Toxicology Mechanisms and Methods

ISSN: 1537-6516 (Print) 1537-6524 (Online) Journal homepage: https://www.tandfonline.com/loi/itxm20

Ornithine Decarboxylase Activity in Tissues from Rats Exposed to 60 Hz Magnetic Fields, Including Harmonic and Transient Field Characteristics

L. J. McDonald, L. I. Loberg, D. L. McCormick, J. R. Gauger, R. E. Savage Jr., H. Zhu, W. G. Lotz, R. Mandeville, R. D. Owen, L. W. Cress & A. B. Desta

To cite this article: L. J. McDonald, L. I. Loberg, D. L. McCormick, J. R. Gauger, R. E. Savage Jr., H. Zhu, W. G. Lotz, R. Mandeville, R. D. Owen, L. W. Cress & A. B. Desta (2003) Ornithine Decarboxylase Activity in Tissues from Rats Exposed to 60 Hz Magnetic Fields, Including Harmonic and Transient Field Characteristics, Toxicology Mechanisms and Methods, 13:1, 31-38, DOI: 10.1080/15376510309826

To link to this article: https://doi.org/10.1080/15376510309826

Published online: 30 Sep 2008.

Toxicology Mechanisms and Methods, 13:31–38, 2003

1537-6524/03 $12.00 + .00
DOI: 10.1080/15376520390178148

Taylor & Francis
healthsciences

Ornithine Decarboxylase Activity in Tissues from Rats Exposed to 60 Hz Magnetic Fields, Including Harmonic and Transient Field Characteristics

L. J. McDonald
Cellular Biochemistry & Biophysics Project, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

L. I. Loberg, and D. L. McCormick
Life Sciences Research Section, IIT Research Institute, Chicago, Illinois, USA

J. R. Gauger
Electromagnetic and Electronic Systems Section, IIT Research Institute, Chicago, Illinois, USA

R. E. Savage Jr., H. Zhu, and W. G. Lotz
National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Cincinnati, Ohio, USA

R. Mandeville
Biophage, Inc., Montreal, Quebec, Canada

R. D. Owen, L. W. Cress, and A. B. Desta
U.S. Food and Drug Administration, Center for Devices and Radiological Health, Rockville, Maryland, USA

Ornithine decarboxylase (ODC) activity is used widely as a biomarker for tumor promotion in animal model systems. Several previous studies have reported increases in ODC activity in tissues of rats exposed to 60 Hz magnetic fields. The goals of this study were to confirm these findings and to determine whether ODC activity is increased in tissues of animals exposed to magnetic fields containing complex metrics. Three experiments were conducted in male F344 rats. Each study included a sham control group and a group exposed to pure continuous 60 Hz fields (0.2 mT). Additional groups included animals exposed to randomly time-varying 60 Hz fields (range of 0.02 to 0.2 mT); intermittent 60 Hz fields (2 mT) with on-off cycles ranging from 5 s to 5 min; pure continuous 180 Hz fields (2 mT); 60 Hz fields with a superimposed 3rd harmonic (total field strength, 2 mT); 60 Hz fields with superimposed third, fifth, and seventh harmonics (total field strength, 2 mT); 60 Hz fields (2 mT) with superimposed transients; and randomly time-varying 60 Hz fields (range of 0.02 to 0.2 mT) with superimposed transients. After 4 weeks of exposure (18.5 h/day), eight animals per group were euthanized within 1 h of magnetic field deactivation. Homogenates of liver, kidneys, spleen, and brain were prepared from each animal, quick-frozen, and shipped for analysis by four independent laboratories. No consistent pattern of differences in the ODC activity among experimental groups was found either within a laboratory or among laboratories. The results do not support the hypothesis that exposure to extremely low frequency magnetic fields stimulates ODC activity.

Keywords Intermittent Exposures, In Vivo, Multilaboratory Analysis, ODC

Received 15 June 2001; accepted 22 February 2002.
The in vivo animal exposures were supported by EMF-RAPID grant R03-ES-08854, administered by the U.S. National Institute of Environmental Health Sciences (NIEHS). The tissue preparation at IITRI, the ODC assays conducted at IITRI, the ODC assays conducted at Institut Armand-Frappier, and the statistical analyses performed at IITRI were supported by NIEHS contract N01-ES-07093. The ODC assays conducted at NIOSH were supported by Y1-ES-0032. The ODC assays conducted at the FDA were supported by Y1-ES-0066. A preliminary report of these data was presented at the 1998 meeting of the Bioelectromagnetics Society, St. Petersburg Beach, FL.
Address correspondence to Russell E. Savage, Jr., DART, NIOSH MS C-27, 4676 Columbia Parkway, Cincinnati, Ohio 45226-1998, USA. E-mail: ras6@cdc.gov

Ever since the provocative report by Wertheimer and Leeper was published in 1979, a substantial research effort has been made to address the issue of whether exposure to the 50 to 60 Hz electric and magnetic fields (EMFs) of electrical wiring and equipment is associated with increased risk for human cancer

and with childhood leukemia in particular. This effort has consisted of both epidemiological and experimental studies. The experimental studies have included both in vitro and in vivo efforts, and have been designed to identify a plausible biological basis to support suggestive epidemiological findings.

Most in vivo studies have been designed to determine whether or not EMFs are carcinogenic in animal models; these models have included complete carcinogenesis and tumor promotion and copromotion systems. An enzyme system that is a critical regulator of neoplastic development in both in vitro and in vivo models is the polyamine biosynthetic pathway, whose products are necessary for cell growth and proliferation. Ornithine decarboxylase (ODC, EC 4.1.1.17) is the first and rate-limiting enzyme in the polyamine biosynthetic pathway. ODC is a key regulatory protein, is closely regulated, and is stimulated by a number of growth factors, toxicants, and tumor promoters and by some complete carcinogens (Russell 1981). Inhibition of the enzyme has been clearly demonstrated to be an effective strategy for cancer chemoprevention, using agents such as the suicide ODC inhibitor alpha-difluoromethylornithine (DFMO) (Takigawa et al. 1983). Thus, stimulation of the enzyme may be a sensitive molecular marker for tumor- and growth-promoting agents.

Several investigators have described the induction of ODC in cells exposed to EMFs with an extremely low frequency (ELF) component (Byus et al. 1987; Litovitz et al. 1991, 1993, 1994, 1997a, 1997b; Penafiel et al. 1997). However, attempts to replicate these findings have been largely unsuccessful because of differing methodologies and other less tangible factors (Azadniv et al. 1995; