BPNL/NIST WORKSHOP ON
EXPOSURE PARAMETERS FOR IN VITRO STUDIES
WITH ELF MAGNETIC AND ELECTRIC FIELDS

Mariott Riverwalk Hotel – Salon A
San Antonio, Texas

Sunday, June 10, 1990
8:45 a.m. to 2:00 p.m.

Chairpersons:

Marvin Frazier
Battelle Pacific Northwest Laboratories

Martin Misakian
National Institute of Standards and Technology

8:45 a.m.
Introductory Remarks

9:00 a.m.
David Krause, Catholic University, "Magnetic and Electric Field Contributions to Bioeffects During In Vitro Studies Using ELF Magnetic Fields"

9:30 a.m.
Douglas Miller, Battelle PNL, "The Role of Electric Field Dosimetry and Bioeffect Mechanisms In Relating In Vitro to In Vivo Exposures"

10:00 a.m.
Ross Gunderson, University of Wisconsin–Parkside, "ELF Electric Field Measurements in Ionic Media"

10:30 a.m.
Coffee/Tea Break

11:00 a.m.
Marvin Frazier, BPNL and Martin Misakian, NIST, "Examination of Experimental Designs For In Vitro Studies Using ELF Magnetic Fields"

11:40 a.m.
Discussion (problems, solutions, needed research)

12:00 noon
Lunch

1:00 p.m.—2:00 p.m.(room is available if more time is needed)
Continuation of discussion

EXAMINATION OF EXPERIMENTAL DESIGNS FOR IN VITRO STUDIES USING ELF MAGNETIC FIELDS

Marvin Frazier and James Morris
Battelle Pacific Northwest Laboratories

Martin Misakian
National Institute of Standards and Technology

ABSTRACT

The exposure parameters for several experimental configurations that can be used for conducting in vitro studies with ELF magnetic fields are examined. Many of the biological parameters and constraints that must be considered with the different experimental arrangements are also discussed.

1. INTRODUCTION

There have been many laboratory studies since the mid 1970's examining the possibility of biological effects to cells in vitro due to power frequency magnetic fields. These studies have typically used Helmholtz coils to produce an approximately uniform magnetic field for exposure purposes. Significantly, the enclosures containing the cells and liquid growth medium have varied in shape and size. Because the geometry of the liquid volume has a major impact on the exposure parameters, the enclosure, which determines the shape of the liquid volume, must be considered an important part of the exposure system. This paper examines the exposure parameters for several experimental configurations that have been used during in vitro studies and notes some of their advantages and limitations. Section 2 of the paper considers the exposure conditions associated with the different configurations, and Sections 3 and 4 note the biological impacts and constraints that must be considered for the different configurations. Extensive use is made of the equations developed by McLeod et al.[1] for determining the current density and electric field induced in the liquid growth medium by the magnetic field. The text in Section 2 is a contribution of NIST and has been prepared with support from the Department of Energy's Office of Energy Storage and Distribution. Sections 3 and 4 are a contribution from BPNL and have been prepared with support from the Department of Energy under contract number DE-AC06-76RLO 1830.

2. EXPERIMENTAL GEOMETRY AND EXPOSURE PARAMETERS

In vitro studies with power frequency magnetic fields have often been conducted using circular plastic or glass petri dishes and rectangularly shaped vessels as the enclosures. When the enclosure containing the liquid and biological cells is introduced into the magnetic field, an electric field and an associated current are induced in the liquid by the time-varying magnetic field. Thus, the cells are exposed to three candidate parameters that may individually or in combination cause a biological effect, i.e., the magnetic field or flux density (B), the induced electric field strength (E), and the induced current density (J). While all the cells will experience essentially the same magnetic field, the exposure to E and J will depend on the size and shape of liquid volume, the direction of the magnetic field with respect to the liquid volume, and the location of the cell in the liquid volume.

cross sectional shape of the volume. The cells will all experience the same magnetic field because the magnetic field is uniform throughout the liquid volume. However, the electric field and current density experienced by the cells can vary from zero to the maximum value depending on the location of the cell. If the approximate region the cells occupied during exposure to the magnetic field were known, their exposure to a more limited range of E and J values could be determined using equations (3) and (4). Such information is in general not known when experiments are conducted with cells in suspension.

Our ability to describe the exposure conditions for the cells improves if the cells can be cultured on the bottom surface of the enclosure. Then the vertical components of E and J vanish and approximately uniform values of E and J in the horizontal direction can be produced along the bottom surface of the liquid if the liquid depth is small compared to the width, i.e., $2h \ll 2a$. Figure 2(a) shows normalized values of E or J calculated at the bottom surface of the liquid volume for $2a = 6$ cm and $2h = 0.2$ cm. It can be seen for this example that the region of approximately uniform E or J extends almost to the side edges where E and J rapidly decrease to zero. Increasing the depth of the liquid by 1 mm decreases the uniformity, but because the liquid depth, 2h, is still much smaller than the side dimension, 2a, the uniformity is still very good as shown in figure 2(b). While the 1 mm difference in depth has had a small effect on the uniformity of E and J in this example, the increase in magnitude of E and J is not insignificant, amounting to 50% over most of the area. This sensitivity of E and J to liquid depth for the rectangular configuration may be an important consideration when attempts are made to replicate the results of an earlier in vitro study. The biological significance of liquid depth is noted in Section 3.C.

It should also be noted that cells cultured on the bottom surface of the enclosure will experience an "uneven" exposure to the approximately uniform E and J values described above because a portion of the underside of the cell, which is in contact with plastic or glass, will not be exposed to the electric field or current density in the liquid. This problem can be reduced if the cells can be cultured in agar that has the same conductivity as the liquid. However, the use of agar can introduce undesirable biological effects (see Section 3.B) and cause significant changes in the exposure conditions. For example, if liquid 2 mm deep covers a layer of agar 2 mm thick on the bottom of a rectangular enclosure 3 cm wide, and if cells are cultured near the upper surface of the agar, they will experience little or no exposure to E or J except near the side edges.

Summarizing the above discussion, the rectangular configuration allows for the exposure of large numbers of cells to uniform values of B, E, and J if they are cultured on the bottom surface of the enclosure. The values of E and J are sensitive to liquid depth and can be calculated using equations (3) and (4). The equations for determining E and J are not elementary, but can be readily evaluated with a computer. The use of agar can significantly influence the exposure parameters and also have biological consequences that are discussed in Section 3.B.

2.B Cylindrical Geometry: Magnetic Field Perpendicular to Axis

As noted earlier, the case of a cylindrical liquid volume with the magnetic field perpendicular to the cylindrical axis can be treated by imagining the cylinder being cut into rectangular sections and using the theory for rectangular geometry. Thus, the above

mechanisms involving the magnetic field and/or induced electric field and current density. For example, the observation of a biological effect at the *same* level in all sections of the enclosure would suggest a mechanism involving direct magnetic interaction, whereas an effect that differed between sections could be indicative of a mechanism involving the induced electric field and magnetic field combination. For the latter case, a single experiment could provide data for a range of E or J exposure levels.

Summarizing this section, the cylindrical configuration with the magnetic field parallel to the cylinder axis results in induced electric fields and current densities that are easily visualized, and elementary equations for calculating E and J that are independent of liquid depth. In addition, the amount of information pertaining to dose-response during a single experimental run can be maximized, and it may be possible to distinguish between a purely magnetic field bioeffect and a bioeffect that involves the electric field or current density-magnetic field combination. The configuration in this section does not permit exposure of large numbers of cells to the same E and J values. The use of agar does not have a significant effect on E and J values, but can have biological effects as discussed in Section 3.B.

5. REFERENCES

1 B. R. McLeod, A. A. Pilla, and M. W. Sampsel, "Electromagnetic Fields Induced by Helmholtz Aiding Coils Inside Saline-Filled Boundaries," Bioelectromagnetics, vol. 4, pp. 357–370 (1983).

2 M. Misakian and W. T. Kaune, "An Optimum Experimental Design For In Vitro Studies Using ELF Magnetic Fields," Bioelectromagnetics, in press.

