Are Magnetic Fields in Incubators Confounding Cell Culture Studies?

Squashing the Cheshire Cat

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Lucas Portelli just ran over the Cheshire cat. He didn't know it was there. He's too young to appreciate how this fictional feline has held sway in the EMF-health controversy.

A little background for newcomers: the Cheshire cat is a metaphor for the lack of reproduciblity of EMF effects observed in some laboratories —but not others. It's a favorite of those who see the study of EMFs as pathological science. The effects come and go, like the Cheshire Cat. Sometimes you see them, sometimes you don't. EMF effects are not thought as being robust. Or more plainly, they are not to be believed.

But what if there was an unregognized confounding factor that was playing havoc with the EMF experiments? Portelli may well have found such a confounder. He is warning that the fields generated by the equipment used in many experiments may be greater than those under study. Think of trying to measure ambient sound levels with an instrument that was powered by a loud generator. The background noise would drown everything else out.

In a systematic measurement survey, Portelli has shown that the ambient static and time-varying magnetic fields in laboratory incubators are large and variable: He found that they can differ by a factor of a hundred or more within and between incubators.

"These variations can be observed within the same incubator in locations that are centimeters apart," he writes in a paper published in *Bioelectromagnetics* earlier this month. Such magnetic fields could be a "potential confounder" of cell culture studies, he warns.

The measurements were carried out as a part of his doctoral thesis at the University of Colorado in Boulder under the guidance of his

thesis advisor, Frank Barnes, and with the assistance of Ted Schomay, an undergraduate.

"There are strong classical and fundamental reasons why these fields shouldn't matter," Portelli told *Microwave News,* "but when you take the time to put the experimental data all together, they suggest otherwise." Magnetic fields "may become another factor that we may just have to control," he said.

While Portelli's main objective is to convince the research community to add magnetic fields to the list of variables —such as light, temperature and pH— that can influence cell biology experiments, Portelli may have also helped settle a long-standing controversy, the often-reported inability of researchers to repeat experiments showing biological effects of weak EMFs. As he writes in his paper: the ambient fields, by inhibiting and/or accelerating biological processes such as growth rates, may provide "a possible explanation for some of the conflicting results in the literature."

"This is a very, very important paper," said Carl Blackman of the Environmental Protection Agency (EPA) in a telephone interview from Research Triangle Park, NC. "Despite a number of warnings, inadequate attention has been paid to ambient fields and they continue to be a problem," he said.

Portelli's paper raises important questions about *in vitro* experiments, said James Lin, the editor of *Bioelectromagnetics.*"With the huge degree of variation, there's no guarantee that the shams [controls] will have the same ambient fields," he said. "Are we back to square one? Maybe so. In some sense, it's a game changer," added Lin, who is with the University of Illinois in Chicago and is a member of ICNIRP.

“The Cheshire Cat Phenomenon”

Back in 1978, H.B. Graves of Penn State University drew parallels between the inconsistent results of EMF experiments and the Cheshire Cat from Lewis Carroll's *Alice in Wonderland* in a paper titled "Biological Effects of 60 Hz alternating Current Fields: The Cheshire Cat Phenomenon":

> [T]he Cat has the disconcerting habit of appearing without warning and then vanishing … That same phenomenon often occurs and should be expected to occur in many studies on biological effects of ELF [EMF] fields.

At the time, Graves was working for the Electric Power Research Institute (EPRI), which had —and continues to have— an interest in showing that EMF effects are illusory and that most of EMF science is junk (see the "The Real Junk Science of EMFs.") Some year later, Graves went to work for LeBoeuf Lamb, a high-profile law firm that defends electric utilities from EMF-related health claims.

The stigma of unreplicable results stuck and gained currency within the EMF community, even among those who believed that EMFs do indeed cause biological effects. Lab-induced EMF effects have been "like a Cheshire Cat with a tendency to disappear when attempts are made to replicate them," Richard Phillips, a former

editor of *Bioelectromagnetics* and senior manager at EPA, told the *Journal of the National Cancer Institute* nearly 25 years ago.

Early Warnings

Sweden's Kjell Hanson Mild raised the incubator issue in a paper presented at the 1991 annual meeting of the Bioelectromagnetics Society. Mild and coworkers at the National Institute of Occupational Health in Umeå, reported that there may be "highly intense background 50-Hz magnetic fields" in commercial incubators used for cell culture work. This should be "taken into account in future studies of biological effects," they warned.

Two years later, David Blask of the Mary Imogene Bassett Research Institute in Cooperstown, NY, offered a concrete example of how incubator fields could confound an EMF experiment. He was studying how weak magnetic fields could block the action of melatonin in inhibiting the growth of MCF-7 breast cancer cells. He found that the ambient fields inside the incubators "had a significant effect" on the cell growth of the controls. "This could explain variability in results within a lab and between labs," Blask told a conference of EMF researchers in Savannah, GA in November 1993. Blask has since moved to Tulane University in New Orleans.

As it turned out, despite the warnings of possible confounding from Mild and Blask, among others, the idea that EMF effects were so small and variable as to be non-existent continued to be dominant. For instance, in a report to Congress in 1999 at the close of the Congressionally-mandated EMF RAPID $45 million research program, the director of the National Institute of Environmental Health Sciences (NIEHS) wrote that mechanistic studies "fail to demonstrate an consistent pattern across studies, although sporadic findings of biological effects have been reported." The lack of support from *in vitro* studies led NIEHS to discount the many epidemiological studies linking power-frequency EMFs to childhood leukemia. Not long afterwards, NIEHS advised that there was no leukemia risk for children living next to power lines. In the face of criticism, the institute later backed off and withdrew this opinion (see *MWN,* MA03, p.1).

Have EMF researchers —the few that remain— learned the lesson to be wary and to control the ambient fields in their experiments? "It does not seem so," Mild wrote in a recent e-mail exchange." We are getting new researchers in to the area and some of them don't have the proper background in physics," How much of the *in vitro* literature is reliable? "Hard to say," replied Mild. There must be studies out there that are influenced by background incubator fields —and that applies not to just EMF experiments, but pure cell biology studies as well, he said.

When asked the same question, Henry Lai, the coeditor of *Electromagnetic Biology and Medicine,* painted a dismal picture of the field: "It's very difficult to separate the good work from the bad work. It's a big concern." Lai recently retired after a career in EMF research at the University of Washington in Seattle. "Most of those working on EMFs —more than 80%— don't know about the potential confounding posed by the ambient fields in incubators," he

said. "The researchers come and go. They publish and move on." Lai blames the lack of funding which does not allow most researchers to stay in the field for very long.

Portelli's Measurement Survey

Portelli's survey shows how important it is to take nothing for granted. "[Our] data point to the fact that in the absence of direct measurements, there is little room for educated guesses about the 'best' location to perform experiments in a specific incubator," he, Shomay and Barnes write in *Bioelectromagnetics.*

Here's some of what they found:

- All incubators tested had points that exceeded natural (0.1 μT/1 mG) and household or "habitation" (≤0.4 μT/4 mG) values by at least a factor of three (see figure below);
- Time varying magnetic fields can vary as much as 240 μT (2.4 G) Worth repeating, 2.4 Gauss;
- Nearly 93% of all the 567 locations measured in 21 incubators present magnetic fields that fall above 0.4 μT (4 mG);
- Nearly 40% of all the point tested in the 21 incubators fall outside of the natural geomagnetic field range (23-65 μT/230-650 mG);
- Some of the time-varying fields were intermittent, not continuous.

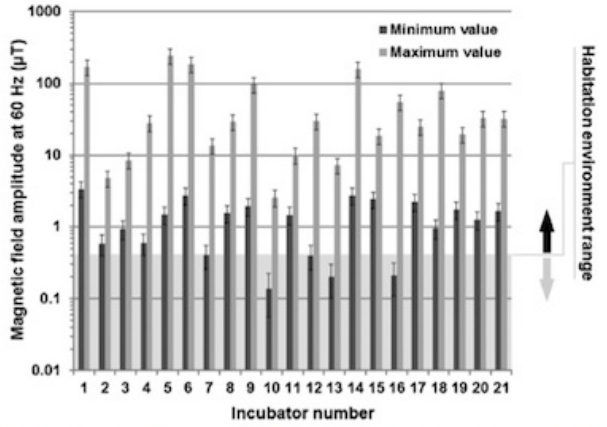


Fig. 3. Maximum and minimum TVMF amplitudes measured at 60 Hz in each of 21 CO_2 cell culture incubators. As a comparison, the TVMF in habitation environments is typically ≤0.4 μT at 50 or 60 Hz, as depicted by the shaded area and light arrow. The black arrow represents magnitudes outside this range. Error bars represent ±0.05 μT (±24.2%) and correspond to errors associated with the measurement system and protocol.

