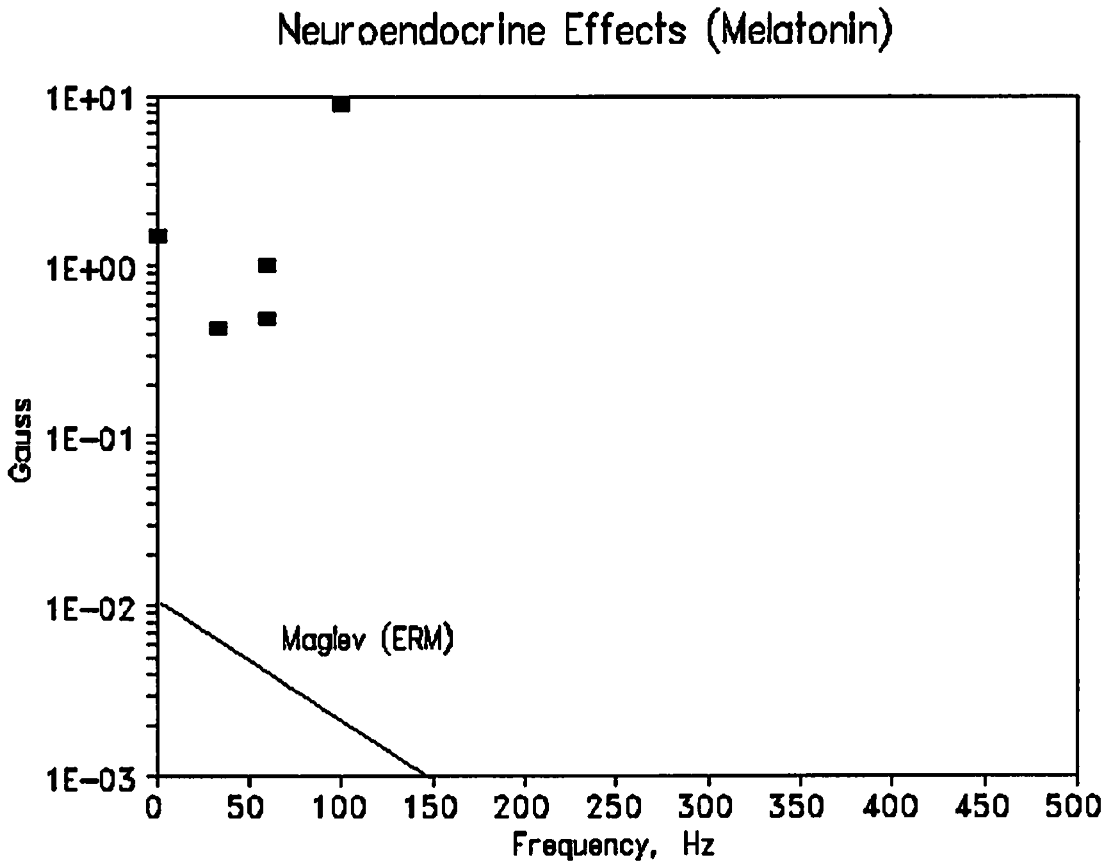

FIGURE 7-1. REPORTED EFFECTS OF ALTERNATING MAGNETIC FIELDS ON NEUROENDOCRINE SYSTEMS OF ANIMALS, IN COMPARISON TO THE ENVELOPE OF MAGNETIC FLUX DENSITY ONBOARD THE TR07 MAGLEV.

8. PHYSIOLOGICAL EFFECTS

Sections 7 through 9 discuss reports of bioeffects which involve a variety of physiological responses in an integrated neuroendocrine system. It should be realized that many of these responses involve several of the subject categories outlined in this report and, therefore, do not easily fit within the report structure. Cross references to other sections are made where appropriate. In this section, we describe some of the acute and short-term effects of ELF EMF (which may or may not have long-term health consequences). There are some other effects of ELF EMF discussed in this section of the report which could clearly be termed "physiological" since they fall within the range of normal physiological variation and are not generally considered a health hazard.

8.1 ACUTE PHYSIOLOGICAL EFFECTS IN HUMANS

There are a number of reports in the literature describing general health complaints of workers exposed to ELF fields, some of which, if valid, indicate a potential negative impact of ELF EMF exposure.

The first reports of adverse human health effects of lower level ELF EMF exposures came from the Soviet Union in the early 1960s. Several studies of workers in high-voltage switchyards (exposed to electric fields up to 26 kV/m) reported subjective symptoms of central nervous system dysfunction, and physiological symptoms such as hematological changes, sinus arrhythmia and tachycardia, and abnormalities in electroencephalographic patterns (see, for example, Asanova and Rakov 1966). Similar effects were reported in Soviet workers exposed to static magnetic fields in the manufacture of permanent magnets.

Although the body of Soviet studies reported such acute behavioral and health effects as listlessness, excitability, headache, loss of sex drive, drowsiness, and fatigue among electrical switchyard workers; other studies on substation and transmission line workers in Canada, England, France, West Germany, Italy, and the United States have failed to confirm these effects (Sulpor 1979). In order to investigate the possibility of chronic health effects resulting from high-voltage exposure, epidemiologic data on 53 substation workers, with more than 5 yr of exposure to electric fields of 400 kV, were compared with data for a matched reference group of 53 nonexposed workers at the same power stations in Sweden (Knave *et al.* 1979). The investigation included effects on the nervous system, the cardiovascular system, the blood, and fertility. The results showed no significant differences between the exposed and reference groups as a result of long-term electric field exposure. In an acute health effects study of 26 experienced Swedish linemen, Gamberale *et al.* found no significant effects on reaction time, complex reaction time, perceptual speed, short term memory, encephalographic recordings (EEG), or blood hormone levels (levels of thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, prolactin, cortisol, testosterone, and neopterin) which could be related to measured field exposure. Mean exposure of linemen to ELF EMF during a simulated work day was 2.8 kV/m (range of 2.1-3.6 kV/m) for electric fields, and 23.3 μT (range 14.7-24.8 μT) for magnetic fields (Gamberale *et al.* 1989).

Thus, most of the studies designed to follow up reports from the Soviet Union of acute and long-term negative health effects from EMF exposure failed to confirm these effects. Health effects reported in the Soviet studies may be attributable to other factors in the work environment, such as exposure to solvents.

8.1.1 Heart Rate

There were reports of static and time-varying magnetic field effects on respiration and heart rate in the Soviet literature for some time (for example, see, Medvedev, Urazaev, and Kulako 1976), but the effect has only recently been systematically investigated in the West. Intermittent exposure of healthy human volunteers to combined electric and magnetic fields (9 kV/m, 20 μT), in a sophisticated environmental exposure system at the Midwest Research Institute, has been shown to produce statistically significant alterations in cardiac interbeat interval (slowing of heart rate) and psychological effects (alterations in evoked response) which seem to depend primarily on the magnetic component of the EMF (Graham *et al.* 1988; 1991a). Furthermore, there is significant variation in the response to intermittent magnetic fields in different individuals, depending in part on the individual's heart rate prior to exposure and the change in systolic blood pressure (Graham *et al.* 1991b; 1992). These effects fall within the range of normal physiological variations (comparable to the effects of mild exercise or fatigue). Such effects would only be of concern if they have subtle negative effects on human performance that might enhance the risk of accidents, produce a much stronger effect in interaction with other stressors, or produce more extreme effects in particularly susceptible individuals. At the present time there is no evidence that these physiological effects constitute a health hazard. However, in contrast to the dramatic but poorly documented reports of psychophysiological responses in the earlier literature, these effects represent well documented human responses to ELF magnetic fields.

8.2 MAGNETIC FIELD SENSING

There is a substantial body of literature on magnetic field sensing and its behavioral consequences in non-human vertebrates and invertebrates. In many organisms, the magnetoreceptive sensory apparatus is based on ferromagnetic compass-needle-like particles consisting of uniformly magnetized (single-domain) crystals of magnetite (Fe3O4). Recent technical developments in the ultrasensitive Superconducting QUantum-Interference Device (SQUID) magnetometer have given researchers the ability to detect and characterize nanogram levels of fine-grained magnetite within large tissue volumes (Kirschvink and Nesson 1989).

Species of bacteria and algae have been found to have magnetite-containing organelles, called magnetosomes, which provide the cells with a degree of magnetotaxis. Magnetotactic bacteria collected in New England have been reported to swim towards the north pole in the ambient magnetic field, be that the geomagnetic field or a localized experimental field, and reversal of those fields was associated with an immediate "U-turn" reversal in the direction in which the bacteria were swimming (Frankel and Blakemore 1989). Geomagnetic sensitivity extends over a range of phyla. There is evidence for geomagnetic sensitivity in bacteria, eukaryotic algae, honeybees, sharks and rays, sockeye salmon, tuna, homing pigeons, migratory birds, salamanders and newts, wood mice, and cetaceans (Kirschvink 1989). Magnetic sensitivity has been linked in behavioral studies with the geomagnetic navigational abilities of animals (the ability to discriminate subtle variations in the magnetic field strength as a directional cue). Concern over possible adverse effects of installations, such as the U.S. Navy's ELF Communications System, on insect behavior or the migration of birds has made these effects a focus of environmental impact studies (Zapotosky 1990). In general, they indicate minimal effects resulting from the intermittent fields used for communication on the distribution of biota.

Work on magnetic field sensitivity in laboratory rodents has been conducted preliminary to behavioral experiments (Smith, Clarke, and Justesen 1990). Indications of behavioral responses to artificial magnetic fields, specifically altered direction finding, changes in circadian activity levels, decreased emotional reactivity, and reduced morphine-induced analgesia, have been observed in the European woodmice, gerbils and hamsters, rats, and mice (Olcese, Reuss, and Semm 1988). The involvement of nocturnal activity in many of these behaviors has focused attention on the pineal gland as a possible target of EMF interactions.

Some animals have remarkably sensitive organs for detecting electric fields in an aquatic environment. The platypus (*Ornithorhynchus anatinus*), a nocturnal diving monotreme, has been trained to attack a 1.5 V miniature alkaline battery by reinforcing the behavior with a piece of shrimp. Sensitivity in one series of trials indicated 100% success in detecting a 2 mV/cm gradient 10 cm away, with occasional search bouts with a gradient of 300 μV/cm at 30 cm. The threshold values for this response were 500 μV/cm for a male, and 500, 100, and 50 μV/cm for three females (Scheich *et al.* 1986). Although in many cases the physiologic basis for the ability to sense weak electric fields is unknown, in other cases specialized sensory organs are well characterized. One of the best understood is the electroreceptor of elasmobranch fishes, the ampullae of Lorenzini, which mediates behavioral responses to environmental electric fields as weak as 5 nV/cm (Kalmijn 1992) and changing field gradients as weak as 6 nV/cm/s (Brown *et al.* 1979). The adaptive function of this electrical sensitivity may be related, in some cases, to the detection of weak electrical fields given off by the neural activity of prey species. In other cases, it may be associated with the detection of compass direction by using the weak fields induced in tissues as the animal moves through lines of force in the geomagnetic field.

Mammals have generally been thought to show low sensitivity to electric fields and virtually no ability to detect magnetic fields. For example, in an assessment of rat sensitivity prior to behavioral experiments, Smith *et al.* found rats were unable to detect a 7, 16, 30, 60, or 65.1 Hz sinusoidal magnetic field at flux densities of 200 to 1900 μT (2 to 19 G), or square wave magnetic fields at the same frequencies at flux densities of 290 to 1360 μT (Smith, Clarke, and Justesen 1991). Creim *et al.* found that rats showed no evidence of avoidance behavior in a magnetic field of 3.03 mT (30.3 G), which produces approximately the same internal body currents as exposure to a 100 kV/m, 60 Hz electric field, previously shown to produce avoidance behavior in rats (Creim *et al.* 1985).

Of greater interest in assessing the possible human health impact of ELF magnetic fields is the question of human sensitivity to weak magnetic fields. Gamberale (1990) has reviewed the physiological and psychological effects of exposure to extremely low-frequency electric and magnetic fields in humans. The threshold for perception of an ELF electric field is approximately 5-15 kV/m, and perception of magnetic fields (as magnetophosphenes) occurs in excess of 10 mT at frequencies over 10 Hz. In other experiments, humans have detected magnetic fields as low as 3 mT for 10 Hz pulsed fields, and as low as 0.5 mT for "sweeping magnetic fields." Subjects in preliminary experiments for behavioral studies conducted as part of the New York State Powerlines Project were unable to detect a magnetic field at any of the field strengths tested (up to 40 μT), and 90% of the subjects had a threshold of perception of 9 kV/m for the electric field while in the seated test position (Graham and Cohen 1985). There are reports of individuals who claim an unusually high sensitivity to low level EMF, and at least one study indicates that highly sensitive individuals show frequency-specificity in the EMF to which they are most sensitive (Rea *et al.* 1991). In another case, a highly "sensitive" individual showing severe clinical symptoms exhibited no real sensitivity to EMF when tested in a blinded protocol (Gyuk, Dietrich, and Wisecup 1989).

In contrast to these threshold assessments based on conscious perception, there is a curious report of human magnetic sensitivity from an investigator at the Zoology Department of the University of Manchester. Baker conducted a study in which subjects were blindfolded and reoriented in a rotating chair or by a ride in a van, then they were tested for their ability to estimate the direction of "home" (relative direction) and the relation of that direction to the compass direction. Results showed that humans had a weak but significant ability to perceive and name compass directions when blindfolded. Homeward orientation by inexperienced human subjects in van experiments (h = 0.205) compared favorably with the ability shown by inexperienced homing pigeons (h = 0.15-0.25). Magnets on the back of the head had little if any influence on compass orientation, but magnets on the forehead or between the ear and the eye had a significant influence on orientation and navigation. A weaker effect was seen when magnets were located behind the ear. Exposure to magnets for only 10-15 min before a trial resulted in an improvement in orientation ability in both chair and walking experiments that lasted for at least 1.5 h (Baker 1988). Similar anecdotal reports of magnetic sensitivity have been associated with dowsing (the ability to sense the presence of water below ground) (Sheppard and Eisenbud 1977). One report claimed to have localized the magnetic sensing organ in the kidney area. This was accomplished by shielding areas of experienced dowsers' bodies and following the sensitivity to microwatt level radiofrequency EMF (Harvalik 1978).

Claims of low-level human magnetic sensitivity have not generally been accepted, but that view may change as a consequence of a new anatomical finding by Kirschvink *et al.* of magnetite structures in human tissues, including the brain and in a variety of tumor materials. Using an ultrasensitive superconducting magnetometer in a clean-lab environment, saturation remanent magnetic moments of pia and dura revealed the presence of between 1 and 10 million crystals of single-domain magnetite per gram, or volume concentrations between 4 and 100 parts per billion. Particle extracts from solubilized brain tissues examined with high-resolution transmission electron microscopy and electron diffraction further identified the material as magnetite, with a bimodal crystal size distribution. Smaller prismatic crystals (approximately 50 nm in diameter) showed a morphology strongly resembling those precipitated in the magnetosome chains of magnetotactic bacteria and fish. The larger magnetite particles found in human brain material were 200 nm in diameter. Kirschvink has proposed a possible mechanism for low-level magnetic field effects based on calculating the force needed to move these larger particles (a minimum ELF EMF component with an amplitude of 140 μT at 60 Hz) (Kirschvink and Kobayashi-Kirschvink 1992). While the present physiological evidence for the ability of humans to sense magnetic fields of any amplitude is weak, this anatomical finding will add credibility to any future claims of magnetic field sensing ability in humans; and, suggests the possibility of an evolutionary continuum in physiological sensitivity to weak electromagnetic fields.

9. BEHAVIORAL AND NEUROLOGICAL EFFECTS OF ELF EMF

9.1 BEHAVIORAL RESPONSES IN HUMANS

Laboratory experiments, mainly by scientists in the Federal Republic of Germany, have documented effects of ELF EMF on human hematopoiesis, behavior, and the nervous system (Gamberale 1990). Exposure to 2.5 V/m 10 Hz electric fields was reported to shorten the circadian rhythm by more than one hour, and shielding from the geomagnetic fields prolonged the circadian rhythm by about 20 min. In these experiments, exposure to higher intensity static magnetic and electric fields (up to 600 V/m) had no effect on circadian rhythm. In another series of experiments, human volunteers were exposed to 1-20 kV/m for periods of up to 5 h with only minor changes within the normal physiological range in some blood cell variables and a slight positive effect on fatigue in a psychomotor task. Other experiments involving exposure to 50 Hz magnetic fields (0.3 mT) and exposure to electric fields of up to 20 kV/m were negative. Some field-related effects have been reported in studies from the United States. Subjects exposed for 6 h to 9 kV/m and 19 μT showed changes in evoked potentials after acoustic and optic stimuli, and a field-related decrease in resting heart rate. In studies conducted in England, exposure to a 50 Hz field, equivalent to 36 kV/m, produced changes in mood and a decrement in performance of a reasoning task. A recent study which evaluated the acute effects of exposure to ELF EMF in line workers (mean exposure to 2.8 kV/m and 23.3 μT), showed no significant effects attributable to field exposures. Gamberale concluded that the general impression to be gained from this research is that ELF EMF exposure does not constitute a health hazard for the general public or for the occupationally exposed worker, at least with respect to acute physiological and behavioral effects.

Animal experiments (discussed in Section 7) have indicated that ELF EMF can function in a manner analogous to a strong light stimulus, blocking or delaying the normal nocturnal peak of pineal melatonin synthesis. The only study to examine this effect directly in humans was an electric blanket study conducted at Battelle Pacific Northwest Laboratories. Wilson and coworkers exposed 42 human volunteers to fields from several different types of electric blankets producing different ELF EMFs, and followed the long-term effects on nocturnal melatonin peaks as measured by urinary 6-hydroxy melatonin sulfate (6-OHMS) excretion (Wilson *et al.* 1988; 1989). Results indicated a greater pineal-suppressive effect among certain subjects using a blanket design (continuous polymer wire) that has a shorter duty cycle, and consequently, subjects the user to 50% higher magnetic field levels (Wilson and Anderson 1989). This result is consistent with observations that rats show a greater inhibition of nocturnal melatonin peaks when exposed to interrupted (60 s on/ 60 s off) 60 Hz EMF than when exposed to a steady 60 Hz field (Wilson *et al.* 1990). A possible role for suppressed nocturnal melatonin peaks in behavioral effects, like seasonal depressive disorder ("low-melatonin syndrome"), is discussed by Wilson (1988). Epidemiologic studies suggesting an association between residential proximity to power line fields and increased incidence of depression and suicide are discussed in Section 12.2 of this report.

There are a variety of reports of human psychological responses to ELF EMF which are largely unreplicated by other groups and consequently difficult to evaluate. Persinger and coworkers have reported a range of mood and performance differences associated with weak magnetic field exposures. In one study, a group of students were evaluated for their suggestibility before and after an approximately 15 min exposure to either sham, 1 Hz, or 4 Hz magnetic fields applied across their midsuperior temporal lobes (field strength at the distance of the temporal cortex was approximately 0.2 μT, at the frontal lobes about 20 μT, and at the hippocampus about 50 μT). Subjects who had been exposed to the 4 Hz field were reported to show a significant decrease in heart rate compared to other groups, and to show more imaginings and more references to vestibular experiences (self or entity rising or floating); thus, supporting the hypothesis that weak brain frequency fields may influence certain aspects of imaginings and alter suggestibility (De Sano and Persinger 1987). Persinger has also published a number of retrospective analyses showing that rapid change in geomagnetic field strength (temporal variations of 10 μT/s) can be correlated with psychological effects such as increased psychiatric admissions and reports of hallucinations (Persinger 1987b). This concept is supported by an independent study reporting a correlation between the occurrence of visual hallucinations and a measure of geomagnetic activity (Spearman coefficient of 0.64; $p<0.05$, two-tailed test). Although the authors of this report indicate that a cause-and-effect relationship is not necessarily implied, they discuss a possible mechanism involving magnetic influences on the pineal hormone melatonin (Randall and Randall 1991).

Based on subjective clinical observations of 30-50 patients who displayed neurotic labile personality characteristics associated with highly active geomagnetic days, Grunner treated 28 patients suffering from vasomotor headaches with a homogeneous 9.64 mT field. All patients reported that the intensity of the headache either declined or disappeared with exposure to the field, and that there was a statistically significant increase in forehead skin resistance during exposure to the field but not during sham field exposure (Grunner 1989).

9.2 ANIMAL BEHAVIOR EXPERIMENTS

A number of long-term ELF EMF exposure studies have been conducted using behavioral endpoints. Extrapolating from animal behavior to human performance or psychological effects is always problematic, but perhaps less so for non-human primate animal models. A number of studies have been conducted in the baboon colony at the Southwest Foundation for Biomedical Research in San Antonio, TX. When a social group of eight male baboons was exposed to a 30 kV/m, 60 Hz electric field over three, six-week periods (12 h/day, 7 days/wk), behavioral categories of passive affinity, tension, and stereotypy were most responsive (Coelho, Easley, and Rogers 1991a). The observed behavioral differences indicated a stress response to the electric field, but it is not clear if these effects are harmful or permanent. More recent experiments by the same group have examined the effects of combined electric and magnetic field exposure. Initial analyses indicated that the behavioral responses of the animals exposed to the 6 kV/m, 50 μT electromagnetic field did not produce the levels or pattern of behavioral changes observed in earlier 30 kV/m or 60 kV/m electric field experiments (Coelho, Easley, and Rogers 1991b; Orr and Rogers 1991).