3 D. Krause, private communication.

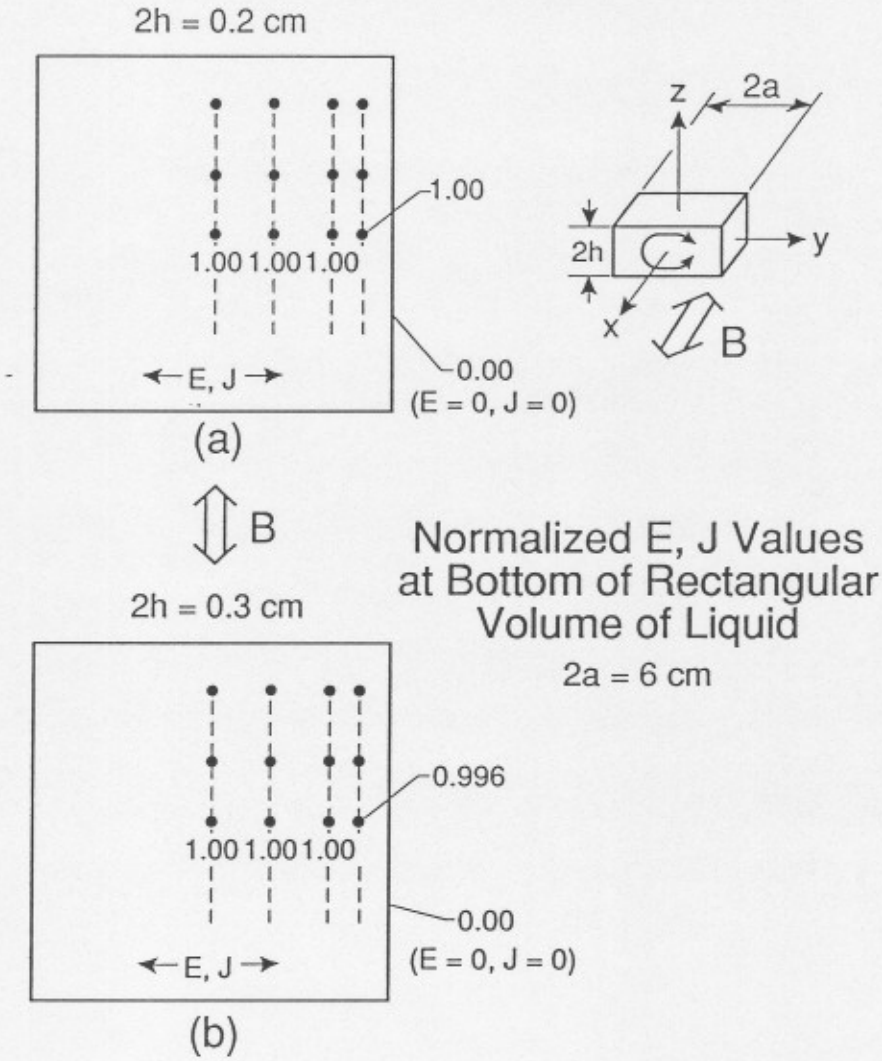
2h = 0.2 cm
1.00
1.00 1.00 1.00
E, J
0.00
(E = 0, J = 0)
(a)
B
2h = 0.3 cm
0.996
1.00 1.00 1.00
E, J
0.00
(E = 0, J = 0)
(b)
z
2a
2h
y
x
B
Normalized E, J Values
at Bottom of Rectangular
Volume of Liquid
2a = 6 cm

Figure 2

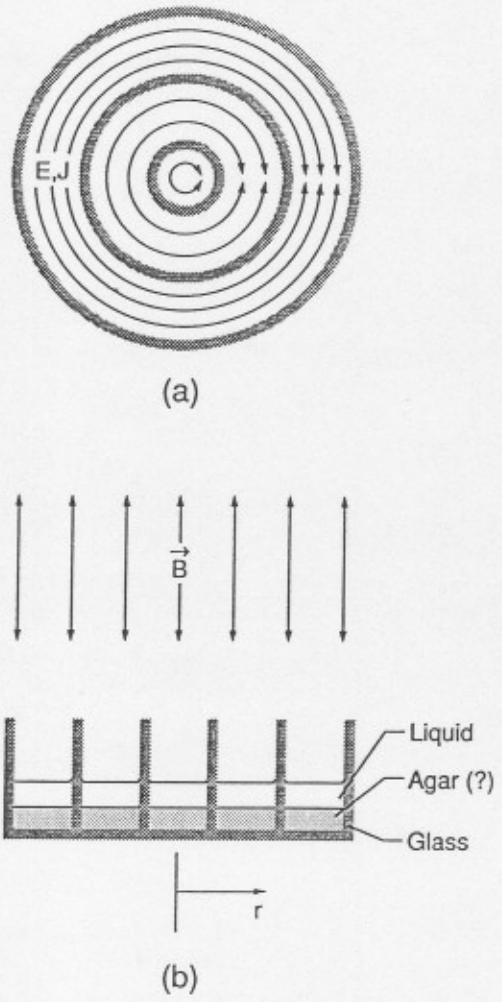

Figure 5. Experimental design for short term in vitro studies with cells in suspension.