Source: L.A. Portelli, T.E. Schomay and F.S. Barnes, *Bioelectromagnetics,* online March 1, 2013.

Portelli dismantled a few of the incubators and identified fans and resistive (resistance?) heaters as the main cause of the high EMF.

People "Simply Ignored" the Problem

Allan Frey, one of the founders of the Bioelectromagnetics Society, was probably the first to raise the incubator issue some 40 years ago. "I gave a paper at a meeting in the 1970s," he told us. "I discussed how incubators can generate a complex field that would

affect the specimens. I pointed out that the assumption that they would have no effect was unjustified. I concluded that all such data was confounded and that no valid conclusions could be drawn from such research. The people doing the work simply ignored what I said."

Industry funding sources played along and let the conflicting —and confounding— results stand. This sowed the seeds of doubt and confusion. With very few exceptions, no one made the effort to find out why one lab was seeing an effect, while another did not. As a result, the study of EMF health effects was largely dismissed as a fool's errand.

But as Abe Liboff, a former coeditor of *Electromagnetic Biology and Medicine,* points out there were people who were aware and who avoided the problem. "I built my own incubator," he said from Boca Raton where he is now retired and writing a book. Liboff tells the story of visiting a lab in the mid-1980's and seeing a metal cart near an incubator and warning the researcher there that it could affect the static magnetic field in the incubator because it was ferromagnetic. "He didn't believe me," Liboff recalled.

Liboff expressed concern that people may use Portelli's paper to question the existence of low-level effects. He says that many other studies that did not involve incubators show that they exist beyond question. "One needs to look beyond ELF cell culture studies and look at the full context of what has been done, otherwise the exercise would be like yesterday's mashed potatoes: cold, lifeless and useless," he said.

Time will tell whether Portelli's paper will help settle the long-standing controversy over low-level effects. After conceding that "people are slow to accept new ideas," Frank Barnes, Portelli's mentor at the University of Colorado, added, "I will be happy if Lucas's work gets used as a guide for future studies."

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Dong Su, another graduate student —working on a master's degree at McGill University under the direction of Paul Héroux— carried out his own survey of magnetic fields in lab incubators last year. Su measured both ELF and VLF (5 Hz – 2 kHz) fields in 46 different incubators used for cell culture studies. Among his findings are that all 46 had "an average field greater than 0.2 μT [2 mG]; among them, 39 (85%) had an average field greater than 1 μT [10 mG]."

While the fields are lower than those found by Portelli, Su reached a similar conclusion:

> Although many researchers have pointed to significant impacts of MFs [magnetic fields] on the results of *in vitro* experiments, this critical issue has been overlooked by both incubator manufacturers and the large majority of bioscience researchers. This oversight will at the very least introduce extra variations in experimental outcomes performed in incubators with uncontrolled MFs. The situation is particularly dire in that MFs vary not only from

one unit to the next, but also at different locations within the same incubator.

Su includes the name and model numbers of all 46 incubators and their measured magnetic fields.

In an interview with *Microwave News,* Héroux explained that many biologists still do not appreciate the importance of controlling ambient magnetic fields. "Some scientists at McGill forbade Dong Su access to their labs, insisting that magnetic fields did not affect their cell cultures," he said. "In addition, some reviewers in our department were skeptical about the significance of magnetic fields in biology." Héroux is with the Department of Epidemiology, Biostatistics and Occupational Health at McGill's medical school in Montreal.

Su and Héroux's paper was published by arXiv, an open access, Web-based publisher run by Cornell University, last November. For more on Héroux's latest research, see "A United Theory of Weak Magnetic Field Action."

in vitro studies, cell biology, Lucas Portelli, Frank Barnes, Carl Blackman, James Lin, Kjell Hansson Mild, David Blask, Henry Lai, Allan Frey, Abe Liboff, H.B. Graves, EPRI, Richard Phillips, Paul Héroux, Su Dong, pathological science,

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