A more controversial and problematic body of research literature exists regarding the ability of ELF EMF to alter conditioned behavior in rats. A number of research workers have reported the ability of weak ELF electric or electric and magnetic fields to modify behavior, mostly functioning as a non-specific stressor degrading performance on learning tasks or increasing arousal. Le Ruz and Colin reported a reduced threshold to excitement and lower motor activity in newborn Wistar rats exposed to a video display terminal field (Le Ruz and Colin 1991). Rudolph *et al.* (1985) reported that short-term (4 h) exposure to a weak, 50 Hz electromagnetic field which reversed the natural horizontal component of the earth's magnetic field 50 times/s, produced an increase in rearing behavior and ambulation that was interpreted as an increase in "non-specific excitability level" when the field was applied at the beginning of the light phase but not the dark phase. A similar sensitivity to the onset of field exposure was shown in mice given short, 4 h exposures to 60 Hz electric fields (30-50 kV/m), determined to be a "startle" response or field awareness during the first hour of field onset at all circadian phases tested (Groh and Readey 1989). The most significant circadian-phase-dependent response to electric-field exposure seen in these animals, phase advance of the activity rhythm peak (acrophase), occurred in mice adapted to a long light entrainment schedule (LD 16:8) at the dark-to-light and light-to-dark transitions.

Lovely (1988) has reviewed reports on the behavioral effects of exposure to ELF electric and magnetic fields, primarily in the frequency range of 1-300 Hz, and offered the opinion that the toxicological orientation of much of the published research in this field is based on the missions of power industry and Department of Energy research programs to evaluate the health effects of 50 and 60 Hz fields. Indices of detection (ELF-induced arousal responses and changes in the level of activity following ELF exposure) or aversive behavior have been reported in rats after exposure to 50 or 60 Hz electric fields, but remarkably few robust behavioral effects have been reported. Those that have been reported probably relate to the animal's perception of an electric field rather than to any neurotoxic effects.

In order to investigate possible long-term effects of EMF exposure on behavior, Sprague-Dawley rats were exposed or sham-exposed to a combined 60 Hz, 30 kV/m electric field and a circularly polarized 60 Hz, 0.1 mT magnetic field produced using two sets of three vertical and three horizontal coils fed by currents 90 degrees out of phase (Salzinger *et al.* 1990). Animals were exposed from the day of conception to eight days after parturition, and the effect of this perinatal exposure on the operant behavior of the rats was evaluated. When observed as adults, rats responded at consistently lower rates after a period of conditioning than did sham-exposed controls. Sienkiewicz *et al.* have also reported small but statistically significant behavioral differences in CD1 mice exposed for the period of gestation to a 20 mT, 50 Hz magnetic field (Sienkiewicz, Robbins, and Saunders 1991). These behavioral effects, while not necessarily indicative of a deleterious effect of ELF EMF exposure, are a lasting effect showing greater robustness over time and changing conditions than some of the short-term studies. For some time, Adey, Bawin and their coworkers have suggested that ELF EMF has the potential to interact with electrical activity of similar frequency in the central nervous system, producing various alterations in circadian rhythms and electroencephalogram rhythms through changes in the ion conductivity of neural membranes (Adey and Bawin 1979).

Thomas, Schrot, and Liboff (1986) reported that operant behavior in rats could be affected by a combination of a 60 Hz magnetic field and a very small static magnetic field (26 μT, about half the geomagnetic field intensity). Rats exposed to this combination for 30 min consistently exhibited changes in the rate and pattern of response during the differential reinforcement of the low rate (DRL) component of a multiple fixed ratio DRL reinforcement schedule. By contrast, there were no measurable changes following exposure to the static field alone, or to the oscillating field alone. These authors have suggested a frequency/intensity specific ion cyclotron resonance (ICR) mechanism for the effect, as well as a range of other bioeffects, specific in this case for lithium. Smith (1988) supported a lithium-based mechanism for behavioral effects with a review of evidence indicating that lithium, which has been used as a therapeutic agent in behavioral disorders, is a normal trace constituent of animal and human tissues. However, Stern and Laties (1990) were unable to replicate these results using similar methodology.

Using ELF fields shown to have effects on Ca^{++} ion efflux, Lovely *et al.* have reported the ability of such fields to disrupt radial arm maze (RAM) memory in rats. The exposure system produced uniform (±5%) ELF and DC magnetic fields (vertical and horizontal) within the RAM. Field strengths were a DC magnetic field of 26 μT (260 mG) combined with a 60 Hz magnetic field of 50 μT rms (Lovely *et al.* 1989). Preliminary analysis of errors/group using a repeated measures analysis of variance (ANOVA) approached significance ($p<0.086$), with the exposed group making more errors and, when combined with results of a similar previous study, indicated a significant increase in errors made by the exposed groups ($p<0.015$) (Creim *et al.* 1990). With additional experiments, results were not always consistent and often indicated substantial trends between groups rather than clear-cut statistically significant differences (Lovely *et al.* 1991; 1992). Lovely *et al.* determined that because RAM task difficulty was minimal, rapid learning (a "floor effect") made discrimination of EMF exposure effects difficult. When rats were pretrained under sham-exposed conditions, no significant deficits were observed. However, when rats were always exposed to ICR magnetic field conditions, they made significantly more errors than sham-exposed controls ($p=0.055$). These results were interpreted as indicating that ICR fields affect learning as opposed to working memory.

Other studies have shown ELF EMF behavioral effects under specific frequency/intensity conditions not necessarily conforming to ICR conditions. Greater variability in time-dependent behavior (task performance following a tone stimulus) was observed in rats exposed to a 65.8 Hz electric field compared to a range of other frequencies tested from 45 Hz to 78.9 Hz (Stell *et al.* 1987). Changes in short-term memory in 54 random-bred male rats during development of a delayed reaction conditioned reflex, were reported after exposure to an alternating magnetic field (AMF) with a frequency of 5 and 8 Hz and field strength of 0.005 mT. The degree of changes in conditioned reflex activity depended, to some extent, on the frequency of AMF. The number of correct responses after exposure to 5 Hz AMF decreased to 81.0 ± 4.35, compared with 70.7 ± 2.0% after exposure to 8 Hz AMF ($p = 0.01$) (Sidiakin *et al.* 1987).

Magnetic fields have been reported to have interactive effects with opioids and, by implication, with endogenous opioid receptors. Ossenkopp and Kavaliers reported that time-varying magnetic field exposure inhibited the analgesic and locomotory effects of morphine in CF-1 and C57BL mice, respectively. When CF-1 male mice were chronically exposed for 5-10 days to a rotating magnetic field (RMF: 0.15-9.0 mT, produced by an apparatus consisting of two horseshoe magnets rotating about their major axes which generates a very heterogeneous time-varying field of about 0.5 Hz), the day-night rhythm in analgesia was eliminated by reducing the nocturnal levels to those found during the day. Acute exposure to the RMF for periods of 60 min, significantly reduced both day-time and night-time levels of morphine-induced analgesia, and was also found to inhibit effects of the mu, kappa, and delta agonists, but not the prototypic sigma agonist. Mice receiving daily injections of morphine develop tolerance to the effects of the morphine, but brief exposures (30 min) to the RMF prior to daily injections of morphine resulted in significantly reduced levels of tolerance. Exposure of both CF-1 and C57BL mice (subjected to immobilization for 30 min) to RMF significantly reduced the stress-induced analgesia in CF-1 and hyperactivity in C57BL. Ossenkopp and Kavaliers have suggested that these biomagnetic effects may be mediated by alteration of pineal function after magnetic field exposure, or by changes in central calcium levels in brain tissue (Ossenkopp and Kavaliers 1989).

Finally, there are screening studies which report a lack of behavioral effects for fields which simulate normal occupational ELF EMF exposure. One example is the report by Davis *et al.* of a lack of behavioral alterations in mice exposed to either a 1.5 T DC field or a 60 Hz, 1.65 mT (rms) AC field (Davis *et al.* 1984).

9.3 NERVE GROWTH AND MEMBRANE REACTIVITY

Due to the typical interactions of neural and neuroendocrine tissues with electric charges, one would expect to observe a response of these tissues to ELF EMF (Anderson 1989). As has been pointed out previously, ELF EMF strong enough to induce electric fields in peripheral nerves or central nervous system tissue close to the action potential, are several orders of magnitude higher in intensity than maglev or other common environmental ELF EMFs. On the other hand, factors such as membrane nonuniformity, membrane nonlinearity, intracellular currents, effects of pericellular currents tangent to the membrane, effects of tissue microstructure on pericellular currents, and membrane dielectric properties can increase the effective field level at the neurone membrane. For example, Sheppard and Adey have calculated that as frequency approaches DC, the field-generated transmembrane voltage in large invertebrate cell axons or dendrites increases greatly from 3% of the applied ELF EMF voltage at 60 Hz, to 16% at 10 Hz, to 99% at 1 Hz (Sheppard and Adey 1992).

Some changes in nervous tissue have been reported in environmental fields of relatively high intensity. Neural tissues from rabbits exposed for 23-24 h/day under a 50 or 60 Hz 400 kV line (an electric field intensity estimated at 14 kV/m, and magnetic field intensity at 1.2-6.0 μT) showed variable loss of Nissl bodies in large nerve cells of the cerebellum, hippocampus, superior colliculus, and certain thalamic regions with concomitant loss of microtubules and the appearance of lamellar structures. Similar changes were observed in rats and mice. In laboratory preparations, exposure of frog sciatic nerve to a 50-1000 nA electric current at 16 Hz for 17 h decreased the number of microtubules and increased number of axonal filaments in the neurons. Similar but less extensive changes were also seen at 50-60 Hz (Mild 1988). Persinger, on the other hand, reported only minor changes in the degranulation of brain mast cells in albino rats exposed to weak rotating magnetic fields, which he considered within the normal physiological range (Persinger 1987a).

DC electric currents are known to stimulate and direct elongation of nerve axons *in vitro*. Enhanced nerve growth and regeneration has also been reported *in vivo* in operated animals exposed to pulsed EMF similar to fields used to promote healing in bone and soft tissues. This literature on enhanced nerve growth (healing) is discussed in the context of other low-level pulsed EMF effects in Section 10.3 of this report.

9.4 GRAPHIC ANALYSIS OF BEHAVIORAL/NEUROLOGICAL EFFECTS IN RELATION TO TR07 FIELDS

Figure 9-1 summarizes several reported behavioral effects of alternating magnetic fields. Most have occurred at flux densities considerably higher than those associated with the TR07 maglev; however, three reported effects occurred at flux densities within the envelope.

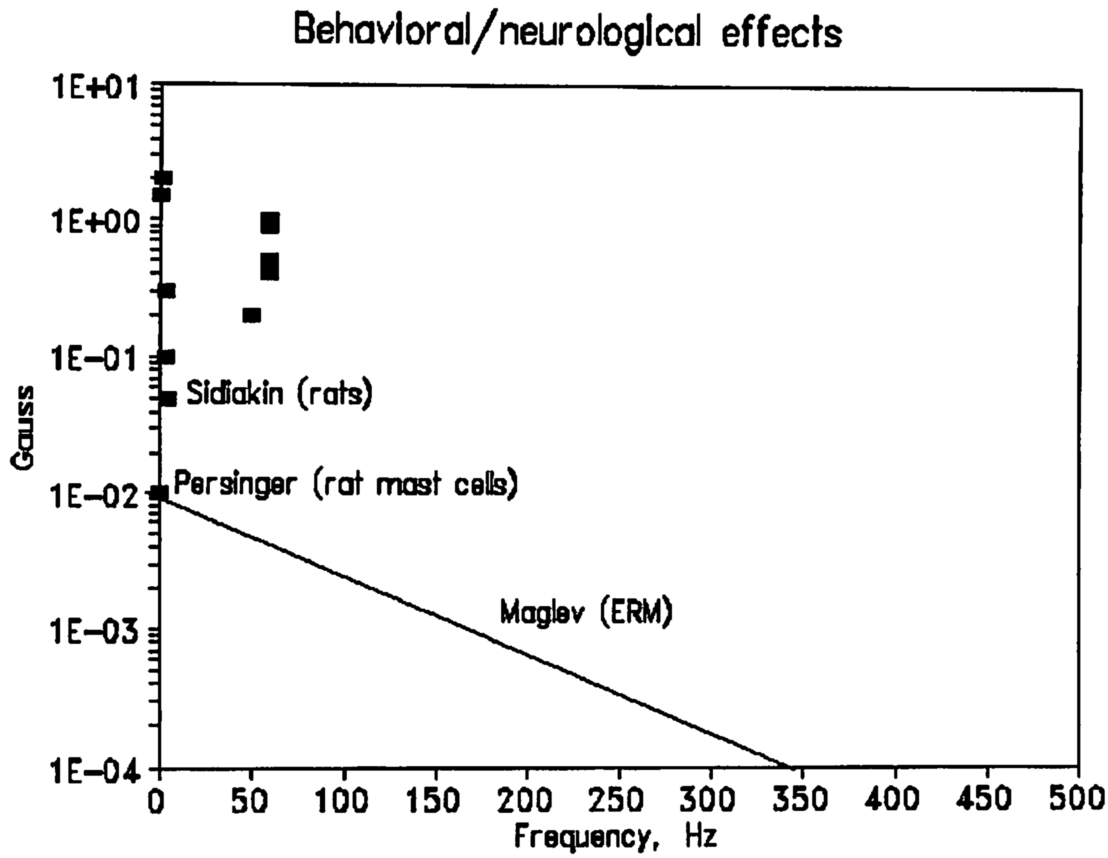

FIGURE 9-1. REPORTED BEHAVIORAL/NEUROLOGICAL EFFECTS OF ALTERNATING MAGNETIC FIELDS

10. CELL PROLIFERATION AND GENE EXPRESSION

10.1 IN VITRO EFFECTS ON CELL GROWTH

There are currently a few reports on the effects of ELF EMF on proliferation in normal cell lines. In one such study, Parola *et al.* described the transformation of normal chicken embryo fibroblasts exposed to sinusoidal 100 Hz EMF at 0.7 mT (7 G) for 24 h (Parola, Porat, and Kiesow 1988). A doubled growth rate, reduced adenosine deaminase activity and increased membrane lipid microfluidity were the criteria of transformation. These changes resembled those of viral transformation by RSV and did not result from thermal effects. Danielova *et al.* examined the effect of 3.125, 12.5, and 25 Hz pulsed magnetic fields (25 mT) on a variety of cell cultures, including lymphocyte hybridoma and mouse macrophage cell lines, either in monolayer or cell suspension. Cells were exposed for 1 h at 37°C, once or repeatedly, at 1-day to 7-day intervals. Growth characteristics of cells were not significantly different following one passage; but with repeated exposure, serially passaged stable cell lines were obtained with a higher proliferation index than control cells; morphological changes were also observed (Danielova, Jerabek, and Holubova 1990). In experiments conducted as part of the New York State Powerlines Project, Winters reported that DNA synthesis in human skin fibroblasts was elevated after exposure to a 60 Hz magnetic field (Winters 1986). This effect has not been seen in fibroblasts exposed to 50 Hz fields at 1-2 mT (Cridland and Saunders 1992).

Along with calcium flux changes discussed in an earlier section of this report, ion cyclotron resonance (ICR) conditions have also been reported to affect proliferation of cells. Ross examined the effect of an ELF sinusoidal magnetic field and DC field, tuned or detuned for calcium ICR conditions, on a rabbit ligament fibroblast cell line (RLF) derived from the medial collateral knee ligament (Ross 1990). Exposure to 100, 75, and 42 Hz sinusoidal signals, with the vertical DC field scaled so that tuning conditions for Ca^{++} were maintained, produced mean decreases in proliferation of 7.7%, 5.1%, and 6.3% respectively, significant at $p<0.01$ in each case. When the density of ELF was varied (ELF frequency = 100 Hz, 0.13 mT DC vertical field), inhibition of proliferation occurred at amplitudes below 0.5 mT p-p, while stimulation of proliferation occurred starting at 0.5-0.6 mT p-p through 1.0 mT (highest value tested). Ross argued that the linear dependence of response on signal amplitude in both inhibitory (0.1-0.5 mT) and stimulatory (0.6-1.0 mT) amplitude ranges indicates a very specific cell-signaling effect, possibly involving two separate molecular pathways.

Schimmelpfeng *et al.* detected changes in the cell cycle distribution of mouse fibroblasts cultured as monolayers or as multicellular spheroids after exposure to 50 Hz, 2.0 mT magnetic fields by means of flow cytometry (Schimmelpfeng and Dertinger 1991). However, Arron *et al.* investigated the effects of calcium ion cyclotron resonance field conditions (16 Hz, 21 μT) on multicell spheroids of V79 (Chinese hamster lung fibroblasts) cells and found no effect on the proliferation rate (Arron, Bremner, and Sherar 1991). Adolphe *et al.* examined the effect of exposure to a 50 Hz, 0.5 mT magnetic field for 24-72 h on the *in vitro* growth of two normal cell lines, L929 mouse fibroblast cells and a skin keratinocyte line established from a neonatal C3H x C57Bl/6 F1 mouse, and the human cancer cell line HeLa. They reported no modification of growth rate or viability of the cells (Adolphe *et al.* 1987).

Using a transformation growth assay, Lyle *et al.* found a weak indication of promoter activity by a 60 Hz electric field on C3H10T½ fibroblasts pretreated with the carcinogen MCA, but the magnitude of the response was not statistically significant (Lyle *et al.* 1988b). Focus formation of daughter mutant cells (UV-TDTx10e) in the presence of C3H10T½ mouse fibroblast cells, induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA; 50 ng/ml), was approximately doubled by exposure to 60 Hz sinusoidal magnetic fields (0.1 mT, in 1 h periods, 4 times daily for 28 days). These foci arise as a result of an impairment of intercellular communication that is associated with transformation, and represent a measure of the ability of the transformed phenotype to be expressed in the presence of normal cells. No focus formation was seen in exposed or sham-exposed cultures not treated with TPA, or in cultures treated with TPA only at concentrations of 10, 30, or 100 ng/ml (Cain, Thomas, and Adey 1991). In contrast, Frazier *et al.* could find no effects of 60 Hz 10 V/m electric and magnetic fields (0.01, 0.075, or 0.6 mT) on growth rates and transformation frequencies of C3H10T½ cells, whether untreated, subjected to initiation with ionizing radiation (^{60}Co), or subjected to initiation and promotion with 100 ng/ml TPA (Frazier, Reese, and Morris 1989; 1990). However, it should be noted that in the study of Cain *et al.*, there was a particularly critical dependence of the effect on TPA concentration (Cain, Thomas, and Adey 1991).

Exposure of cell lines to a 60 Hz electric field has been reported to produce changes in levels of the enzyme ornithine decarboxylase (ODC), similar to those produced by exposure to the tumor promoter TPA. Byus and coworkers reported that exposure to a 60 Hz field increased ODC activity in CEM and P3 cell lines, and stimulated (with brief exposure) or inhibited (with longer exposure) ODC activity in H35 cells (Byus and Adey 1988; Byus *et al.* 1992). In the latter cells, there does not appear to be any change in the amount of ODC mRNA that is present, suggesting that the effect stems from increased translation, decreased degradation of ODC protein, or stimulation of enzyme activity (Byus, Hawell, and Adey 1990; Byus *et al.* 1992). Cain *et al.* (1988; 1989; 1990) demonstrated that the time course of the ODC response of mouse C3H10T½ fibroblasts to a 60 Hz electric field (suppressed activity followed by stimulation) was similar to the response of the same cell line to TPA. Meanwhile, Krause *et al.* (1991; 1992) have detailed some effects of frequency shifts and field strength on ODC response in L929 fibroblasts.

Other studies, however, have suggested only weak or no stimulatory effects on growth of neural and skeletal cells in culture (Rodan 1987), or on CHO or NIH-3T3 mouse fibroblast cell lines (Frazier, Reese, and Morris 1988). There was no consistent enhancement of the plating efficiency of CHO cells by 60 Hz electric and magnetic fields, alone or in combination (Brayman and Miller 1988).

There are many other reports of enhanced proliferation in clearly transformed tumor-derived cell lines. These studies are not relevant to transformation, but may be applicable to processes involved in promotion and tumor progression. Enhancement of growth rates by both pulsed and simple ELF fields has been described (Phillips, Winters, and Rutledge 1986; Akamine *et al.* 1985), together with stimulation of ornithine decarboxylase (60 Hz fields: (Byus, Pieper, and Adey 1987; Cain, Thomas, and Adey 1990, 1991); 50 Hz fields: (Mattsson, Mild, and Rehnholm 1992) and of protein kinase C (60 Hz fields: (Phillips, Haggren, and Adey 1990; Thomas *et al.* 1991), modulation of protein phosphorylation by pulsed EMF (Ryaby, Jones, and

Pilla 1986; Jones and Ryaby 1987), and suppression of retinoic-acid-induced differentiation of murine embryonal carcinoma cells (Akamine *et al.* 1985). However, clonogenic assays are notorious for their variability; and Cohen, who repeated the work of Phillips *et al.* in human colon cancer lines, was unable to show consistent growth enhancement (Cohen 1987).

Liboff *et al.* recently examined the effects of ion cyclotron resonance (ICR) field conditions on proliferation of the human lymphoma cell line, HSB-2 (ATCC CC1 120.1), following 24 h exposure to 60 Hz magnetic fields, as a function of both the DC field strength and the AC intensity. They reported a sharp peak in proliferation relative to controls (mean ratio 1.36) at precisely 51.1 μT, corresponding to the third harmonic for the K^+ ion at 60 Hz, and found that when the exposure frequency was changed from 60 Hz to 16 Hz the peak DC field shifted from 51.1 μT to 40.8 μT, corresponding to the predicted first harmonic at 16 Hz (Liboff and Jenrow 1991; Liboff, Jenrow, and McLeod 1992). Nirsimloo *et al.* (1989) extended their previous observations that the proliferation of spontaneously transformed guinea pig keratinocytes can be enhanced by therapeutic pulsed magnetic fields; reporting significant ($p<0.05$) increases in proliferation compared to the control level, with a 0.9 mT field at 16.7, 25, 33.3, 50, and 60 Hz (maximal effects were at 33.3 Hz (18%) and 50 Hz (24%), and at 0.25 mT and above at 50 Hz (maximum increase over control of 23% at 2.5 mT) (Nirsimloo, Smith, and Dyson 1990). N-18 neuroblastoma cells were exposed to AC and DC magnetic fields in a combination which satisfied ICR conditions based on the hypothesis that fields tuned to calcium would stimulate growth, and fields tuned to potassium would inhibit growth (Smith *et al.* 1992). Contrary to expectations, cell proliferation was increased significantly only for the static field value of 30.0 μT, close to the resonance point for cobaltous ions (Co^{++}; 30.7 μT) and ferrous ions (Fe^{++}; 29.1 μT), while cell process formation was significantly depressed in 25.0 and 30.0 μT static fields. Therefore, in spite of attempts to summarize proliferative field effects using theoretical mechanisms such as cyclotron resonance, the data appear to present inconsistent patterns for field conditions characterizing ELF EMFs which stimulate proliferation of normal or tumor cell lines *in vitro*.

It is not possible to clearly interpret these results in the context of neoplastic growth *in vivo* (see Section 13.3 of this report). Any association between malignant transformation and EMF exposures requires more studies with untransformed (i.e., normal) cell lines. The data obtained with transformed lines, while suggestive overall of an interaction leading to proliferation, is of limited relevance to questions of carcinogenicity. Transformed cell lines already exhibit abnormal features (such as, loss of senescence and of a finite life span *in vitro*) suggestive of abnormal differentiation and loss of growth control, and may, therefore, not behave like fully normal cells experiencing carcinogenesis *de novo*.

10.2 IN VITRO EFFECTS ON GENE EXPRESSION

To the extent to which differentiation and cell division are mutually exclusive processes, gene expression can be an indication of the termination of the cell's potential for proliferation. However, many of the experiments described in this section indicate that ELF EMF increases the activities of housekeeping genes (like the histones) or genes associated with increased proliferative activity (like the oncogenes); so, in that sense, ELF EMF effects on gene expression can be seen as an extension of the effects on cell proliferation.

In a recent study in which cells were exposed to either 60 Hz, 0.1 mT continuous fields or pulsed EMF, total RNA fluorescence, as determined by laser flow cytometry and used as an indicator of transcription, increased in Daudi lymphoma cells. However, it was unaffected in HL-60 promyelocytes, and was actually reduced in mitogen-activated human lymphocytes, reflecting a wide range of behavior by different cell types in their response to EMF (Swicord *et al.* 1992). The same group also reported a differential response of RNA fluorescence between intact Daudi lymphoma cells (increase) and their isolated nuclei (decrease) when exposed to 60 Hz, 0.1 mT continuous EMF (Ning *et al.* 1992). Enhanced transcription, as evidenced by increased incorporation of tritiated uridine pulses, has also been described in human leukemic cells (HL-60) exposed to a 60 Hz, 1.5 mV/m, 1.0 mT field (Greene *et al.* 1989).

Another report described enhanced transcription of the *c-myc* oncogene in cell cultures from Drosophila salivary gland and transformed human (HL-60) cell lines (Goodman and Henderson 1987a; Blank *et al.* 1991). Other changes seen in these cell lines, apart from *c-myc*, involved changes in beta-actin, *c-src*, beta-tubulin, histone H2B, and actin transcript levels, as well as changes in unidentified polypeptide patterns (Goodman *et al.* 1992). Support for these findings *in vivo* was provided by an earlier study showing similar enhancement of *c-myc* and *v-ras* in regenerating livers of rats exposed to pulsed ELF EMF (Barbiroli *et al.* 1985). Goodman *et al.* also reported that exposure of *Escherichia coli* to weak EMFs increased the expression of several proteins including the a subunit of DNA-dependent RNA polymerase (Goodman *et al.* 1991). Enhanced transcription has also been reported in human leukemic cells (HL-60) exposed to 60 Hz fields (Greene *et al.* 1989). Results of this type have led some workers to suggest that stimulated DNA synthesis and transcription could upset normal growth and development, ultimately leading to cancer (Liboff and Kaplow 1984). Other studies, however, have suggested only weak or no stimulatory effects on growth, or found no increase in total or oncogene RNA synthesis in cultured cells (Parker and Winters 1988). In spite of evidence for selective increases in gene transcription, including transcription patterns which involve oncogenes, none of the results suggests coordinated expression of oncogenes leading directly to neoplastic growth.

In over a decade of reported experiments, Goodman *et al.* have established that specific fields stimulate characteristic transcription patterns, and, to some extent, have shown that these stimulatory effects occur in specific frequency and intensity "windows" (Wei, Goodman, and Henderson 1990; Litovitz *et al.* 1990). The most detailed results have been reported for the pattern of gene expression stimulated in dipteran salivary gland polytene chromosomes which show distinctive patterns in response to various pulsed clinical EMF signals (discussed in Section 10.3) and sinusoidal fields (Goodman, Bassett, and Henderson 1983; Goodman and Henderson 1988; Goodman *et al.* 1992).

Jones *et al.* found that pulsed EMF tended to increase tyrosine hydroxylase, an indicator of cell differentiation, in Cloudman S91 melanoma cells: results which contrast with the proliferative effects mentioned above (Jones, Pedley, and Ryaby 1986). Later work by the same group indicated a more signal-specific response: continuous pulsed EMF exposure reduced tyrosine hydroxylase activity, while alternate 6 h periods (6 h on, 6 h off) increased levels of tyrosine hydroxylase and reduced mitosis (Jones and Ryaby 1987). Similar results were obtained with the mouse embryonal carcinoma cell line F9, which showed a mean reduction of 34-45% in cell number relative to controls, depending on the age of the culture when exposed to a vertical 50 Hz, 300 mG sinusoidal magnetic field (Mattsson, Rehnholm, and Mild 1991). Such results raise the possibility that ELF EMF may be used to selectively stimulate or inhibit growth of cells, and therefore could be used in medical practice to direct the growth and differentiation of normal and neoplastic cells (Bassett 1991).

New molecular biology techniques are being used to follow transcriptional changes stimulated by ELF EMF. Phillips *et al.* have used a nuclear run-off assay to assess changes in the transcription of early activation genes in human T-lymphoblastoid cells exposed to a 0.1 mT sinusoidal 60 Hz magnetic field. They observed increased transcription of the genes encoding *c-myc*, *c-fos*, and *c-jun* products, and protein kinase C; while they detected no effect of magnetic field exposure on the transcription of the genes encoding transferrin, insulin receptor, metallothionein, ornithine decarboxylase, and actin (Phillips *et al.* 1991). The new technology is expected to reveal some of the molecular details of field interactions with chromatin (Phillips 1992) once certain technical problems are overcome in producing gene/regulator constructs that are active enough to be detected following *in vitro* EMF exposure (Phillips *et al.* 1992).

10.3 BONE HEALING AND EFFECTS ON BONE CELLS IN VITRO

Low-level pulsed electromagnetic fields (PEMFs) have been approved for use in the treatment of bone nonunion, and their clinical efficacy has been documented by a number of controlled studies (Bassett 1985; Pilla 1992). More recently, such PEMFs also have been applied to soft tissue wound healing, and recovery from spinal cord injury (Ellis 1987; Lee 1992). Clinical PEMFs are usually produced using magnetic field coils (Helmholtz coils) applied to the skin surface over the nonunion. Compared to power line and other environmental EMF, clinical PEMFs are characterized by a relatively strong magnetic field which induces a weak electric current in the tissue, similar in magnitude and waveform to the piezoelectric currents induced in bone by mechanical deformation.

Different PEMF signals have been developed for specific clinical applications (e.g., human bone nonunions, equine veterinary use). A typical PEMF signal (produced by a Bi-Osteogen coil system) consists of a series of quasirectangular pulse bursts of 4.8 ms duration repeated at 15 Hz, each pulse of 230 μs, repeated 21 times/burst. This signal produces a peak magnetic flux density of 2.2 mT (flux density change of 310 T/s), and would be expected to induce an electric field density on the order of a few $\mu A/cm^2$ (Murray and Farndale 1985). The electric current due to the external electric field is negligible. Clinical signals have been developed based on theory, bioelectric measurements on bone tissue, and clinical results. It is not clear which signal parameters are responsible for the evident biological effects. However, since pulsed EMF, whether delivered by external coils or implanted electrodes, produced essentially equivalent acceleration of bone repair in a rabbit fibula osteotomy model, it would appear that electrical dosimetry at the cell membrane is critical, and the magnetic component does not play a direct role in healing (Pilla *et al.* 1992).

Much of current research into the cellular effects of PEMFs is done in an attempt to establish *in vitro* correlates and a mechanism for promoting bone healing. For example, Ozawa *et al.* reported that rectangular, 3-ms pulsed electric fields induced DNA synthesis in growth phase, but not confluent, MC3T3-E1 mouse osteoblast-like cells, apparently through a calcium-dependent process (Ozawa *et al.* 1989). Other studies have used 60 Hz EMFs, and, in some cases, comparison has been made of the effects of different types of fields on patterns of biosynthesis (Goodman and Henderson 1988).

Several reported effects of both PEMFs and ELF EMFs appear to involve changes in cell membrane physiology or response. Biophysical experiments with *Physarum* (slime mold) amoeboid cells indicated signal-specific changes in surface charge and molecular constituents (Greenebaum, Goodman, and Marron 1987), while cultured fibroblasts showed altered motility reflecting changes in extracellular adhesion proteins as a result of exposure to 60 Hz magnetic fields (Winters *et al.* 1988a, 1988b; Winters and Darnell 1989). Furthermore, using the same type of sinusoidal waveforms, Phillips found changes in the distribution of transferrin receptors in Colo 205 cells (Phillips 1986). Pulsed fields have also been shown to alter parathyroid hormone receptor-associated antigens in bone cells (Luben 1987), and related cyclic adenosine monophosphate levels (Jones and Ryaby 1987), as well as lectin binding sites in human lymphocytes (Gigante *et al.* 1987). Work by Luben and coworkers has established a series of molecular events following stimulation, based on the observation that PEMF from clinical fracture treatment devices decreased signal transduction by the parathyroid hormone (PTH) receptor in cultured bone cells by as much as 90% (Luben 1992). Based on the hypothesis that a range of other cells might be affected by PEMF through a common mechanism involving other members of the G protein linked receptor family (e.g. adrenergic, muscarinic, serotoninergic, and peptidergic receptors), Luben has demonstrated significant inhibition of binding of 125I-cyanopindolol, a specific ligand for the beta-adrenergic receptor, and of adenylyl cyclase activation by isoproterenol, in bone cells and pineal cells but not in fibroblasts (Luben 1991; 1992).

Additional evidence for PEMF effects at the cell membrane includes changes in cell surface glycosylation (Fisher, Dulling, and Smith 1986), in cyclic AMP levels in fibroblasts exposed to prostaglandin E2 (Farndale and Murray 1986), and in bone cells exposed to parathyroid hormone (Adey 1986). The fact that both PEMFs and ELF EMFs produce membrane-associated effects makes the data more convincing. It has been suggested that there is a common mechanism for such membrane effects involving enhanced divalent cation flux through the membrane by cyclotron resonance with electromagnetic energy of a specific frequency and intensity (see Section 4). However, some recent results suggest that the membrane may not always be involved in the response to ELF EMF. Goodman *et al.* have reported that transcription can be stimulated in membrane-free S30 extracts of *Escherichia coli* by a sinusoidal 72 Hz, 1.5 mT peak magnetic field (Goodman *et al.* 1992). There are also reports of effects of weak magnetic fields, tuned to Ca^{++} parametric resonance frequency, stimulating the phosphorylation of myosin in a membrane-free solution containing calmodulin, and the kinase of myosin light chains (Shuvalova *et al.* 1991).

As might be expected for an agent with applications in healing, enhanced rates of cell division, usually measured by incorporation of ^{3}H-thymidine into DNA, have been reported in: bone organ cultures, in response to specific PEMF signals (Jackson *et al.* 1982); bovine articular cartilage plugs (Ryaby *et al.* 1992a); human foreskin fibroblasts (Liboff, Strong, and Wistar 1982); Chinese hamster V79 cells (Takahashi *et al.* 1986); and CHO cells (Goodman *et al.* 1985). Enhanced growth and differentiation were reported for mouse limb bud organ cultures exposed to PEMF (Hinsenkamp and Rooze 1982) and for regenerating liver in partially hepatectomized rats (Ottani *et al.* 1984b). PEMFs were also found to have a stimulatory effect on cell cycle progression of transplanted bone marrow in mice (Cadossi, Iverson, and Hentz 1987).

Changes have also been reported at the level of gene expression, often involving effects that show quantitative or qualitative differences in response to specific PEMF signal characteristics. Stimulation of specific transcription or a general quantitative increase in RNA synthesis was reported in dipteran salivary glands (Goodman and Henderson 1987a, 1987b, 1988; Goodman *et al.* 1989), a system in which single pulses or sine waves repeating at 72 Hz were most effective, in HL-60 cells (Goodman, Wei, and Henderson 1991), in Daudi lymphoma cells (Swicord *et al.* 1992), and human leukemia cell lines (Phillips and McChesney 1988). Stimulation of collagen production was reported in cultured fibroblasts (Murray and Farndale 1985), and of proteoglycan synthesis in bovine cartilage explants (Aaron and Ciombor 1992). Stimulatory effects have also been reported for liver regeneration following partial hepatectomy (Battini *et al.* 1991; Ottani *et al.* 1984a), rat skin flaps (Delport *et al.* 1984; Albertini *et al.* 1990), peripheral nerve regeneration and neurite growth (Albert *et al.* 1985; Murray, O'Brien, and Orgel 1984; O'Brien, Murray, and Orgel 1984), chicken embryonic femora (Noda *et al.* 1982), and rat osteosarcoma (Noda *et al.* 1987).

It has not been demonstrated consistently that sinusoidal ELF EMFs produce growth-stimulatory effects similar to those described for PEMFs. Stimulation of general metabolism has been reported in whole animals exposed to 50 Hz fields (Tomashevskaia and Dumanskii 1981; Marsakova 1982). At the cellular level, both stimulatory and inhibitory effects of 60 Hz magnetic fields were described on protein synthesis in human chondrosarcoma cells (Winters, Liboff, and McLeod 1987), and on DNA synthesis in regenerating corneal epithelial cells (Basu *et al.* 1987). Exposure of neonatal bovine fibroblasts to a 10 Hz current as low as 1 $\mu A/cm^2$, caused up to a 30% reduction in extracellular matrix synthesis when the field was applied parallel to the long axis of the cell (McLeod, Lee, and Ehrlich 1987).

Thus, in contrast to membrane interactions, where PEMFs and sinusoidal ELF EMFs appear to act similarly, the two types of EMF differ markedly in their effect on parameters of cell growth. One possible source of this latter discrepancy could be a frequency specificity for continuous wave EMF. A number of recent reports describe cell kinetic or metabolic effects that are contingent upon the use of fields with frequencies or intensities associated with the resonance of specific ions. For example, Ross (1990) exposed rabbit ligament fibroblasts to combined DC and ELF (100, 75 or 42 Hz) magnetic fields tuned or detuned for calcium cyclotron resonance conditions. Cellular proliferation was inhibited at peak-to-peak ELF amplitudes below 0.5 mT, while stimulation was seen above this value; detuning from the cyclotron resonance conditions abolished these effects. Peaks seen in the 60 Hz-induced proliferation of HSB-2 lymphoma cells were associated with K^+ cyclotron resonance harmonics (Liboff and Jenrow 1991; Liboff, Jenrow, and McLeod 1992). Fitzsimmons *et al.* described a similar specificity with a very narrow frequency window for stimulation of proliferation and release of mitogen activity in a human osteosarcoma cell line, TE-85, when exposed to an EM field tuned to calcium (static field 20 μT, ELF field 15.3 Hz at 40 μT) (Fitzsimmons *et al.* 1991). When guinea pig keratinocytes were exposed to sinusoidal magnetic fields of a range of frequencies and amplitudes, maximal enhancement of proliferation occurred at 33.3 and 50 Hz, and at an amplitude of 2.5 mT (Nirsimloo, Smith, and Dyson 1990). Among recently-reported changes in metabolic activity in response to specific field characteristics are increased thymidine and sulfate incorporation by bovine cartilage plugs exposed to 15.3 (Ca^{++}) and 25.4 (Mg^{++}) Hz magnetic fields, with 76.6 Hz (Ca/Mg) only affecting sulfate uptake (Ryaby *et al.* 1992a); and the increased levels of both neural cell adhesion molecule (NCAM) and microtubule-associated protein-1 seen in PC12 phaeochromocytoma cells exposed to 76.6 Hz, but only of NCAM when exposure was at 15.3 Hz (Ryaby *et al.* 1992b).

10.4 METHODOLOGICAL FACTORS IN CELL CULTURE EXPERIMENTS

In vitro experiments constitute a substantial body of data supporting, or contradicting, reports of significant biological effects of weak ELF EMF. The reliability of these *in vitro* results depends to a large extent on the care used in working with cultured cells. For this section of the report, Dr. Joseph Tumilowicz has reviewed some of the potential methodological problems which he has identified in a representative sample of EMF experiments performed with cell cultures. This section focuses on problems with monolayer cultures, and augments the analysis of medium conditions (which apply to both adherent and nonadherent cells *in vitro*) discussed previously in Section 6.4.

The derivation of cell lines and isolation of tissue fragments are critical elements in many of the experiments, and usually few details are given in the reports. Tissues are most susceptible to contamination during dissection and preparative procedures to digest matrixes and free cells. Specimens obtained from slaughterhouses require particularly careful dissection of externally contaminated portions to expose and isolate uncontaminated tissue. In such cases, the use of antibiotics is recommended at twice the concentration often employed in established cell cultures. As soon as outgrowth begins to appear, antibiotics should be discontinued. Reasons for cultivating cells without antibiotics were noted in the discussion about lymphocyte cultivation. Additives like the antibiotic, Gentamicin, can induce toxic effects, including chromosome damage, on different cell types, (Leonard and Botis 1975). Monitoring of cultures for microbial contaminants becomes increasingly imperative in experimental designs that involve the introduction and manipulation of electrodes or atypical implements within cultures. Cultures should then be monitored regularly for the presence of contaminants, even if no contamination is grossly evident.

Some authors in the field of bioelectromagnetics, who work with cell cultures, may be unjustifiably confident about the identity of isolated cells as osteoblasts, chondroblasts, chondrocytes, etc., based only on anatomical origin of derivation. Others appear to recognize that, in the absence of characterizations by various criteria (e.g., ultramicroscopic anatomy, detection of specialized markers, or specific reactivities with monoclonal antibodies), it is necessary to inform readers that results pertain to, for example, osteoblast-like cells rather than definitively to osteoblasts.

It is important for researchers to record the approximate number of doubling times elapsed since the primary culture, or the passage number at commencement of an experiment. The composition of a population of cells may change sharply in newly derived cultures as differentiated and undifferentiated or blast-like cells of the same lineage predominate in turn. The presence of cells of other lineages, in the typically heterogeneous mixtures of cells in primary cultures, further complicates identification and characterization. Continuous or immortal cell lines are also heterogeneous, but probably less so histiotypically, and more so karyotypically, than primary lines; having adjusted to a particular set of *in vitro* conditions. Effects of exposure of human diploid fibroblasts in the 10th passage to electromagnetic fields may not be the same as effects on cells entering the senescent phase. It must not be overlooked that the number of passages undergone by a cell line in a researcher's laboratory should be added to the number of passages transited elsewhere.

Confusion in comparing results can be created by similar designations of cell lines. For example, Ngo, Blue, and Roberts (1987) identify a cell line as V79, obtained from Dr. Blakely of Lawrence Berkeley Laboratory, who according to a review of the history of this cell line (American Type Culture Collection catalogue, 7th ed., p. 56, 1992), was not involved in derivative work with V79 or its clone, V79-4. Because this cell line was not obtained from a recognized repository, researchers setting out to repeat the work of Ngo *et al.* may wonder whether the V79 of Ngo *et al.* is actually V79-4, isolated by Chu in 1968, and presumably in circulation since that time. Incomplete information about a cell line regarding normalcy may also mislead about the significance of results. In associating the literally correct designation, normal origin, with the mouse fibroblast line, L929, and with a mouse keratinocyte cell line, Adolphe *et al.* convey the impression that the cell lines were normal at the time of the experiment (Adolphe *et al.* 1987). L929, obtained by Adolphe *et al.* from the American Type Culture Collection, is karyotypically abnormal (American Type Culture Collection catalogue, 7th ed., 1992) and no karyotypic evidence is presented regarding the keratinocyte line.

Possible variations in the defined and partially defined components of medium, accumulating phototoxic products, and inattention to maintenance of sterility can affect reproducibility of results in experiments with adherent cells, just as with detached or suspended cells as discussed in Section 6.4. Colacicco and Pilla found that the composition of medium markedly influenced the effect of pulsed electromagnetic fields on calcium uptake (Colacicco and Pilla 1984). Some components, like bicarbonate, were indispensable; phosphate was potentiating, and magnesium was stimulating. Jones *et al.* proposed that their findings could be altered by undefined components of different serum batches (Jones, Pedley, and Ryaby 1986).

Having little catalase activity, bovine fetal serum and calf serum should be protected from fluorescent light to prevent formation of phototoxic products. Wang and Nixon identified hydrogen peroxide as the phototoxic product in medium exposed to fluorescent light, and reviewed some of the intracellular actions of hydrogen peroxide (Wang 1978). They noted that base destruction, single strand breaks, double strand breaks, and cross linking of DNA were caused by hydrogen peroxide. An increased level of sister chromatid exchanges was reported by Estervig and Wang in cultured human cells incubated in media exposed to fluorescent light.

The sister chromatid exchange was eliminated by catalase (Estervig 1979). Based on the calculations of Burki and Lam, cultures and media should be shielded not only from fluorescent light, but from daylight entering through laboratory windows (Burki 1978). This is extremely important in EMF experiments assessing effects on genetic mutations or focus formation.

Cells are commonly detached from plastic or glass surfaces by an enzyme like trypsin. Cell membranes are damaged to some degree by trypsin, depending on concentration, exposure time, and temperature. Thus, time should be allowed for restoration of membrane integrity before use of cells, particularly in experiments dealing with questions of transport and ionic fluxes. In such experiments, it may be useful to inform others about the elapsed time between trypsinization and commencement of exposure to electromagnetic fields.

All plastic vessels used in cell or tissue culture tend to accumulate and retain static charge, which varies with fluctuations in humidity and is enhanced by synthetic clothing and laboratory coats. Failure to eliminate static charge may cause clumping on one side of the well in multi-welled plates, peripheral or uneven growth across a surface, and wicking of fluid up vessel walls. Interaction of electromagnetic fields with such charged foci may produce different results in exposed cells compared with unexposed cells. Static charge can be eliminated by wiping the top and bottom of a vessel or plate with a damp cloth. Comparison of growth on glass (hard or soft) and plastic surfaces in the same experiment may be misleading, because characteristics of growth in the presence of the same medium may be different on each type of surface. Furthermore, glass containers and cover slips must be thoroughly cleaned before sterilization and use to eliminate possible toxic substances included during manufacturing and processing. It is also possible that a sterilization procedure, like autoclaving, may introduce toxic products to the cleaned glass surfaces. This is of particular importance in EMF studies which employ specialized glass exposure chambers like U-tubes, concentric ring culture dishes, and microscopic chambers.

11. DC MAGNETIC FIELD EFFECTS

This review has focused on research conducted with ELF EMF characteristic of the emissions of the German TR07 maglev system, the only maglev system for which detailed measurements are available (see Section 3). Levels of DC magnetic fields are low with the TR07 system, but may be substantially higher than other common EMF sources with other transit systems, such as the Japanese superconductor maglev and certain conventional high-speed rail systems. For this reason, the following section reviews reported bioeffects with high-intensity static magnetic fields.

11.1 PURE STATIC MAGNETIC FIELDS

As discussed previously in Section 8 of this report, the older Soviet literature contains reports of adverse health effects in workers exposed to industrial levels of static (DC) as well as time-varying (AC) magnetic fields. Many of the reported physiological effects reported for high-intensity AC magnetic fields (e.g., magnetophosphenes) are apparently produced as a function of induced electric fields, and would therefore not be expected from exposure to static magnetic fields (a possible exception occurring if a subject is moved rapidly through an inhomogeneous static field, in effect producing a time-varying field). Soviet studies on workers employed in the fabrication of permanent magnets reported symptoms such as bradycardia, tachycardia, and a decrease in arterial blood pressure along with irritability, fatigue, occasional dizziness, altered appetite, and headache. Animal studies were reported to confirm that static magnetic fields can affect central nervous system activity, appetite, behavior, aging, and heart action, as well as produce changes in various organs and tissues, including blood (Ketchen, Porter, and Bolton 1978). As also noted previously, similar studies in the West have failed to confirm these findings (Sulpor 1979). A recent evaluation of the health status of eleven volunteers who were intermittently exposed to 4 T (40 kG) fields over a one year period showed no evidence for field-related pathology, but there was evidence for field-related sensory effects - vertigo, nausea, magnetophosphenes, and metallic tastes. These effects were of mild intensity and were often absent (Schenck 1991). There is some possibility that a static magnetic field could interact with sensory structures (such as the inner ear and the retina) or with the recently discovered magnetic particles in the brain (discussed in Section 8.2) in ways which are not yet understood.

Behavioral effects of extremely high intensity static magnetic field exposure have been documented in a number of animal studies. deLorge (1978) reviewed some of the early work with non-human primates in high static magnetic fields. In one study, three squirrel monkeys (*Saimiri sciureus*) were conditioned to respond in a visual vigilance task and were subsequently exposed to a direct current magnetic field in the core of a water-cooled Bitter magnet. Response was greatly suppressed by fields of 7.0 T or more and a threshold seemed to exist between 4.6 and 7.0 T. A second experiment in a superconducting magnet in which eight squirrel monkeys were trained on several operant tasks revealed similar suppressive effects at magnetic field strengths of up to 9.7 T. In addition, two of the monkeys regurgitated when exposed to the higher fields. All of these effects were reproducible.

A series of experiments were conducted at facilities for large-scale experimentation on magnetic field effects in whole-animal, cellular, and molecular systems at the Lawrence Berkeley Laboratory. Results indicated a strong effect of direct current magnetic fields on the electrocardiogram, with a sixfold increase in the T-wave amplitude occurring in a 2 T field (Tenforde *et al.* 1979). T-wave amplitude changes were reported in magnetically-induced blood flow potentials, and blood pressure changes were measured in adult male Macaca monkeys exposed to stationary DC fields ranging in strength up to 1.5 T (Gaffey *et al.* 1982; Tenforde *et al.* 1983), or in juvenile female *Papio* baboons at field strengths up to 1.6 T (Gaffey, Tenforde, and Dean 1980). A study conducted to evaluate the possibility of induced cardiac dysrhythmia in healthy human subjects exposed to a 2 T magnetic field, found no measurable effect on electrocardiogram (ECG) recording; on cardiac excitability, vulnerability, or contraction; or on blood pressure, but reported a similar magnetically-induced change in the T-wave region of the ECG recording related to the flow of blood through the field (Jehenson *et al.* 1988). This T-wave amplitude effect was found to be promptly reversible with no residual effect, and is considered to impose no health risk.

Rosen and Lubowsky demonstrated an effect of strong static magnetic fields on the excitability of striate cortex in decerebrate adult cats. In all animals subjected to a 1.2 T (1200 G) field, there was a significant decrease in both amplitude and variability of the evoked response stimulated at the left optic tract beginning 50-95 s after the field was turned on and persisting after the field was turned off for 200-280 s. In one animal, an 80 mT (800 G) field was associated with increased amplitude and variability of the evoked potential with optic tract stimulation. Rosen and Lubowsky suggested that the field alters the ionic environment in the nerves, possibly by altering calcium transport, or affects neurotransmitter availability at synapses in the central nervous system (Rosen and Lubowsky 1987).

A number of studies involving static magnetic field exposures of laboratory animals have indicated no biological effects or small effects of questionable long-term significance (Tenforde 1992). As noted in Section 5.5, little or no effect of strong DC fields has been reported in experiments with developing vertebrates. CD-1 and LAF-1 mice exposed to a homogeneous 1.5 T static magnetic field for 72 h showed no statistically significant behavioral differences in a passive-avoidance training procedure using an electric footshock stimulus (Tenforde *et al.* 1981). A variety of blood chemistry parameters associated with bone metabolism were evaluated in mice exposed to 1 T magnetic fields for 30 min per day for 10 consecutive days, and no significant changes were found (Papatheofanis and Papatheofanis 1989). Although earlier reports indicated an effect of 0.4-8.0 T static magnetic fields on the thermoregulatory ability of mice, Tenforde and Levy found no body temperature effects on Sprague-Dawley rats, LAF-1 mice and BALB/c mice, within either a homogeneous DC field of 7.55 T intensity, or in magnetic field gradients up to 60 T/m (Tenforde and Levy 1985). Continuous exposure for 5 days or 8 h/day exposure for 10 days to a 1.50 T static magnetic-field was also found to have no influence on the circadian periods or the waveforms of seven physiological and behavioral variables in adult female LAF-1 mice (Tenforde, Levy, and Veklerov 1986). Exposing homing pigeons to strong magnetic fields or placing small permanent (DC) magnets on their heads has been reported to negatively effect their homing ability on overcast days (Benvenuti and Ioale 1988; Walcott, Gould, and Lednor 1988), but others have suggested these effects are experimental artifacts (Moore 1988).

On the other hand, severe effects on spermatogenesis were reported in mice which had been exposed to 0.4-1.6 T constant magnetic fields for 3 h to 30 days (Mastriukova and Strzhizhovskii 1982). Merlo Pich *et al.* (1991) reported that a week of continuous exposure to a 1.0 T field significantly reduced peripheral square entries ($p<0.01$) as well as rearings ($p<0.05$) in weaning mice, but had no effect on body weight. Chronic exposure of rats to a static horizontal 0.6 T magnetic field during the last few weeks of conditioning inhibited the performance of avoidance responses, and behavioral carryover of this effect of exposure during learning occurred in a manner similar to effects of stressful physical stimuli, such as loud noise and low temperature (Nakagawa and Matsuda 1988). Conditioned rats exposed after 15 weeks of training showed only slightly increased lever-pressing frequency during the exposure week, indicating that the exposure was not strong enough to affect a well conditioned response in rats. Exposure of rats continuously to a vertical magnetostatic field of 2800 Oe (approximately 280 mT) for a period of 20 days was reported to produce delayed reinforcement operating responses and decreased weight (Laforge *et al.* 1978). NMRI mice exposed to a 3.5 T magnetic field for a mating period of 8 days showed reduced mating activity as a consequence of magnetic field exposure, but no effect on fetal or parental mortality was evident (Zimmermann and Hentschel 1987). Intrauterine exposure of mice to a 6.3 T static magnetic field for 1 hr/day between days 7 and 14 of gestation was reported to produce no significant effects (Murakami, Torii et al. 1992). These reports indicate magnetic field effects which are comparable to the effects of other stressing agents, and support the suggestion that a magnetic field may act at most as a moderate stressor. Occasional unreplicated reports of more extreme effects, such as (Mastriukova and Strzhizhovskii 1982) are hard to accept in the context of other reports in the literature.

The effects of extremely weak static magnetic fields have not been examined, except in the context of ion cyclotron resonance (ICR) fields involving combined exposure to AC and weak (on the order of geomagnetic fields; 50 μT) static fields. These effects have already been discussed in the context of cell level effects on calcium ion flux (Section 4) and activation of lymphocytes (Section 6), and whole animal effects, such as teratologic effects (Section 5), and alterations in the secretion pattern of pineal melatonin (Section 7). These results indicate that bioeffects may be possible with static magnetic fields of geomagnetic field intensity (the normal horizontal component reversed or reoriented) down to the nT level.

11.2 MRI FIELDS

Recent studies have addressed the problem of possible bioeffects from magnetic fields of the type generated by magnetic resonance imaging systems (MRI). MRI devices produce a combination of a high intensity static magnetic field (on the order of 2 T), gradient magnetic fields of 6 T/s or less, and radiofrequency magnetic fields in a complex time sequence (Shellock and Bierman 1989). Since some features of MRI fields may resemble components of those generated by maglev systems, these experiments are of interest.

In some cases, animal studies have been conducted which involve exposure to just the static magnetic field component of MRI fields, but in most experiments, animals were exposed to some combination of time-varying magnetic and radiofrequency fields characteristic of MRI devices, or to the clinical devices themselves. Concern exists that MRI fields may alter the permeability of the blood-brain barrier (BBB), and results of experiments to evaluate this possibility have produced contradictory results. For example, Ross *et al.* (1990) reported no effect of MRI conditions on permeation of 125I-labelled bovine serum albumin through the BBB of rats, and Preston, Butler, and Haas (1989) found no effect on permeation of 14C-sucrose. However, Prato *et al.* (1990) found that rats injected intracardially with radio-labelled diethylenetriaminepentaacetic acid ([153Gd]DTPA, a chelate which has low BBB permeability) between two sequential 23.2 min MRI exposures, showed significantly greater (29%, p=0.006) brain retention of [153Gd]DTPA than did sham exposed rats (n=22) 1 h after the end of the last 23.2 min exposure. Ravnborg *et al.* (1990) assessed the effects of a transcranial magnetic pulse with 163 μs rise time, a 4 ms decay, and a peak intensity of 1.9 T in anesthetized rats, and found no effect on BBB permeability.

Chuvpilo (1982) noted differences in the levels of sodium fluorescein in several brain regions following exposure of anesthetized rats to a constant 0.4 T magnetic field for 3 h. Garber *et al.* (1989) followed tritiated mannitol permeation in groups of adult male rats that were exposed to MRI procedures at 1.5 T, 0.5 T and 0.3 T. Increased brain mannitol associated with gradient fields may reflect increased blood-brain barrier permeability or brain blood volume. Brain mannitol concentration was significantly increased at 0.3 T and 0.5 T but not at 1.5 T. At 0.3 T, exposure to gradient field fluctuations, as used for imaging, increased brain mannitol concentration, while exposures to the static main field and pulsed radiofrequency energies did not. Persson *et al.* (1991) found an increase in the extravasation of Evans Blue and albumin with all components of the MRI fields, but the strongest effect was obtained with pulsed 915 MHz microwaves at 215, 50, 16, and 8 Hz modulation frequencies. These results suggest that pulsed electromagnetic fields have greater potency to open the blood brain barrier. At the present time it is not possible to determine if these differences in experimental results are due to differences in experimental methodology, or in the type of EMF exposure. Questions have been raised regarding possible effects of anesthetics, imperfect temperature control, stress, or artifacts associated with tracers like Evans Blue.

Following reports of muscular twitches in volunteers exposed to high speed magnetic field intensity changes (peak dB/dt rates of 86 T/s) while evaluating an MRI system under development, Cohen *et al.* investigated possible effects of imaging fields on peripheral nerve stimulation. For the operating clinical units, calculations indicated an upper limit on the volume average dB/dt of 36 T/s (with localized peaks within the patient bore of 75 T/s). Over 100 patients and volunteers have been scanned in the system, and only three instances of perceived peripheral nerve stimulation have been reported (Cohen, Rohan, and Rzedzian 1991). In general, the risks of neural stimulation are predictable, and clinical MRI exposures fall at or below the limits.

Minor temperature elevations well within a normal thermoregulatory range have been reported in human subjects exposed to MRI fields (Shellock 1991). Even in localized poorly perfused regions of the head where thermal effects are expected to pose the greatest problem (e.g., the eye), normal MRI procedures do not seem to produce harmful thermal effects (Athey 1989).

Exposure of mice to nuclear MRI fields resulted in marked reduction in both day- and night-time morphine-induced analgesia (Ossenkopp and Kavaliers 1989). In contrast to results in animal studies, MRI fields from a General Electric 1.5 T Signa scanner or a Teslacon 1500 G (0.15 T) resistive system did not suppress plasma melatonin concentrations in human subjects (Schiffman *et al.* 1992; Prato *et al.* 1989). These results suggests that human neuroendocrine physiology may be less sensitive to MRI fields than some animal models.

Controlled studies of the effects of MRI fields on performance on a battery of standard cognitive tests were conducted in order to investigate possible behavioral hazards associated with MRI. Sham-imaged subjects were placed in the scanner with all magnetic fields off, and a cassette tape was used to reproduce the sounds of the imaging procedure. No apparent effects on cognitive function were seen immediately after, and 3 months after imaging (Sweetland *et al.* 1987). Eleven volunteers were exposed in the head region to a static magnetic field from a 1.5 T Signa MR system and short latency somato-sensory evoked potentials (SEPs) were recorded with a Cadwell Quantum-84 EMG machine using specially constructed magnetic field insensitive electrodes. There were no statistically significant differences between the N20 or the P25 latencies, or in the amplitudes from N20 negative peak to P25 positive peak, or the SEPs recorded at baseline compared to those measured during exposure to the 1.5 T static magnetic field (Hong and Shellock 1990). A review of side effects associated with MRI indicated that there are a few sensations, but no permanent effects, with exposures to fields with DC magnetic field components of up to 4 T (Shellock 1992). These results support the regulatory conclusion that MRI field levels are safe for normal patient exposures, but there is an ongoing examination of the possible adverse effects of MRI as part of the routine regulatory process applied to medical devices (Shellock and Bierman 1989; Budinger 1992).

12. CLINICAL AND EPIDEMIOLOGIC RESULTS

Epidemiologic and acute health effects studies involving ELF EMF exposure mainly include studies of exposures to electric power delivery systems and industrial and home electrical appliances, with a limited number of studies of workers exposed to current electric rail technology. As discussed in an earlier section of this report, maglev-type fields apparently differ substantially from fields produced by power delivery systems in that they have a larger proportion of components below 60 Hz, and the possible biological significance of these differences is unknown. In this section of the report, we will review in detail those studies involving exposure to present electric rail technology and high level occupational exposure to power-frequency fields. In the following section, we will present a general review of occupational and residential epidemiologic studies relating ELF EMF exposure to increased cancer incidence (the epidemiologic endpoint of greatest interest in these studies).

12.1 ACUTE HEALTH EFFECT STUDIES OF WORKERS EXPOSED TO ELF EMF

Because risk estimates associated with exposure to power frequency EMF suggest, at most, a small increased risk of cancer and other diseases, it may be extremely difficult to conduct a meaningful epidemiologic study on the relatively small human populations exposed to electrical railroad EMF if the increased risks involved are of a similar magnitude. However, there have been a limited number of health surveys and epidemiologic studies which have been conducted with workers exposed to exceptionally high intensity and variable fields which might be characterized as roughly similar to potential maglev exposures.

A few epidemiologic studies have focused specifically on railroad workers. Generally, conclusions are limited by the relatively small sample sizes in any study of such specific occupational categories, and restricted occupational groups are not usually studied unless a cluster of cases draws attention to a possible occupational association. As far as we can determine, no such cluster has been reported for rail workers.

A cross-sectional epidemiological survey was conducted by Baroncelli *et al.* (1986) of male workers, in the "Electrical Devices" departments of railways in Italy, who are occupationally exposed to ELF EMF from interconnection and conversion substations. Maximum levels of the electric field strength and of the magnetic flux density at 50 Hz were of the order of 5 kV/m and 15 mT, respectively. Exposure of subjects to electromagnetic fields was assessed and categorized by mean weekly duration of exposure to the maximum electromagnetic field strength: 1 h/week; 10 h/week; and 20 h/week. Health examinations were performed on 224 subjects in the 20 h/week exposure category, 153 subjects in the 10 h/week, 117 in the 1 h/week, and 133 in a zero-exposure category. General medical examinations were performed as were three specific analyses: hematologic; electrocardiogram; and psychological. The specific analyses did not reveal any differences between the groups in the investigated systems.

Balli-Antunes *et al.* conducted a relatively large scale retrospective analysis of the incidence of malignancies of the hematopoietic and lymphatic systems (MHLS) among Swiss electric railway engine drivers (RED) (Balli-Antunes, Pfluger, and Minder 1990). These researchers compared the incidence rate to two occupational control groups: group C1, metal construction and machine building workers who had similar chemical (solvent and lubricant) exposures; and group C2, technical personnel, who were exposed to a work environment that was different from RED, but who correspond to the study group more closely than C1 in socioeconomic characteristics. A standardized mortality ratio (SMR) due to MHLS for the period 1969-1983 (1972-1983 for C1 and C2) was calculated, representing an estimated 70,000, 900,000, and 1.56 million man-years for RED, C1, and C2, respectively. The total number of deaths over all age classes, for all causes, was 676, 7029, and 8885; and the number of MHLS deaths was 23, 177, and 207 (for RED, C1, and C2, respectively). The overall proportional mortality ratio (PMR) was 163, 95% confidence interval (CI) of 103-244 (SMR of 171, 109-257 CI) which was a significant increase (chi square of 6.82, $p=0.009$). Comparison against group C1 showed elevated mortality only after age 70, and the overall PMR was 144 (91-217 CI) and the SMR was 108 (69-163 CI), not significant increases. Because of strict health examinations from starting service, RED are expected to have a relatively high proportion of healthy persons (i.e., a relatively strong healthy worker effect) compared to other occupational groups. This is supported by the observation that the absolute mortality in RED is lower than either control group until age 60. The authors estimated that voltage within the engine area is approximately 0.6 kV, producing an estimated magnetic field strength of 100 A/m (125 μT). The estimated magnetic field exposure of RED is therefore orders of magnitude greater than transmission line exposures associated with increased cancer risk in other studies, and in this context the authors consider that their results provide only weak support for the hypothesis that exposure to extremely low frequency electromagnetic fields may be leukemogenic.

A number of epidemiologic studies have included railroad and subway workers within an "EMF-exposed" occupational category, without explicitly calculating risk estimates for rail workers as a distinct occupational sub-category. Wilkins and Hundley (1990) conducted an epidemiologic study of the risk of neuroblastoma in the children of men with presumed occupational exposure to electromagnetic fields. Their primary occupational category replicated the grouping in an earlier study by Spitz and Johnson (1985) and included electric power and telephone linemen, power station operators, electrical engineers, welders, and electronic repairmen. In this occupational grouping, they found an OR of 1.6 (0.3-9.1 95% confidence interval (CI)). However, a category which also included railroad and telecommunication workers, electricians, and repairmen had an OR of 1.9 (0.4-9.7 CI). An epidemiologic study using data from death certificates of adult white male Maryland residents who died of brain tumor during the period 1969 through 1982 was conducted by Lin *et al.* (1985). Men in an occupational category characterized as "definite exposure to EMF" (electric and telephone servicemen, linemen, foremen and engineers; railroad and telecommunication engineers; electricians, electric and electronic engineers in industry) were found to experience a significantly higher proportion of primary brain tumors (glioma and astrocytoma) than controls (cases = 27, controls = 14; odds ratio = 2.15). In one recent study of occupational associations with male breast cancer, Tynes and Andersen (1990) reported an elevated standardized incidence ratio (SIR) for all electrical occupations (12 cases observed, 5.81 expected; SIR of 207, 107-361 CI) with the highest subgroup SIR for electrical transport workers (railway engine drivers, tram drivers, and railway track walkers; SIR of 396, 108-1014 CI, 4 cases). These studies are included in the tabular summary given at the end of the next section of this report. Comparing these studies to other occupational studies, it is apparent that current electric rail workers appear to be at no obviously greater or lesser risk than other electrical workers.

12.2 SUMMARY OF MAJOR EPIDEMIOLOGIC RESULTS FOR ELF EMF EXPOSURES

More than 50 major studies have examined the possible association between EMF exposure and cancer incidence. They are listed under the two categories of "residential exposure" and "occupational exposure" in Tables 12-1 and 12-2, respectively, which the authors of this report are in the process of revising for a new edition of their review of EMF and Cancer for the Electric Power Research Institute (Creasey and Goldberg 1989). It can be seen that there is wide variation among the studies, but that more than 40 of them describe elevated risk of cancers in exposed populations, with risk ratios tending to cluster around the 1.2 to 1.5 figure.

Most of the epidemiologic studies have focused on the biological endpoint of cancer, and these results will be discussed in the next section of this report. A few occupational and residential studies have attempted to follow up on anecdotal reports of negative behavioral effects by looking at the incidence of depression and suicide.

Perry *et al.* (1981) examined the relationship between suicide and 50 Hz overhead high-voltage lines in the West Midlands, England. Of a total of 651 suicides that occurred between January 1969 and mid-October 1976, magnetic field strengths were measured at the addresses of 590 suicides and compared to measurements made at the addresses of 594 controls. Readings made 1 m above ground, 0.5 m from the front door of the residences, ranged from 10 nT to 1.5 μT (10 μG to 15 mG), with a mean of about 80 nT and a median of about 40 nT. Three hundred and five suicide measurements fell above the median vs. 257 control measurements. The mean and standard deviation for the suicide addresses (86.7 $\pm$ 132 nT) were significantly higher than for the controls (70.9 $\pm$ 110 nT), and the proportion of suicides in the high and very high field regions (above 100 and 150 nT, respectively) was 40% greater than the corresponding proportion of controls. In a subsequent study, Perry and Pearl (1988) investigated possible adverse effects of fields from power lines using an exposure estimate based on proximity of residents to the building power cable in 49 multi-story blocks in Wolverhampton. Using the symmetry of flat lay-outs and the known non-central locations of the power cables, "near" and "distant" locations were established and the assumptions of field exposure confirmed with 50 Hz-magnetic field strength measurements in 110 pairs of high and low field strength flats located in 43 blocks. Field measurements indicated means for all pairs of 0.315 $\pm$ 0.29 μT for near, and 0.161 $\pm$ 0.112 μT for distant exposures. Medical records indicated about 1.7% of the patients (330 cases) were living in the blocks under study. No significant cable proximity effect was found for most categories of illness, including cases of pregnancy and complications, pediatric cases, psychiatric cases, malignant disease, surgery excluding malignancy, and other medical conditions. However, there were suggestions of a "cable effect" associated with myocardial infarct, hypertension, and ischemic heart disease (p=0.168). This preliminary observation was followed with a longer time study involving 113 cases of heart disease (1967-1984), 145 cases of overdose (1980-1985), and 84 cases of psychiatric disorder (1983-1986).

In this extended study, significant cable effects were found for myocardial infarct, hypertension, and ischemic heart disease (p=0.056, 95% confidence interval (CI) of ± 11%); depression (p=0.030, 95% CI of ± 16%); and personality defect, anxiety, agitation, young confused (p=0.026, 95% CI of ± 18%), with the incidence of depression biased toward the cable, and the incidence of the group of psychiatric disorders biased away from the cable. The authors offered no explanation for the apparently contradictory psychiatric responses, but speculated that mechanisms involving fluctuating endorphin levels, serum triglyceride levels, or calcium levels might be involved in "cable-related illness."

These reports of depressive effects of ELF EMF exposure have not been readily accepted by the EMF research community, and those few attempts which have been made to replicate aspects of the studies have reported negative results. For example, Baris and Armstrong examined mortality from suicide in men with occupations likely to involve exposure to electric and magnetic fields, using two independent British decennial supplements of occupational mortality (1970-1972 and 1979-1983; Office of Population Censuses and Surveys). The 1970-1972 data showed an increase in the proportional mortality ratio (PMR) for radio and radar mechanics (PMR = 153, 92-239 95% confidence interval (CI)) and telegraph and radio operators (PMR = 256, 123-471 CI). In the 1979-1983 data, however, there was no significant excess mortality due to suicide in any potentially exposed occupational group, and the authors interpreted these results as indicating no real effect of EMF exposure (Baris and Armstrong 1990). More typically, studies which examined excess mortality from all causes associated with occupational or residential ELF EMF exposure, reported no excess in the number of deaths from suicide (McDowall 1986). However, a recent report by Poole *et al.* confirmed a significant association between self-reported depressive symptoms (OR = 2.4, 1.3-4.2 CI) and residential proximity to a transmission line corridor, even when the responses were controlled for measures of attitudes and awareness of possible negative ELF EMF health effects, or for demographic variables known to be associated with depression (gender, age, education level and marital status) (Poole, Kavet et al. 1993).

TABLE 12-1.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED RESIDENTIAL EMF EXPOSURE AND CANCER INCIDENCE

Investigator and Date	Location of Study	Population Studied	Exposure Estimate	Type of Study	Number of Cases or Subjects	Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Wertheimer and Leeper 1979*	Denver, Colorado	Children living near HCC	Wiring configuration	CC	344	Cancers (all) 3.2 Leukemia 3.0 CNS tumors 2.4
Fulton *et al.* 1980	Rhode Island	Children living near HCC	Wiring configuration	CC	119	Leukemia no increased risk
Wertheimer and Leeper 1982	Denver, Colorado	Adults living near HCC	Wiring configuration - some representative field monitoring	CC	1,179	Cancers (all) 3.2 CNS, breast, & uterus tumors, & lymphoma - largest increase
Tomenius 1986	Stockholm, Sweden	Children living near HV lines	Proximity to HV & monitoring outside house	CC	716	Cancers (all) 2.1 CNS tumors 3.7
McDowall 1986	East Anglia, UK	Persons living near HV lines & substations	Proximity to lines & substations	CC	7,631 (814 deaths)	Cancers (all) 1.04 (.88-1.23) Lung cancer 2.15 (1.18-3.61)
Coleman and Bell 1987	London, UK	Persons living near HV lines & substations	Proximity to lines & stations	CC	771	Leukemia 1.45 For children 1.6
Savitz *et al.* 1988	Denver, Colorado	Children living near HV lines	Proximity to HV, in-house measurements, wiring configuration (wiring best index)	CC	356	Cancer (all) 1.4 (0.6-2.9) Leukemia 1.9 Lymphomas 2.2 Soft tissue sarcomas 3.3
Severson *et al.* 1988	Washington State	Persons living near HCC	Wiring configuration over 15 yr, point in time field measures in & out of house	CC	114	Acute nonlymphocytic leukemia no increased risk
Preston-Martin *et al.* 1988	Los Angeles, California	Adults with leukemia	Electric blanket use	CC	116 AML 108 CML	Acute and chronic myelogenous leukemia no increased risk
Coleman *et al.* 1989	London area & South East, UK	Adults with leukemia	Proximity to electric supply (lines & substations)	CC	771	No significant risk increase, but suggested risk trends: <100 m from line 1.45 (0.54-3.88)
Myers *et al.* 1990	Yorkshire, UK	Children living near HV lines	Proximity to HV and field monitoring, mean 0.15 mG (0.01-4)	CC	374	Cancers (all) and leukemia (all) no increased risk (power to detect OR of 2.5)
Youngson *et al.* 1991	North West & Yorkshire, UK	Adults with leukemia & lymphoma	Proximity to power lines, spot measurements	CC	3,146	No significant risk increase, but suggested risk/exposure trends: <100 m from line 1.26 (0.99-1.60)

TABLE 12-1.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED RESIDENTIAL EMF EXPOSURE AND CANCER INCIDENCE (continued)

Investigator and Date	Location of Study	Population Studied	Exposure Estimate	Type of Study	Number of Cases or Subjects	Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
London *et al.* 1991	Los Angeles, California	Children with leukemia	Wiring configuration, 24 h measurements, self-reported appliance use	CC	232	Leukemia (VHCC) 2.15 (1.08-4.28) Leukemia (0.068-0.124 μT) 1.37(0.65-2.91) Leukemia (>0.124 μT) 1.22 (0.52-2.82) Leukemia (b&w TV use) 1.49 (1.01-2.23)
Feychting, *et al.* 1992	Sweden	Children & Adults living near HV lines	Calculated historical magnetic field levels, spot measurements, 24 h personal measurements, distance from HV line	CC	141 children 546 adults (1637 controls)	Childhood Leukemia (<17 yr old) >0.2 μT historical 2.7 (1.0-6.3) >0.3 μT historical 3.8 (1.4-9.3) 0-50 meters from line 2.9 (1.0-7.3) >0.2 μT spot measurement 0.6 (0.2-1.8) Adult Leukemia (>0.2 μT historical) AML 1.7 (0.8-3.5) CML 1.7 (0.7-3.8)
Lin and Li 1992	Taipei, Taiwan	Children with leukemia	Proximity of school to HV line	SMR	67	Leukemia (all ages) 1.49 (1.17-1.89) Leukemia (0-4 yr old)1.71 (1.24-2.35)
Olsen, Nielsen *et al.* 1992	Denmark	Children with leukemia, lymphoma, or brain tumor	Proximity of residence to HV line and line load	CC	1,707 cases (4,788 controls)	Lymphoma (>0.1 μT) 5.0 (1.2-21) All Cancers (>0.4 μT) 5.6 (1.6-19) All others -- not significant

*Ratio calculated by Aldrich and Easterly 1987

Abbreviations:

CC	Case Control	CNS	Central Nervous System
HCC	High Current Configuration	HV	High Voltage
RFU	Retrospective Follow-up	VHCC	Very High Current Configuration
AML	Acute Myelogenous Leukemia	CML	Chronic Myelogenous Leukemia
SMR	Standardized Mortality Ratio		

TABLE 12-2.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED OCCUPATIONAL EMF EXPOSURE AND CANCER INCIDENCE

Investigator and Date	Site	Workers and Exposure Type	Type of Study	Number of Cases or Subjects	Diagnostic or Occupational Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Wiklund and Eklund 1981	Goteborg, Sweden	Telephone operations	PMR	12 deaths	Leukemia no increased risk
Milham 1982	Washington State	Electrical workers: elevated risk in 10/11 occupations	PMR	196 deaths (438,000 pop.)	Leukemia (all) 1.4 Acute leukemia 1.6
Wright, Peters, and Mack 1982	Los Angeles, California	Electrical workers: greatest exposure & risk for lineman	PIR	35 with leukemia	Leukemia (all) 1.3 Acute leukemia 1.7 AML 2.1
Swerdlow 1983	England & Wales	All occupations	PIR	4284	Eye melanoma elevated for electrical & electronics workers in 3/6 time periods examined
McDowall 1983	England & Wales	Electrical workers: greatest risk electrical engineering & telecommunications	PMR/ CC	98 deaths	AML 2.3 (1.4-3.7) AML - for telecommunication engineers 4.0 (0.8-17.0)
Coleman, Bell, and Skeet 1983	S.E. England	Electrical workers: greatest risk electrical fitters, telegraph operators	PIR	113 with leukemia	Leukemia (all) 1.2 ALL 1.5 AML 1.2
Vagero and Olin 1983	Sweden	Electronics workers: greatest risk in radio & TV industry	RFU	2,864 with cancer (73,102 workers)	Cancer (all) 1.1 (1.0-1.2) Mesopharynx 2.2 (1.1-4.7) Hypopharynx 1.7 (0.9-3.3) Lung (men) 1.4 (1.2-1.5)
Pearce *et al.* 1985*	New Zealand	Electrical workers: greatest risk radio/TV repair, then electricians	PIR/ CC	546 with leukemia	Leukemia (all) 1.7 (1.0-3.0)
Milham 1985*a*	Washington State & California	Amateur radio operators	PMR	24 with leukemia (1,691 deaths)	Leukemia (all) 1.9 AML 2.9 CML 2.7
Lin *et al.* 1985	Maryland	Workers in all occupations, Estimated EM exposure	CC	951 deaths from brain tumors	Definite exposure 1.5 (0.7-3.4) Probable exposure 1.3 (0.6-2.8) No exposure 1.0
Gilman, Ames, and McCawley 1985	USA	Underground miners	RFU	40 with leukemia (6,066 deaths)	Leukemia (all) 2.5 Chronic 8.2 CLL 6.3 Myelogenous 4.7
Spitz and Johnson 1985	Texas	Workers in all occupations with children having neuroblastoma	CC	157 deaths, neuroblastoma in children	Electrical workers 2.1 (1.1-4.4) Electronics workers 11.8 (1.4-98.6)

TABLE 12-2.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED OCCUPATIONAL EMF EXPOSURE AND CANCER INCIDENCE (continued)

Investigator and Date	Site	Workers and Exposure Type	Type of Study	Number of Cases or Subjects	Diagnostic or Occupational Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Olin, Vagero, and Ahlbom 1985	Sweden	Electrical engineers	RFU	108 deaths	Melanoma 3.2 (0.7-9.4)
Vagero *et al.* 1985	Sweden	Telecommunication workers	RFU	139 with cancer	Melanoma 2.5 (1.1-4.9) Melanoma for soldering-workers 3.9 (1.4-8.5)
Milham 1985*b*	Washington State	Workers exposed to EMF in 9 occupations	PMR	317 deaths (12,714 total)	Lymphomas (all) 1.2 Leukemia (all) 1.4 Acute leukemia 1.6
Calle and Savitz 1985	Wisconsin	Workers in 10 occupations involving electricity	PMR	81 leukemia deaths	Leukemia (all) 1.0 For radio & telegraph operators 2.4
Tornqvist *et al.* 1986	Sweden	Workers in electric power industry	RFU	699 deaths (10,061 workers)	Cancer (all) 1.0 Urinary tract cancer Linemen 1.2 (0.8-1.8) Power station operators 1.3 (1.0-1.7)
Stern *et al.* 1986	New Hampshire	Nuclear shipyard workers: elevated risk only for electrical workers, welders	CC	53 leukemia deaths	Leukemia (all) 3.0 (1.3-7.0) Lymphatic leukemia (electrical) 6.0 (1.5-24.5) Myeloid leukemia (welders) 3.8 (1.3-11.5)
McLaughlin *et al.* 1987	Sweden	All job categories	PIR	4,429 brain tumors	SIR 0.9 No apparent relation to non-ionizing radiation in electrical workers
Lin 1987	Taiwan	Electric power company workers	PMR	733 deaths	Liver cancer, brain tumors, & Leukemia/lymphoma - significantly elevated ($p<0.01$)
Thomas *et al.* 1987	Northern New Jersey, Philadelphia, Louisiana Gulf Coast	Electrical & electronics workers - including microwave & radiofrequency	CC	435 brain tumor deaths	All jobs 1.6 (1-2.4) Repairers 4.6 Assemblers 5.6 Soldering 3.4 Electrical engineers 2.2 Technicians 4.1
Tornqvist *et al.* 1987	Sweden	Workers occupationally exposed to low frequency magnetic fields	RFU	325 leukemias	CLL 1.7 (1.1-2.5)
Coleman and Beral 1988	International	Meta-analysis of 11 studies of presumed EMF-exposed occupations & leukemia	CC/PMR	(not specified)	All leukemias 1.18 (1.09-1.29) AML 1.46 (1.27-1.65)
De Guire *et al.* 1988	Montreal, Canada	Workers (9590) in telecommunications company	SIR	10 melanomas	Melanomas 2.7 (1.33-5.02)

TABLE 12-2.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED OCCUPATIONAL EMF EXPOSURE AND CANCER INCIDENCE (continued)

Investigator and Date	Site	Workers and Exposure Type	Type of Study	Number of Cases or Subjects	Diagnostic or Occupational Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Lin 1988	Taiwan	Telephone & telegraph company workers	PMR	374 deaths (129 cancer deaths)	No general increase in cancer deaths Brain tumors 2.4 (0.78-5.59)
Linet *et al.* 1988	Sweden	All job categories	PIR	5,351 leukemia cases	Electrical line workers (SIR) CLL 1.9 (13 cases) ALL 1.4 (8 cases)
Speers, Dobbins, and Miller 1988	East Texas	Workers exposed to EMF	CC	202 deaths	Brain tumors (gliomas) 3.94 (1.52-10.20)
Wilkins and Koutras 1988	Ohio	Workers with children having brain tumors	CC	491 cases	Father employed in: Electrical assembly/repair 2.7 (1.2-6.1) Machinery industry subgroup 3.6 (1.3-10.0)
Flodin *et al.* 1990	Sweden	Electrical workers	CC	86 AML	AML (electrical work) 2.1 (0.7-5.9) (early results for 59 AML cases had OR of 3.8 (1.5-9.5) (Flodin et al. 1986))
Garland *et al.* 1990	All states in USA	US Navy personnel with leukemia	SIR	123 cases (102 verified)	No general increase Electrician's mate subgroup, all leukemia (85% myeloid) 2.5 (1.0-5.1)
Tynes and Andersen 1990	Norway	Electrical workers: greatest risk with long-term employment	SIR	12 men with breast cancer	All electrical work 207 (107-361) All electrical work (>10 yr) 252 (93-549) Electrical transport work 396 (108-1014)
Loomis and Savitz 1990	16 states in USA	Electrical workers: greatest risk of brain cancer in men over age 65, electrical engineers & technicians, telephone workers, electric power work, & electrical work in manufacturing	PMR	2,173 brain cancer deaths; 3,400 leukemia deaths	Brain cancer: All electrical work 1.4 (1.1-1.7) Over age 65 yr 1.9 (1.3-2.7) Leukemia: Engineers, technicians 2.7 (2.1-3.4) Telephone work 1.6 (1.1-2.4) Electric power work 1.7 (1.0-2.7) Other industries 1.7 (1.1-2.5)
Balli-Antunes, Pfluger, and Minder 1990	Switzerland	Railroad workers dying of hematopoietic and lymphatic system malignancies 1969-1983	PMR	23	Versus metal construction and machine building workers 1.44 (0.91-2.17) Versus technical personnel 1.63 (1.03-2.44)
Wilkins and Hundley 1990	Ohio	Workers exposed to EMF with children having neuroblastoma	CC	101	All electrical workers 1.6 (0.3-9.1) Definite EMF exposure 1.9 (0.4-9.7)

TABLE 12-2.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED OCCUPATIONAL EMF EXPOSURE AND CANCER INCIDENCE (continued)

Investigator and Date	Site	Workers and Exposure Type	Type of Study	Number of Cases or Subjects	Diagnostic or Occupational Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Demers *et al.* 1991	USA	Male workers in all occupations diagnosed with breast cancer 1983-1987	CC	227	All EMF exposure 1.8 (1.0-3.7) Electric trades 6.0 (1.7-21) >30 yrs exposure 3.1 (1.2-7.9) >30 yrs exposure & exposure Before age 30 3.3 (1.5-7.3)
Gallagher *et al.* 1991	British Columbia	Male adults with brain & central nervous system cancer	PMR	320,423	All EMF exposure 121 (93-154) Electrical engineers 163 (52-380) Radio/TV work 165 (34-483) Projectionists 408 (84-1192) No increased risk for other electrical occupations; risk for high SES groups (civil engineers PMR = 239 (139-384))
Matanoski, Breysse, and Elliott 1991	New York State	Telephone workers in 5 jobs with EMF exposure	PIR	2 men with breast cancer (50,582 workers)	Central office technicians 6.5 (0.79-23.5) 4 additional cases outside study period: 2 among same group of technicians
Olsen *et al.* 1991	Denmark	Workers in all occupations with children having cancer	CC	1747 children	No elevated risk for childhood cancer with parental employment in electrical occupations
Tornqvist *et al.* 1991	Sweden	Workers in 11 electrical occupations	PMR	133,687 workers	Electrical engineers & technicians Total leukemia 1.3 (1.0-1.7) CLL 1.7 (1.1-5.7) Telephone/telegraph workers Total leukemia 2.1 (1.1-3.6) Ore miners - AML 5.7 (2.1-12.4) Power linesmen - CLL 2.8 (1.1-5.7) Radio/TV repair - glioblastoma 3.4 (1.1-8.0)
Floderus *et al.* 1992	Mid-Sweden/ Goteborg county	All male cases with leukemia or brain tumors 1983-87; EMF exposure in job held longest in decade before diagnosis	CC	250 leukemias, 261 brain tumors	CLL Mean EMF>0.41 μT 3.72 (1.79-7.74) Ever having EMF>0.28 μT 1.27 (0.9-1.81) Brain tumors Median EMF>0.2 μT 1.63 (1.04-2.56) Ever having EMF>0.28 μT 1.27 (0.9-1.78) AML, no increased risk
Guenel, Raskmark *et al.* 1992	Denmark	All actively employed Danes 20-64 yr of age in 1970	SIR	3,932 men; 1,885 women	Leukemia Presumed EMF>0.3 μT 1.64 (1.2-2.2) No significant increases for other cancers

TABLE 12-2.

SUMMARY OF EPIDEMIOLOGIC STUDIES OF PRESUMED OCCUPATIONAL EMF EXPOSURE AND CANCER INCIDENCE (continued)

Investigator and Date	Site	Workers and Exposure Type	Type of Study	Number of Cases or Subjects	Diagnostic or Occupational Risk Examined and Odds Ratio or Risk Ratio (95% Confidence Intervals)
Kuijten *et al.* 1992	New Jersey, Delaware, Pennsylvania	Workers in all occupations with children having astrocytoma	CC	163 children	EMF exposure during pregnancy Definite exposure 1.1 (0.4-3.1) Probable exposure 1.7 (0.7-4.4)
Loomis 1992	24 states in USA	Cases of male breast cancer 19 yr of age or more in 1985-1988	CC	250 male breast cancer cases	Electrical occupations All ages (4 cases) 0.9 Under age 65 (3 cases) 2.2 (0.6-7.8) Telephone worker (1 case) 9.0 (0.9-88.7) Early age and telephone work suggested: too few cases for significance
Tynes, Andersen *et al.* 1992	Norway	Male electrical workers	SIR	37,945 workers	Leukemia 10 or more yr work 1.41 (1.10-1.76) Radio & TV repair 3.18 (1.03-7.43) Power line work 1.90 (1.01-3.24) Brain tumors Railway track walkers 2.20 (1.10-4.18) Male breast cancer Electric transport work 3.96 (1.08-10.14)
Matanoski, Elliott *et al.* 1993	New York City	American Telephone and Telegraph (AT&T) employees	CC	124 leukemia deaths 1975-1980	All exposed workers 1.6 (0.5-49) >median EMF exposure 2.5 (0.7-8.6) With 15 yr latency 6.6 (0.7-58) Central office technicians 3.0 (0.6-15)
Sahl, Kelsh *et al.* 1993	California	Southern California Edison Company utility workers	CC	36,221 workers	EMF exposure estimate based on magnetic field measurements of present-day jobs -- no significant elevation of cancer risks Lymphomas (all electrical workers) 1.25 (0.68-2.31) Leukemia (>5 μT exposure) 1.30 (0.87-1.92)

*Corrected occupational categories as given in *Lancet* 2(8601):48, 1988.

Abbreviations:

ALL	Acute Lymphocytic Leukemia	AML	Acute Myelogenous Leukemia
CC	Case Control	CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myelogenous Leukemia	PIR	Proportional Incidence Ratio
PMR	Proportional Mortality Ratio	RFU	Retrospective Follow-Up
SIR	Standardized Incidence Ratio		

13. CARCINOGENESIS AND ELF EMF

13.1 SUMMARY OF EPIDEMIOLOGIC EVIDENCE

Among the possible biological effects of ELF EMF, the possibility that exposure to ELF EMF may increase the incidence of certain types of cancer in human populations has attracted the greatest public concern. A number of more or less comprehensive reviews and evaluations of the epidemiologic evidence associating EMF exposure with increased cancer incidence have been published over the past few years (Ahlbom 1988; Brown and Chattopadhyay 1988; Coleman and Beral 1988; Czerski 1988; Delpizzo and Keam 1989; Knave 1988; Repacholi 1988; Creasey and Goldberg 1989; Gurvich and Novokhatskaia 1989; Salvatore and Weitberg 1989; Tenforde 1989; Repacholi 1990; Theriault 1991; Savitz and Chen 1990; Environmental Protection Agency 1990; Anderson 1991; Eriksson and Karlsson 1992; McBride and Gallagher 1992; National Radiological Protection Board 1992; Theriault 1992). In this section of the report, we will briefly discuss this epidemiologic evidence, review the results of the few animal tumor studies involving ELF EMF which have been published, and discuss the implications of these results for maglev exposures.

Among the first studies to escalate public concern was the report by Wertheimer and Leeper (1979), describing a two- to three-fold higher incidence of cancer among children living in homes in proximity to distribution lines designed to carry a high current (termed "high-current configuration"). Because the findings were related to lines that carried high current rather than high voltage, the authors suggested the effect was due to magnetic rather than electric fields. This report has been widely criticized for methodological limitations, but it was seminal in stimulating further work in this area.

Although a number of case-control studies were conducted in parallel with or to follow up on Wertheimer and Leeper's report, the first major reevaluation of the study was performed by David Savitz and coworkers in a project funded as part of the New York State Powerlines Project. This study was intended to replicate the Wertheimer and Leeper study in the same geographic area (but with a different case-control population) using substantially improved epidemiologic methods (Savitz *et al.* 1988). Results of this study indicated an increase in risk for several types of childhood cancers associated with residential proximity to power lines which was smaller than the risk estimate determined by Wertheimer and Leeper, but still statistically significant. The odds ratio (OR) for the two highest wire codes compared to the three lowest was 1.5 (1.0-2.3 95% confidence interval (CI)) for total cases, with consistency across cancer subgroups except for brain cancer (OR = 2.0, 1.1-3.8 CI) and lymphomas (OR = 0.8, 0.3-2.2 CI). A limited number of magnetic field measurements were also performed as part of this study. Magnetic fields measured under low power conditions were only weakly associated with cancer incidence (ORs of 1.0, 1.3, 1.3, and 1.5 across four exposure intervals), and for a cutoff exposure level of 0.2 μT (2.0 mG), there was an OR of 1.4 (0.6-2.9 CI) for total cancers and somewhat higher ORs for leukemias (OR = 1.9, 0.7-5.6 CI), lymphomas (OR = 2.2, 0.5-10.3 CI), and soft tissue sarcomas (OR = 3.3, 0.9-12.1 CI). Since the lower confidence intervals for these estimates fall below unity, these risk estimates are considered not significant. Magnetic fields measured under high power use conditions and electric field measurements showed no associations with risk of total cancer. Therefore, results confirmed a low increase in cancer risk related to wire configuration, but the correlation with measured magnetic field levels was not significant.

A third large-scale replicate of the Wertheimer and Leeper study design was conducted in the Los Angeles area (London *et al.* 1991). An objective of this study was to replicate the earlier work basing the study on a more complete set of field measurements rather than the indirect wire code estimates. Interviews were obtained from 232 cases and 232 controls matched to cases (within 1-3 yr depending on case age). An attempt was made to make magnetic field measurements for at least one residence per subject for the "etiologic" period (from conception to the date of leukemia diagnosis for children under 1 yr, diagnosis minus 6 months for children diagnosed at 1-2 yr, or diagnosis minus 1 yr for children diagnosed over age 2). Wiring configuration estimates were made for 219 cases and 207 controls, based on the Denver (Wertheimer-Leeper) classification scheme. Mean 24-h magnetic field measurements were highly correlated with spot measurements in the child's bedroom. Except for static field measurements in the child's bedroom, variability was so large that standard deviations exceeded the means in case and control measurements. No clear associations between leukemia risk and various magnetic or electric field descriptors were seen (arithmetic mean, median, 90th percentile, time over 0.25 μT, etc.). However, an association was observed between the Wertheimer-Leeper wiring configuration and childhood leukemia risk. With the very low and underground categories combined into a single reference group, risk ratios were OR = 0.95, 0.53-1.69 CI for ordinary low wiring configuration, OR = 1.44, 0.81-2.56 CI for ordinary high wiring configuration, and OR = 2.15, 1.08-4.28 CI (p for trend = 0.008) for very high wiring configuration. These risk ratios were not substantially altered by adjustment for potential confounding factors. Cases were more likely than controls to report use of several appliances that produce high magnetic fields: significant risk increases were noted for child's use of black-and-white television (OR = 1.49, 1.01-2.23 CI) and electric hair dryer (OR = 2.82, 1.42-6.32 CI), but risk elevations were not significant for reported use of color television (OR = 1.06, 0.66-1.74 CI), electric blanket (OR = 7.00, 0.86-121.80 CI), dial electric clock (OR = 1.86, 0.97-3.83 CI), or curling iron (OR = 6.00, 0.72-104.80 CI). Among environmental variables, significant risk increases were reported only for incense use (OR = 2.78, 1.30-6.88 CI), and insecticide use inside the home (OR = 2.48, 1.49-4.36 CI). Therefore, authors concluded that their results support an association between childhood leukemia risk and wiring configuration, but not direct measurements of electric and magnetic fields.

The finding of a stronger association of risk of childhood cancer with wiring configuration than with direct magnetic field measures (24-h or spot measurements) was an unexpected result which raised concern in the public and research communities. The results suggest two contradictory interpretations. If there is a true etiologic relation between residential exposure to higher electric and/or magnetic fields and childhood leukemia, wiring configuration may be a better indication of long-term field exposure than spot or 24-h measurements; or the biologically relevant aspect of EMF exposure in relation to leukemia risk was not measured (e.g., some other attribute is important, such as abrupt changes in magnetic field intensity at a specific frequency). Alternatively, there may be no true association between EMF exposure and leukemia risk, and the observed association is due to bias or a confounding factor. London *et al.* point out that observed risk was adjusted for a wide variety of confounding factors thought to influence leukemia with little effect on the results, and a new unknown factor would have to be very strongly associated with wiring configuration to produce the odds ratios observed (London *et al.* 1991).

A possible explanation for the stronger association with wiring configuration can be found in results of a Swedish study reported by Feychting and Ahlbom (Feychting and Ahlbom 1992). This study utilized historical estimates of exposure in the vicinity of power transmission lines, and found good agreement between contemporary estimates and measurements, but a tendancy for greatly increased power usage in later years which produced a substantial mismatch between historical and contemporary exposure patterns. Therefore if it more closely reflects long-term exposure patterns, wire configuration may provide a better estimate of historical exposure than contemporary magnetic field measurements.

Many of the occupational studies with high risk ratios are based on small numbers of subjects, and the extent of their exposure to EMF and other agents is very uncertain. To define more completely valid ratios in this range would require prohibitively large subject accrual for rare cancers, and in other cases would be made difficult by confounding factors which are typical of many occupational settings. A number of studies are in progress to address these issues with larger sample sizes, detailed dosimetry during typical work situations, and prospective studies of ELF EMF-exposed populations.

In the results of epidemiologic studies of the general population conducted to date, presumptive EMF exposure appears to be associated primarily with leukemias, nervous system tumors, melanoma, and male breast cancer (Tables 12-1 and 12-2), which represent a heterogeneous group of malignancies. The barely detectable increase in the incidence of childhood tumors associated with ELF EMF exposure may only reflect the technical limitations of these studies. Childhood cancers have a relatively short latency period, making it possible to get some meaningful indication of residence at the critical time of tumor induction or growth. Male breast cancer would be an example of a neoplasm with an extremely low background frequency that might facilitate statistical demonstration of an increased incidence; the same would apply to melanoma in the eye. A statistically significant increase in other types of cancer having a longer latency period or a higher background incidence in the general population is harder to detect with epidemiologic methods. The detection of an increased incidence of childhood, hematopoietic and other rare cancers in EMF epidemiologic studies may therefore reflect methodological limitations rather than indicating a tissue-specific biological response to EMF. Wertheimer and Leeper (1987) have suggested an association between EMF exposure and particular cancer subtypes and age-incidence patterns, but there is not much data to support this hypothesis. If the observed lack of specificity proves to be correct, it suggests that ELF EMF exposure increases cancer incidence through a very general mechanism such as promotion, or that relatively subtle variations in field characteristics (frequency, waveform, rate of change of field strength) or biology result in signal-specific effects on a variety of target cell types. Clearly much more work needs to be done to relate specific field characteristics to biological effects, so that further speculation at this time seems premature.

13.2 ANIMAL EXPERIMENTS

After an effect has been suggested by epidemiologic evidence, the usual course of experimentation is to model the effect in an animal system in order to develop a plausible biological mechanism and to suggest measures for the epidemiologic work that might refine exposure metrics to more directly relate the suggested effect to the cause (Matanoski 1992). Previous large scale animal screening studies with ELF EMF have indicated little or no carcinogenic activity, but experiments have recently been initiated to look more directly at EMF as a cancer promoter.

On the basis of studies in animal models, primarily skin cancer, carcinogenesis is traditionally viewed as a two-stage, multistep process (Berenblum and Shubik 1947; Berenblum 1954). The first stage, initiation, is thought to involve a mutational event that sets up the potential for malignant growth that is passed on to subsequent daughter cells. For an overt cancer to develop, a series of further steps are needed which are grouped loosely together as the promotion stage. Promotion involves changes that enhance the survival or proliferative advantage of the initiated cells and lead to the expression of the malignant phenotype. There is a significant number of independent changes, both genetic and epigenetic, comprising these steps in the carcinogenic pathway, and the roles played by alterations in gene expression and rate of cell proliferation (itself a carcinogenic factor by virtue of the lack of 100% fidelity during DNA replication) are still being elucidated (Cohen and Ellwein 1991; Cox and Goding 1991).

Recent animal experiments have focused on the concept of ELF EMF as a promoter or copromoter of cancer. Epidemiologic results suggest a non-specific effect on cancer incidence which involves a range of cancer types, something which is not characteristic of chemical initiators, which tend to act on limited number of cells and consequently produce a limited range of tumor types. The timing of exposure to ELF EMF and tumor incidence reflects, at least in some cases, a short latency period characteristic of action at a late step in the carcinogenesis process (Wertheimer and Leeper 1979). In general, results of *in vitro* experiments indicate that ELF EMF is not mutagenic (Creasey and Goldberg 1989). These results then support the concept that ELF EMF, if it has any effect on cancer incidence, is more likely to be acting at the promotion level than at the tumor initiation level (Goldberg and Creasey 1991).

A group from the Swedish National Institute of Occupational Health evaluated the ability of 50 Hz magnetic fields (flux densities of 0.5 to 500 μT) to enhance the incidence of preneoplastic liver foci in Sprague-Dawley rats subjected to a partial hepatectomy followed by a subcarcinogenic dose of dimethylnitrosamine (Rannug, Holmberg, and Mild 1990). They reported that there was no systematic pattern in foci development associated with magnetic field exposure. Baumann *et al.* (1989) reported no effect on the growth of transplanted mammary carcinoma (or on reproductive hormone levels) in retired breeder Wistar-Furth rats exposed to 0.1-2.0 mT 2000 Hz magnetic fields. Another recent study failed to show any promoting activity of a 50 Hz, 30 mT magnetic field on the induction of adenocarcinomas in rats exposed to 9,12-dimethylbenzanthracene at 5 mg, followed by magnetic field exposure for 91 days (Buntenkotter *et al.* 1990).

Makinodan *et al.* reported plans to evaluate the carcinogenicity of chronic exposure (20 h/day) to 60 Hz, 10 and 1000 mT, circularly polarized magnetic fields in C57BL/6 mice (Makinodan *et al.* 1990). They have not yet reported any results with magnetic field exposure alone, but recently reported preliminary results indicating that they have altered their experimental design and are now using low-level ionizing radiation to enhance the basal leukemia rate (Makinodan *et al.* 1992). They report that their assays show good sensitivity for the T cell differentiation markers which will be used to follow lymphatic leukemia in these animals.

In contrast to these reports of no effect, there are a few reports in the literature of strong carcinogenic/promotional effects of ELF EMF in animal tumor systems. Several years ago, Leung *et al.* (1988b) reported that high voltage electric field exposure (a 60 Hz (40 kV/m) electric fields with no magnetic field component) significantly increased the incidence of mammary tumors in rats exposed to 7,12-dimethylbenz(a)anthracene compared with sham-exposed rats. Similar findings of significantly enhanced mammary tumorigenesis with shortened latency in rats exposed to household-frequency (50 Hz) magnetic fields have been reported more recently by Beniashvili, Bilanishvili, and Menabde (1991), using methylnitrosourea (MNU) as the inducer. Virgin female rats (55-60 days old; strain bred at the Oncology Research Center, but not otherwise specified) were exposed to MNU (50 mg/kg iv) and a variable (AC) magnetic field (50 Hz frequency) or a static (DC) magnetic field (0.2 Oe [approximately 20 μT] intensity). Four hundred animals were divided into 10 groups. They were exposed to various combinations of EMF only (0.5-3 h/day) or a single MNU exposure followed by daily EMF exposure, and followed for up to 2 yr. Animals exposed for 3 h/day to AC or DC fields alone had a significant increase in tumor incidence and decreased mean latent periods to mammary tumor development. There was no increase in tumor incidence in animals exposed to EMF for 0.5 h/day. EMF exposure also affected the morphological spectrum of tumors produced by MNU, with a preponderance of malignant cancers developing in EMF/carcinogen-treated animals as compared to animals treated with carcinogen alone.

Fam and Mikhail followed cancer incidence in three generations of SW-ICR that were continuously exposed to a 25 mT (250 G), 60 Hz magnetic field. In their initial reports they indicated that there was no significant increase in tumor incidence (Fam and Mikhail 1990), but by the third generation they were reporting that 45% of the exposed animals had developed malignant lymphoma, (characterized by thymic tumor, generalized lymphadenopathy, hepatosplenomegaly, and massive tissue infiltration of bowel, lung, liver, bone marrow and kidney), 30% of the exposed animals developed marked lymphoid hyperplasia, and only the remaining 25% did not show evidence of disease (Mikhail and Fam 1991). They recently reported results with another group of 41 CFW mice continuously exposed to a 25 mT, 60 Hz EMF from conception until the age of 363-418 days, which were compared with a group of 36 unexposed controls. They confirmed their earlier report, indicating that 78% of the exposed animals vs. 6% of the animals in the control group developed premalignant changes or malignant lymphoma (Mikhail and Fam 1992).

Results of several groups investigating the promotion effect of ELF EMF seem to present contradictory results as experimental approach evolves. Mevissen *et al.* examined the effects of a 50 Hz, 1 μT (10 mG) magnetic field on the development of mammary tumors in female rats pretreated with 7,12-Dimethylbenz(a)anthracene (Mevissen *et al.* 1992). The initial results indicated that magnetic field exposure had no detectable effect on tumor incidence, while measurements of serum melatonin levels in the same animals showed that the exposure conditions were sufficient to significantly reduce nocturnal melatonin levels. A later report from the same group indicated suggestions of an effect in at least one replicate of the experimental design (Mevissen, Stamm et al. 1993), and the most recent reports from this group (Mevissen, Loscher et al. 1993; Loscher, personal communication) indicate a much stronger (highly significant) promoter effect. Among other changes in the experimental design, Mevissen and coworkers have enlarged their exposure facility to allow simultaneous exposure of 100 rats per group, which overcomes some limitations of small sample size. In a recent experimental series (Loscher *et al.* 1993), they found a 50% increase in tumor incidence with exposure to a 100 uT (2 G) 50 Hz field. The mean number of rats with tumors was significantly increased ($p<0.03$) for weeks 8-13, and the mean tumor size was significantly increased by 13 weeks ($p=0.013$). There was no significant increase in the number of tumors per rat in tumor-bearing animals. The authors attribute the increased level of significance primarily to the larger sample size, noting that previous studies showed the same trend of effects, but were not statistically significant. Previous experiments, which used sample numbers on the order of 30 per group, would not reveal increases in tumor growth of less than 100%.

McLean *et al.* (1991; 1992) have looked at the copromotional effect of 60 Hz magnetic fields (2 mT, 6 h/day, 5 days/week for 23 weeks) in the mouse-skin model, with 7,12-dimethylbenz-anthracene as initiator and suboptimal levels of phorbol esters as promoters. Early results suggested some stimulation of tumor development but no effect on overall tumor yield. More recent results indicated a weaker effect of magnetic field exposure, and a possible confounding promoter action from exposure to fluorescent light (McLean, Thansandote et al. 1993). This result would appear to explain away the copromoter effect of magnetic fields reported earlier, but experiments were done with only 48 animals per group and, as noted above, are therefore subject to some statistical uncertainty. A number of other studies are currently in progress with no results yet available. A group from Battelle Laboratories is looking at the effect of 5 or 500 μT continuous, or 500 μT interrupted 60 Hz magnetic fields on the proliferation and spleen colonization of a transplantable large granular lymphocytic leukemia cell line in male Fisher F344/N rats (Morris *et al.* 1992; Sasser, Morris *et al.* 1993). Mandeville *et al.* have developed and characterized an exposure system for a chronic exposure study of Fischer F344/N rats in 60 Hz linear (single axis) sinusoidal continuous wave magnetic fields of different intensities (< 0.5 μT (sham), 2 μT, 20 μT, 200 μT, or 2 mT) (Mandeville *et al.* 1991).

Other frequencies of EMF have been investigated for therapeutic purposes, and results indicating that EMF may be used as a means to inhibit the growth of preexisting tumors have been reported in several different experimental systems. Inhibition of growth was reported for spontaneous mammary tumors in C3H mice and spontaneous leukemia in AKR mice exposed to pulsed magnetic fields (12, 100, 460 Hz: Bellossi, Bernard-Griffiths, and Le Gall 1988; Bellossi and Desplaces 1990, 1991), reticulosarcoma in rats (fluctuating EMF less than 200 kHz; (Iur'ev and Krasnogorskaia 1980), and for benzopyrene-induced tumors in BALB/c or C3H, and grafted B16 melanoma in C57/Yellow mice exposed 8 h/day to a square-wave pulsed magnetic field of 0.8 Hz and 10 mT (de Seze *et al.* 1992). On the other hand, Salford *et al.* (1992) could find no evidence of any alteration in the growth rate of a rat brain glioma model exposed to continuous 91.5 MHz microwave radiation, or the same microwave modulated at 4, 8, 16, and 200 Hz in 0.5 ms pulses or at 50 Hz in 6 ms pulses, while the growth of P388 leukemia in mice was not affected by 60 Hz magnetic fields (Thomson, Michaelson, and Nguyen 1988). EMF (100 Hz) was found to inhibit the growth of HeLa and mouse mammary tumor cell lines *in vitro* (Rius, Alvarez-Rodriguez, and Valladares 1985), while others have also reported a decreased proliferation of HeLa and U 937 cells 24 h after exposure (20 h) to square-wave pulsed magnetic fields of 0 to 1.6 Hz and 0-180 mT (Tuffet *et al.* 1992). Other studies have found no such effect, either in HeLa cells in culture exposed to 50 Hz ELF EMF (Adolphe *et al.* 1987), or in human promyelocytic HL-6 cells exposed for 6 to 72 h to AC magnetic fields of 1 μT up to 25 mT. These studies with tumor lines are of questionable relevance to carcinogenesis *per se*, representing, rather, a test of tumor progression and growth. As a therapeutic approach, these experimental results have not been replicated by more than a single group of investigators, and reported field conditions are not consistent enough and biological systems are too variable to define clearly therapeutic field conditions. The data do not allow a clear definition of which fields enhance and which fields inhibit tumor growth. The findings do suggest, however, that sinusoidal ELF fields, unlike pulsed fields, do not consistently exhibit growth-stimulating actions.

At the present time, the results of animal model studies may be summarized as inconclusive. Reports are largely negative, but in this context negative reports are not very meaningful. There is little or no clear evidence from epidemiologic studies as to which aspects of EMF exposure, if any, are linked to increased cancer incidence. For example, it is not certain if a common assumption in chemical carcinogenesis studies, that dose can be increased to increase the statistical probability of an effect, is valid for EMF studies. Nor is it clear which aspect of "dose" (e.g., average magnetic field intensity, number of on/off events, peak field intensity, etc.) should be scaled up. To avoid these problems, many tumor model systems and large numbers of animals could be screened. However, large-scale *in vivo* experiments are time consuming and costly, and few have been funded. This area of EMF research is therefore likely to progress slowly unless strong results from one or more groups stimulate greater activity.

13.3 IN VITRO EXPERIMENTS

The effect of ELF EMF on cell proliferation and gene expression was discussed in some detail in a previous section of this report (Section 10), and the reader is referred to that section for details of experiments and results. Experimental results generally indicate consistent effects on stimulating proliferation of bone and certain soft tissue cells by pulsed and DC electric fields, and a similar pattern of stimulation by time-varying or pulsed magnetic fields, presumably through the induction of weak electric fields in the tissues. Proliferatative effects of other fields, especially of specific combinations of sinusoidal and DC fields which satisfy various ion cyclotron resonance conditions, are less certain, as stimulatory, inhibitory, and null effects have been reported by different investigators (for similar but not identical field conditions and biological systems).

In vitro experimental results present a confused picture with respect to possible cancer-promoting effects of ELF EMF. There is evidence in both normal cells and established cell lines (which are considered by most workers to be already partially transformed) that certain ELF EMFs can increase proliferation rates, alter cell cycle parameters in the culture, enhance or inhibit the expression of certain genes, and stimulate biochemical responses, such as increased ODC activity, which are also simulated by known chemical promoters (Adey 1988b). On the other hand, the degree to which *in vitro* responses parallel responses *in vivo* are not clear. Those physicians involved in promoting pulsed EMF treatment for enhanced bone healing argue that there is a distinct difference between these fields and those that might promote malignant growth (Bassett 1988), and suggest that it may even be possible to control cancerous growth with appropriate pulsed fields (Bassett 1992). Although there are strong indications that both proliferation and growth inhibition can be produced by different types of EMF, the critical field and biological parameters are not clearly defined. Whole animal systems may also respond differently to ELF EMF than isolated cells. Although there has been only limited clinical use of pulsed fields, there have been no reports, of which we are aware, indicating increased cancer incidence or other abnormal growths associated with these treatments.

13.4 THE EVIDENCE FOR THE CARCINOGENICITY OF ELF EMF

Epidemiologic studies have suggested a weak association between exposure to time-varying extremely low frequency electromagnetic fields (ELF EMF) and increased risk of various types of cancer. The magnitude of the relative risk increase is small in most cases, and a number of other studies have failed to demonstrate any adverse health effects. Experimental work relevant to the possible mechanism of an EMF-cancer association has been limited to date, and the body of literature is difficult to interpret because of conflicting results. It is generally accepted, however, that nonionizing radiation has no mutagenic effect, and therefore, most work has been based implicitly on the hypothesis that EMF energy acts as a cancer promoter. Relatively few studies have directly addressed carcinogenicity in animal systems, but a number of these are now underway.

The epidemiologic evidence presents a consistent, if rather puzzling, picture (with the relationship of risk to wire code but not to measured magnetic fields). The epidemiologic studies are somewhat controversial evidence for human health risk, however, because of inherent technical limitations. As discussed by Savitz, Pearce, and Poole (1989), Tenforde (1989), and Wilkening and Sutton (1990), there is little consistency in the reported associations between different "electrical" occupations and cancer incidence. In most cases, insufficient attention has been paid to coincident exposure to other known carcinogenic factors such as organic solvents. Furthermore, most studies suffer from methodological problems that include incomplete or qualitative dosimetry; absence of consideration of the harmonics and other features, such as time-variation, of fields generated by specific equipment or appliances and by grounding currents; sample populations too small for meaningful analysis of small but potentially important differences; problems in the selection, composition and matching of control groups; and reliance on study designs that involve weaker statistical end points, such as proportional mortality ratios. Many of the earlier studies exhibit features from this list, such as lack of measured exposures, problems with the control groups, and insufficient allowance for confounding factors (Carstensen 1987; Michaelson 1987). Others acknowledge the methodological weaknesses, but still maintain that these studies have yielded results indicative of elevated risk (Savitz and Calle 1987). Even when experiments are well designed, cost and difficulty in obtaining a sufficient sample size and valid exposure classifications may make accurate estimate of a low risk impractical. In their case-control study of 436 childhood cancer patients, Nasca *et al.* (1988) found no statistically significant risk associated with paternal exposure to electromagnetic fields, but also estimated the lowest detectable risk at a minimum odds ratio of 2.47-2.83. Inconsistent results in the association of tumor risk with paternal exposure to ionizing radiation in this study underscores the sensitivity problem.

The two major epidemiologic studies replicating the initial report of Wertheimer and Leeper (1979) have all found a stronger correlation with wiring configuration as a surrogate for actual field measurements than for the magnetic field measurement itself. For example, the odds ratio in Savitz's 1987 study, calculated on the basis of the wiring configuration scheme, might be 5.4 for residents of homes classed in the highest current exposure category, but is only 1.3 for the highest exposure category (houses over 0.2 μT) based on magnetic field measurements under low house power (Savitz *et al.* 1988; Wachtel, Barnes, and Savitz 1987). Similarly, in the case-control study carried out by London *et al.* (1991) in 232 children with leukemia in Los Angeles, the odds ratio was 2.15 on the basis of very high voltage configuration, falling when 24 h field monitoring was the metric, to 1.37 and 1.22 for 0.068-0.124 and >0.124 μT fields, respectively. While it may appear that actual measured field would be the preferred metric, the major variations in exposure that continuous monitoring has disclosed (Deadman *et al.* 1988) show point-in-time measurements may be inadequate. Even mean field estimates, based on 24 h continuous EMDEX measurements, may not truly reflect biologically-relevant exposure, since they do not take into account detailed time variations and on-off switching. Indeed, in a recent 24 h personal EMDEX dosimeter study in pregnant women, a rate of change metric that did reflect these time variations did not correlate with estimated geometric mean exposure (Lee *et al.* 1992a). Furthermore, residential exposures are greatly affected by the existence of field gradients from distribution lines and grounding currents (Donnelly and Agnew 1991), which makes the locations at which measurements are being made very critical. Wiring configuration, as an indicator of both average long-term potential exposure and the capacity of the system to deliver variations and surges in current, thus appears to be a plausible parameter to use (Savitz *et al.* 1988; Savitz, Pearce, and Poole 1989).

Nevertheless, the results of recent EMDEX surveys still have been in conflict in terms of whether wiring code is (Kaune and Zaffanella 1992) or is not (Rankin, Bracken, and Senior 1992) a good predictor of magnetic field exposure. It has been suggested that inconsistencies in the epidemiologic findings reflect the operation of factors other than the magnetic field. These include the likelihood of large ground currents flowing through water pipes and giving rise to electrolytic corrosion with release of high levels of ions, especially copper, which has been associated with tumorigenesis (Kavet 1991); indoor air pollutants (Easterly 1992); or greater residential mobility on the part of those living in high-configuration, as compared with low-configuration, homes (Jones *et al.* 1992). The ongoing effort to measure field strengths, taking into account their time-varying quality, and various possible confounders in residential and occupational situations, will hopefully help resolve this problem, at least for the United States. In other countries such as Denmark (Kaad Jensen and Folkersen 1992), differing arrangements of the electrical supply systems may make wire codes less applicable.

Least satisfactory of the exposure surrogates is occupational classification, where it has been found that there is wide variation in exposure to magnetic fields among workers with the same job title (McDermott and White 1992a; 1992b). While exposure to EMF may be inferred from such categorization as "electrical worker", this occupation may also involve exposure to chemical carcinogens such as organic solvents, and not all electrical workers would be in close proximity to high-voltage lines or working with them when power is on. Some occupational classifications may not even suggest the degree of exposure to EMF. Bowman *et al.* (1988) recorded the highest magnetic field exposure for forklift operators, an occupational category generally not considered "electrical." This may contribute to the conflicting findings in studies based on deaths from a single disease for all occupations. Examples of this are the studies by Lin (1985), in which the risk for brain tumors correlated with probable and definite exposure to EMF (OR 1.3 and 1.5 respectively); Spitz and Johnson (1985), in which the ORs for neuroblastoma in electrical and electronics workers were 2.1 and 11.8, respectively; and McLaughlin *et al.* (1987), where there was no relationship between brain tumor deaths and EMF exposure. Stern has indicated that electric welders are exposed to higher EMFs than those recorded for any other worker category, and spend 30-50% of their working day with this exposure (Stern 1987). Leukemia was not associated with this classification (risk ratio (RR) of 0.92), but respiratory cancer was (RR = 1.39). This latter risk, however, was ascribable to exposure to welding fumes rather than EMF. A similar conclusion was reached by Wilkins and Koutras who noted an association between childhood brain tumors and paternal employment in metal-related jobs (which included electrical assembly and repair) at the time of birth (Wilkins and Koutras 1988). The net effect of any errors in exposure classification, assuming they are non-differential, is to bias the estimate of risk toward the null (no effect) conclusion (Sahl 1988).

Problems with controls have beset even the best epidemiologic studies. That of Savitz (1988), for example, disclosed the highest odds ratios in the group with the greatest inferred exposure, but it was precisely this group in which compliance of the controls was least satisfactory, thus reducing the validity of the findings. In the studies of Wertheimer and Leeper, case/control exposure evaluation was not blinded and thus was subject to bias. Many of these factors have been discussed by Cole (1987) and Tenforde (1989). Although animal data are clearly needed to help clarify the situation with regard to epidemiology, in particular by establishing whether or not the association in humans is biologically plausible, further well-designed epidemiologic studies are also needed. Several studies are currently underway at sites other than those used in previous surveys, an important consideration given the widely different wiring and transmission systems throughout the world.

There are many studies of ELF EMF bioeffects that relate indirectly to potential cancer-causing mechanisms, as we have discussed in this and other reviews of the EMF bioeffects literature (Creasey and Goldberg 1989; Goldberg and Creasey 1991). Cancer is a biological "endpoint" which may be affected through physiological systems, such as the immune system or the neuroendocrine system, as well as by direct effects on cell proliferation and differentiation. These effects have been discussed previously in this report. Several studies have examined effects of ELF EMF on the growth of transplanted or chemically induced tumors, and others have followed small populations of animals chronically exposed to EMF over a few generations, most of them without evidence of increased cancer incidence or growth. Increased cell growth, cell division, and transcription have been reported for a number of cell types *in vitro* and *in vivo*. Reported effects on the immune system of intact animals and on cells of the immune system *in vitro* range from gross inhibition of immune function to enhanced immune activity. There have been reports of neuroendocrine effects of ELF EMF that could be interpreted as simple stress responses. Disturbances in circadian rhythms, primarily associated with altered pineal function, have also been observed, and these are currently the object of considerable attention because of their relevance not only to cancer promotion, but also to postulated behavioral effects of EMF. Each of these bodies of results can be used to support potential carcinogenic mechanisms (Goldberg and Creasey 1991), but this does not, in itself, confirm that ELF EMF exposure is a risk factor for cancer. The definitive answer to that question can only be provided by a whole-animal tumor model which is shown to reproducibly respond to specific EMF conditions with some sort of dose and/or frequency and/or intensity relationship. Results of these animal experiments could then be used to better define potentially harmful EMF exposures in the environment, and refine the "exposed group" criteria in epidemiologic studies in order to more accurately assess potential human risk.

Perhaps the single most important unresolved issue is the nature of the dose-response to EMF. Increasingly, many bioeffects have been reported to occur only at specific combinations of frequency and intensity, and are attributed to the magnetic rather than the electric component of the field, possibly in interaction with the static geomagnetic field. In addition, the time-varying nature of the signal has also been described as a determinant of biological activity in a number of systems. If such effects prove to be real, they imply a complex dose-response relationship, and raise the possibility that a much higher risk of cancer is associated with a subset of the "exposed group" that experiences the critical specific range of exposure conditions. At this point, it is not possible to assume that a population exposed to a higher average intensity field is necessarily being exposed to a greater risk of cancer than a group exposed to a lower intensity field, especially if other field characteristics (*e.g.*, peak field intensity, frequency, waveform, and number of on/off cycles) differ. The major effort now being devoted to improved dosimetry (measuring and producing well-characterized electric and magnetic fields in biological specimens) is a necessary prerequisite to experimental work that will enable bioeffects to be defined in terms of internal field characteristics. Improvement in dosimetry may also help resolve the problem of conflicting experimental results that currently characterize research in this field and prevent any definitive assessment of EMF risk from being made.

14. HYPOTHETICAL UNIFYING BIOLOGICAL MECHANISMS

This section discusses some hypothetical biological mechanisms to explain how weak ELF EMFs could produce some of the bioeffects discussed in the previous sections. These bioeffects are described with few citations, and the reader is referred to earlier sections of this report for references to the research.

Hypothetical mechanisms have been organized on the basis of their biological level of action, and it should be recognized that they are not mutually exclusive. For example, mechanisms which operate at the level of tissue interactions may have a basis in the molecular biology of the cell (for example, in the transcription of new surface and extracellular glycoproteins), at the membrane level (signals which stimulate that transcription), and at the molecular level (EMF-receptor interactions which initiate the stimulus). In this section we have tried to suggest connections between EMF research and the general biomedical literature, but the citations to this literature are intended to be illustrative rather than based on a comprehensive review of the literature.

14.1 HYPOTHETICAL MECHANISMS AT THE WHOLE ANIMAL LEVEL

14.1.1 Neuroendocrine Mechanisms

At the present time, there is moderately strong evidence that ELF EMF has effects on the quantity and timing of nocturnal pineal melatonin release. This conclusion is tempered by the failure to consistently demonstrate EMF effects in laboratory rodent experiments, and by indications in animal and human studies that pineal effects may be produced only by fields with specific characteristics, such as rapidly changing field intensity. If the later tentative conclusion is correct, it means that future epidemiologic research will need to differentiate between effective and ineffective field exposures from environmental sources in order to more clearly define exposed and reference groups.

There are two major biological consequences which could follow decreased nocturnal melatonin levels; an increased rate of tumor growth based on melatonin's oncostatic action, and increased depressive symptoms based on melatonin's role in seasonal affective disorder. More subtle effects of altered circadian patterns of melatonin might include subtle negative effects on mood or task performance, but evidence for this will be more difficult to obtain.

Evidence for ELF EMF effects on steroid hormone levels or their regulatory pituitary-hypothalamic hormones is weaker. Most of the reports of stress effects involve exposures to much higher field strengths than those commonly encountered in environmental situations (including maglev-type rail systems), but a few fall within the range of occupational exposures or near the exposure limits established for ELF EMF. There are reports of individuals who claim a high degree of sensitivity to EMF and report symptoms which could involve neuroendocrine systems. The basis for such sensitivity, if it is real, is unknown. Therefore, although the possibility of ELF EMF stress effects is relatively small, for certain individuals or in interaction with other environmental stressors, ELF EMF exposure could facilitate stress reactions.

14.2 HYPOTHETICAL MECHANISMS AT THE TISSUE LEVEL

14.2.1 Electrical Tissue Gradients

One of the most plausible hypothetical mechanisms of ELF EMF action is a role in simulating or disrupting natural electrical signals in the body. Much of the early work on calcium ion flux in brain tissue was predicated on using ELF-modulated signals that were similar to endogenous electrical signals reported in the electroencephalogram. Similarly, clinical pulsed EMF signals were developed based on electrical signals produced by piezoelectric currents in bone generated by normal movement.

In most instances, the magnitude of magnetic fields produced by environmental exposures is many orders of magnitude below that needed to induce tissue currents comparable to those known to affect electrical transmission in nerves, cardiac muscle, and other electrically responsive tissue. There is a body of literature which indicates that weak DC currents play a role in embryonic development and regeneration, and these currents indicate a plausible mechanism for teratogenic effects of weak magnetic fields, especially those with a high dB/dt.

Small electric currents seem to play a critical role in a range of developmental processes. Vibrating probe measurements of amphibian limbs indicate that they normally produce ionic currents in the medium of 0.01-1.4 $\mu A/cm^2$, but in amputated limbs a relatively intense current of 10-100 $\mu A/cm^2$ is produced at the cut surface, persisting until the regeneration blastema is fully formed (Saunders 1982). Similar currents (22 $\mu A/cm^2$) have been measured in the amputated fingertips of young children who, at least in some cases, are able to regenerate the missing parts. The role of these DC currents in the developmental process (in directing regeneration or as its byproduct) is not clear, but there are some reports indicating that implanting a small (1 nA) DC electric current source in an amputated rat forelimb can improve the degree of partial regeneration of missing tissues (Becker 1985b). More directly relevant is the recent report of Hotary and Robinson, discussed in Section 5.4.3. They measured currents of 108 $\mu A/cm^2$ at the posterior intestinal portal of chicken embryos, and demonstrated that neural tube abnormalities can be produced by reducing this current by 30 $\mu A/cm^2$ using a conductive shunt (Hotary and Robinson 1992).

Time-varying magnetic fields, which induce corresponding time-varying electric fields in biological tissues, are reported to induce abnormalities in chicken embryos as discussed in Section 5.4 of this report. Berman summarized these experiments, pointing out that fields with a repetition rate of 60-100 Hz with a dB/dt of 0.1 T/s or more were associated with reports of teratogenic effects (Berman 1990). A 0.1 T/s field would induce a field in tissue of approximately 0.05 $\mu A/cm^2$ (with no DC component). Pulsed EMF used for bone healing induces currents on the order of a few $\mu A/cm^2$ (Murray and Farndale 1985). Other reports of teratogenic effects with a dB/dt of 10 T/s or more place the induced current in the same order of magnitude as endogenous DC currents, but it is important to note that the current is time-varying not direct current. There are also reports of teratogenic effects with dB/dt as low as 10^{-4} T/s, corresponding to induced electric fields of 10^{-5} $\mu A/cm^2$.

Experimental results reported by Leal, Martin, Litovitz, and others (discussed in Section 10.3) suggests that the importance of the magnitude of ELF EMF is less important than other characteristics such as coherence, orientation, and waveform. It has not been demonstrated that adding a current or changing the direction of endogenous currents with an imposed DC electric field or a time-varying electric or magnetic field can alter development, but it seems to be worth investigating experimentally. Such studies might also help resolve the differences in the biological effects (if any) of DC and time-varying fields, and differences in the effects of different time-varying signals.

14.2.2 Extracellular Matrix and Surface Interactions

There is another body of developmental literature which ascribes morphogenic movements to changes in the cell surface characteristics of developing tissues. Surface changes may involve either changes in the glycoprotein components of the membrane, or production of new extracellular matrix. Patterns of cell migration in gastrulation have been modeled based on differential tissue affinities, and the model has been supported by experimental results in which the appearance of specific surface antigens was correlated with morphogenic movements; and, in which surface components, altered by genetic mutation or chemical treatment of cells prior to aggregation experiments, could alter the pattern of cell movement and association (Monroy 1979). Changes in membrane receptors, lectin binding sites, and extracellular adhesion proteins were reported following exposure to pulsed or sinusoidal ELF EMF (discussed in Section 10.3). This mechanism was suggested for ELF EMF effects in early reports of teratogenesis with changes in glycosaminoglycans in chicken embryos exposed to pulsed magnetic fields (Delgado 1985), but has not been followed up with more recent work. Cell movements could also be evaluated directly in blastodisc cultures exposed to ELF EMF using time-lapse video microscopy.

If a common mechanism is involved, it would be worthwhile documenting changes in surface components using modern molecular probes in the chicken embryo system. It might also be interesting to examine the effects of ELF EMF on some of the established cell sorting experimental systems to see if cell associations can be altered by external ELF EMF. Such results would be interesting, but a lack of response would not be very informative since there is no indication if ELF EMF might act on preexisting surface components or affect only the pattern of new surface component synthesis.

14.3 HYPOTHETICAL MECHANISMS AT THE CELL LEVEL

14.3.1 Membrane-Mediated Cellular Enzymes

From initial reports of ELF EMF bioeffects by Adey and his coworkers, the plasma membrane has been viewed as the primary site of interaction. Changes in ion flux through the membrane could be produced by ELF EMF acting directly on ion channels, surface glycoproteins which may mediate access to the ion channels, or on the ions themselves. For various theoretical reasons discussed below in Section 14.4, structural components of the membrane such as ion channels may be involved in cooperative interactions which would tend to amplify the effects of weak fields. Adey has suggested that intramembranous protein particles may respond to the weak fields in the same fashion as if they are bound to an endocrine factor, thus resulting in a process of amplification of the signal across the membrane barrier, which is typical of the receptor-ligand complex triggering of cytoplasmic and internal membrane effects such as enzyme activation (Adey 1989). In this model, ELF EMF substitutes for or enhances the effects of normal intracellular communication, and the necessary amplification of weak EMF signals occurs by the same mechanism cells used to amplify weak chemical (hormonal) signals. Enzyme systems which are closely coordinated with membrane systems could also be likely targets for such a mechanism.

Recent work has focused on resolving the molecular details of ELF EMF effects with isolated membrane components, at least for a few systems. Stagg *et al.* have reported results with microsomal membrane preparations which indicate an effect of a 60 Hz, 0.1 mT magnetic field on ATP-dependent calcium uptake into microsomes. In a 10 min kinetic study, net $^{45}Ca^{++}$ uptake was 20% less for magnetic field-exposed microsomes, but it is not clear if this represents inhibition of the Calcium ATPase or enhanced leakage from the microsome (Stagg *et al.* 1992). As discussed in Section 10.3, Luben has documented interactions of PEMF with ligand binding to membrane receptor components in bone cells, and has postulated a generalized role for ELF EMF interaction with other members of the G protein linked receptor family.

Litovitz has assembled a series of observations indicating that "field coherence" is critical for ELF EMF effects in a number of experimental systems. Results were initially presented based on the theory that an EMF signal must produce coordinated stimulation of membrane receptors at many locations over the cell surface in order to produce an effect. Litovitz observed that a signal must be applied without shifting its frequency for a period of 1-10 seconds in order to produce an effect (Litovitz, Krause et al. 1991). Application of a second magnetic field which varies in frequency disrupted the response to a coherent field of the same magnitude in two different experimental systems (Mullins, Krause et al. 1993; Litovitz, Farrell et al. 1993). The theoretical explanation initially proposed for this effect has been called into question because the time interval required for coherence is relatively long, but the observations remain.

14.3.2 Cytoplasmic Components

As noted in the experimental results given throughout this report, ELF EMF has been reported to produce effects on cAMP levels, activities of cytoplasmic enzymes such as ODC, and qualitative and/or quantitative changes in patterns of transcription and translation. Until recently, these changes have been considered secondary effects, following and a consequence of initial interactions of ELF EMF with the cell membrane. The possibility of direct interactions with subcellular components has been raised by recent reports of EMF activity in cell-free preparations, but these results are still preliminary and unreplicated. Further details on subcellular events involved in calcium ion influx or efflux will be provided by work just getting underway using fluorescent probes to monitor cytoplasmic calcium levels. At the present time, it seems that subcellular effects of ELF EMF are a possibility, but not enough information is available to suggest a meaningful hypothetical mechanism.

It should be noted that there have been reports for many years in the microwave/radiofrequency research literature of direct resonant interactions with isolated DNA molecules. These reports have been strongly supported by some research, but dismissed as experimental artifact by other research groups.

14.3.3 Gene Expression

As noted in Section 10.2, several investigators have found that ELF EMF (either sinusoidal or pulsed fields) can elicit specific patterns of increased transcription from a variety of cell systems. These experiments initially involved analysis of broad patterns of transcript (RNA by size fractions) or translation product (unidentified polypeptides), but now specific gene probes and antibodies are being used in the analysis. As the technology of molecular biology is applied to experiments of this type, more detailed information will become available on the pattern of gene activity stimulated by different fields.

At the present time, there is no compelling evidence for a specific functional constellation of activated genes: stimulated genes include a range of oncogenes as well as common genes like histone H2B and beta-tubulin. To some extent the pattern is distorted for technical reasons, as investigators have tended to use commercial probes or probes made available by other research workers rather than construct a set of probes appropriate for the functional differentiation of the particular cells being evaluated. At the present time, the question of which genes are activated by bone healing signals or environmental ELF EMF must remain open, because it is not clear if the specific gene activities assessed with probes are a representative sample of the active genes in the system.

The relationship between activated gene transcription and biological effects at the cell or whole animal level is to some degree obvious, but there is a great deal that remains to be discovered about the role of gene expression at higher levels of organization. For example, it is known that many of the oncogenes identified by cloning viral genome segments have homologous counterparts in the normal cell, and many of these are involved in normal cell proliferation, responsiveness to hormonal controls, or differentiation. The simian viral oncogene *v-sis* codes for a protein structurally related to platelet-derived growth factor, *v-erb-B* codes for a protein related to epidermal growth factor, and *c-erb-A* (the cellular gene) is a thyroid hormone receptor (Touchette 1992). It is easy to suggest how gene products of this type could be involved in uncontrolled growth and metastasis of precancerous cells, but it appears that multiple genes are involved and the details of the mechanism are not clear. On the same basis, it is possible to propose a hypothetical mechanism for teratogenic effects based on the activation of inappropriate oncogenes. In Xenopus, a gene called Vgl has been identified which produces a peptide growth factor related to transforming factor beta which is localized in the vegetal pole of the egg and is involved in mesoderm induction (Melton 1991). Other complex bioeffects are more problematic, but may also have a basis in differential gene expression. Recent reports of spatial memory deficits in mice with the alpha calcium/calmodulin kinase II gene inactivated by recombinant genetics techniques ("knockout" mice) demonstrates a potential link between the expression of this structural gene and a complex behavioral pattern (Barinaga 1992). Incidently, since the activity of this kinase in neurons is triggered by calcium, these results could also support behavioral effects based on altered calcium flux in brain tissue.

14.4 HYPOTHETICAL MECHANISMS AT THE MOLECULAR LEVEL

The energy level of the nonionizing radiation involved in most of the reported bioeffects is generally too low to act through such well-established physical mechanisms as heating, dielectric breakdown, particle displacement, or electrophoresis. All of these physical processes require fields that are over 1000 times the maximum power-frequency field which might be induced in human subjects exposed to ground-level transmission-line fields or other common environmental ELF EMF exposures (Carstensen 1987). In these circumstances it is difficult to visualize how cells could respond to a signal which might be below the thermal noise limit for biological membranes. This has been described as "the kT problem," i.e., how can weak EMF be "perceived" by cells against the background of random system thermal energy defined by the Boltzman constant times temperature (Pickard 1988). It is apparent that some mechanism(s) would be needed to select or enhance the signal; a number of such possible mechanisms have been reviewed (Bernardi and D'Inzeo 1989). Since theoretical molecular mechanisms are a major component of the EMF bioeffects literature which was not reviewed in previous sections of this report, this final section will briefly outline some of the theories in the literature.

There has been some speculation as to ways in which cells may select incoming signals of low intensity. First, it should be noted that studies undertaken in suspensions, in which the cells are rounded and with minimal intercellular contact, may provide little indication of the much greater potential sensitivity to ELF in a situation where the cells are confluent, flattened and have extensive gap junctions (McLeod *et al.* 1992). Treated as a single electrically-conductive entity, the current induced in an array of cells connected by gap junctions is several orders of magnitude greater than the current which would be induced by the same field in one of the isolated cells (Pilla, Nasser, and Kaufman 1992). Field effects would therefore be amplified substantially in intact tissues if bioeffects are mediated by the induced electric field rather than by direct action of the magnetic field. Computer simulations of relatively small neural networks (100 neurons), suggest that a 60 Hz signal can be detected with 97% accuracy against a signal-to-noise ratio of 0.001 within about 1200 cycles (Barnes 1992).

Another concept involving enhanced sensitization, termed counterion polarization, has also been proposed as a mechanism whereby sensitivity would be enhanced through cell-to-cell communication involving counterion density distances. Detectable ion currents on or near the cell surface would be produced by fields orders of magnitude lower than those needed to increase transmembrane potentials above noise levels (Polk 1992). Such a mechanism could relate to the small but significant increments in membrane electronegativity seen in human monocyte cells (U 937) exposed to pulsed EMF (Smith *et al.* 1991).

Life evolved in the earth's geomagnetic field, and some organisms have developed field-sensing orientation mechanisms tied to the nervous system (reviewed in Section 8.1.2) that might account for a universal sensitivity to weak artificial magnetic fields (Becker 1985a). One possible mechanism for field-sensitive orientation could involve the presence of intracellular magnetite crystals which have also recently been demonstrated in human brain and have been in a wide range of vertebrates, invertebrates, and prokaryotic cells. Such crystals could enhance tissue sensitivity to EMFs by six or more orders of magnitude if coupled appropriately.

Finally, since ion movements within a cell tend to cancel any potential drop within the cell, most of the potential drop induced by a low frequency field will occur only across the membrane, creating a magnification factor for the membrane field compared with the applied field equal to the cell radius divided by the membrane thickness -- a very significant ratio -- perhaps capable of producing a membrane potential sufficient to evoke a non-linear response (Robertson, Astumian, and Gaigalas 1989). Narrow banding and a process of signal averaging could be important additional elements in overcoming the thermal noise barrier (Weaver and Astumian 1989).

14.4.1 Cyclotron Resonance

One major theory to explain frequency/intensity specific effects of ELF EMF and the sensitivity of such responses to the strength and orientation of the geomagnetic or other DC field components is ion cyclotron resonance (ICR). Because of the central role of this theory in directing a segment of the experimental research in the last decade, ICR theory and evidence supporting it has been discussed in previous sections of this report. According to the ICR model, relatively low energy EMF signals can accelerate the movement of calcium ions through helical channels in the membrane if they are of the correct frequency and intensity to resonate with the gyrofrequency of transport. The ICR hypothesis is supported by data demonstrating ICR AC and DC field combinations which are active in producing frequency/intensity-specific effects on calcium flux in human lymphocytes and marine diatoms, and frequency-specific effects on DNA synthesis in human fibroblasts, coupled with data showing that intermediate (nonresonant) field combinations are not effective.

However, not all experimental results support ICR theory. Results indicating bioeffects under nonresonant conditions or indicating ICR responses for ions of no known biological significance have also been reported, in some cases by investigators like Liboff who have been strong advocates for the ICR model. ICR has also been described theoretically as violating laws of classical physics, in that magnetic fields can alter the direction but not the magnitude of ionic velocity (Halle 1988). Sandweiss (1990) has pointed out an inconsistency between the physically expected and the biologically feasible collision damping times ($<10^{-10}$ versus 0.023 s). Furthermore, if the ICR model were of general applicability, other ions and model systems should exhibit the same phenomenon. However, Liboff and Parkinson (1991) did not observe ICR effects for H^+, Li^+, Na^+, K^+, Ca^{++}, and Cl^- in the Na^+ transport system in turtle

(*Pseudemys scripta*) colonic epithelial tissue. Another mechanistic difficulty is that in a model system involving the binding of calcium to metallochrome dyes and calmodulin, combined weak AC (65-156 μT) and DC (0-299 μT) magnetic fields at 50 to 120 Hz for sine waves or 50 pps for square waves, did not affect binding equilibria (Bruckner-Lea *et al.* 1992). At the present time, the ICR model, at least in its basic form, no longer dominates as a theoretical model to explain window effects. As noted previously however, the experimental observations assembled to support the ICR model can be evaluated independently of the theoretical framework.

14.4.2 Parametric Resonance

Lednev (1990; 1992) has proposed a modification of the cyclotron resonance concept, which he termed "parametric" resonance. This proposed mechanism suggests that biological effects are only seen at well defined harmonics and subharmonics of the resonance frequencies of Ca^{++} and Mg^{++}. Effects would not be expected at the frequencies of other ions; any effect produced at what appears to be the parametric frequency of K^{+}, for example, would actually be the result of the second subharmonic for Ca^{++}. Adair (1992) has produced theoretical arguments against the operation of such a mechanism.

Several other similar mechanisms have also been proposed. The concept of nuclear precessional magnetic resonance was advanced as an alternative to cyclotron resonance by Polk (1989). Chiabrera has described several models based on a classic electrodynamic approach as an extension of the Lorentz force equation for ligands (ions, etc.) in viscous binding sites (Bianco *et al.* 1992). Blank developed a surface compartment model based on voltage-gated membrane channels that provides an alternative mechanism to explain selective effects of specific EMF signals on differential ion flux through membranes (Blank 1987; 1988; 1992).

14.4.3 Ferromagnetic Particles

In conjunction with reports of magnetite crystals in human brain tissue, Kirschvink recently proposed a mechanism in which inclusion of ferromagnetic particles in ion channels would explain both sensitivity to weak magnetic fields and apparent window effects. For a 200 nm magnetite particle suspended in typical eukaryotic cytoplasm, Kirschvink calculated that a minimum ELF EMF component with an amplitude of 140 μT at 60 Hz, held perpendicular to a 50 μT static field, would produce a force sufficient to open an ion gate. Viscous damping at higher frequencies, and membrane deformation at very low frequencies, would yield maximal effects between about 10 and 30 Hz. The dependence of this motion on the relative orientation of the ELF and static field components, and the frequency "window," suggest that magnetomechanical effects could easily be mistaken for ionic resonances (Kirschvink and Kobayashi-Kirschvink 1992).

14.4.4 Membrane Glycoprotein and Lipid Phase Diffusion

Speculation on a possible mechanism for enhancing the effect of the signal has centered on postulating some form of coupling or resonance with endogenous cellular processes to enable weak applied fields to exert bioeffects (e.g., Blackman *et al.* 1989b; Lawrence 1989). Adey has postulated initial interaction between electromagnetic energy and the glycoprotein receptor sites on the cell membrane surface which are involved in transduction of signals to the interior of the cell (Adey 1986). Others have proposed similar interactions for membrane receptors (Chiabrera and Bianco 1987), and the binding of ligands at various binding sites (Kaufman *et al.* 1990; Chiabrera *et al.* 1991; Bianco *et al.* 1992). Gowrishankar *et al.* have calculated that any change in transmembrane potential associated with field-induced redistribution of receptors would be an order of magnitude larger than that due to the induced field itself (Gowrishankar, Han, and Lee 1992). Disruption of cellular communication through gap junctions, and inappropriate cellular responses occasioned by receptor interactions could then lead to a cancer phenotype (Adey 1988a). This effect could be further enhanced if ELF fields generated transient pores similar to those produced by radiofrequency and DC fields (Chang and Reese 1989; Tekle, Chock, and Astumian 1992).

Thermodynamic arguments for similar effects on membrane-bound enzymes have been proposed by Weaver, Astumian, and Tsong, who pointed out that many enzymes can absorb energy from nonequilibrium environmental fluctuations and use it to drive reactions away from equilibrium (Tsong and Astumian 1988; Liu, Astumian, and Tsong 1990). Blank and Soo (1989; 1991) have described modulation of the activity of the ion pump enzyme Na, K-ATPase as a result of applying electric fields at frequencies around 100 Hz. Tsong and Astumian (1990) and Tsong and Markin (1992) have discussed this in terms of the concepts of transduction of high and medium level electric energy through electroconformational coupling (ECC), and of an oscillatory activation barrier (OAB) for low level periodic signals, in which there is resonance transduction between an oscillatory field and the activation barrier of the rate-limiting enzymatic step. From the point of view of detecting ELF, the OAB model is many orders of magnitude more sensitive than the ECC model.

Others have postulated a role for electromagnetic energy in modulating the binding of ions at the cell surface (Pilla *et al.* 1990) or the movement of ions through the cell membrane. Neumann presented theoretical arguments for amplification of electric effects at the membrane surface as a result of local ion accumulation (Neumann 1987). A more generalized suggestion was advanced by Tenforde and Liburdy (1988) who pointed out that there is evidence for phospholipid domain structures in eukaryotic cell membranes existing at pretransition-phase temperatures that are susceptible to magnetic deformation, which could lead to altered transmembrane diffusion.

Several groups have proposed sites of action within the cell. Hsieh and Seto (1984) postulated a thermodynamic mechanism in which substrate availability to cellular enzymes is enhanced by the field energy, increasing the reaction rate. Blank and Goodman (1988) postulated an electrochemical modification of chromatin in an electromagnetic field which opens the conformation to enhance the rate of already activated transcription. Others postulate a broader effect on chromatin conformation mediated by the nuclear divalent ion messenger magnesium (Chiabrera, Grattarola, and Viviani 1982) or through resonant perturbation of the DNA itself (Czerski and Davis 1987).

15. SUMMARY OF DISCUSSIONS OF POTENTIAL BIOLOGICAL HAZARD FROM MAGLEV FIELDS

From the discussions in prior sections, it is clear that many of the reported biological effects of alternating magnetic fields occurred at flux densities that were considerably above those expected within the passenger cabin of the TR07 maglev (or elsewhere within the system). However, several reported effects were in fact associated with flux densities within the envelope provided by the measured magnetic fields, and many other effects are within one or two orders of magnitude of the envelope. Moreover, if one considers the electric fields induced within the human body, many more of the effects would be within the envelope, since most of the biological studies involved small animals and vessels containing *in vitro* preparations whose dimensions are considerably smaller than the human body.

However, for several important reasons, these comparisons have limited value in assessing possible human risks from the maglev exposure. Major factors to be considered include the following:

1. Many of the reports summarized in the figures would not meet normal thresholds for consideration by regulatory bodies or consensus groups. A significant number are abstracts in the technical literature, or were published in unrefereed journals or sources other than traditional peer reviewed scientific publications;

2. These studies are not standard toxicological assays. Many of these reported effects have no apparent connection to hazard;

3. Few of the reported effects have been independently confirmed. Some were subjected to unsuccessful attempts at replication and might be artifacts. Many of the studies had obvious technical problems that might preclude any reliable interpretation of their results; and

4. The only parameter of exposure that is considered in the above comparisons is the flux density of the magnetic field. Many of the studies involved long-term exposure to magnetic fields; by contrast the magnetic field environment of the TR07 maglev is characterized by large and rapid changes within short time periods. Exposure of humans to maglev fields would be expected to range from a few hours or less (passengers) to typical occupational exposures (40-50 h/week), with exposure to any one set of field conditions (frequency/intensity) lasting perhaps several seconds to fractions of seconds.

For these reasons, the association of certain bioeffects with the maglev fields are indicated as possible, but by no means probable. There is no way to determine on a theoretical basis if certain bioeffects will occur under the maglev field conditions: direct experimental evaluation is needed.

As a practical matter, it may be difficult to apply normal standards of risk evaluation, using the strict criteria for hazard assessment outlined above, to the introduction of new electrical technology like maglev trains. Conventional electric power distribution is already subject to constraints of public policy based on legal and environmental concerns that go beyond what could be justified strictly on the basis of the scientific evidence (Florig 1992; Foster 1992). Maglev potentially exposes the public to a similar pattern of ELF EMF as power transmission and distribution systems, but with some unique characteristics of frequency distribution and temporal and spatial variation. As a public policy issue, it therefore will be subject to the range of concerns already raised with regard to 60 Hz EMF, plus the added concern associated with unknown effects produced by lower frequency, highly variable fields. Policy decisions may be assisted by a series of scientific studies showing no substantial bioeffects associated with maglev-like magnetic fields, but the condition of "no risk" is virtually impossible to prove. Probably, the most productive approach is to seek out those biological systems which have been reported in the literature to produce the strongest response to ELF EMF and to evaluate these systems with exposure to maglev-like fields. The alternative approach, testing maglev-like fields in new systems or using standard (chemical) toxicologic protocols, is likely to be less productive.

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*U.S. G.P.O.:1993-301-717:80390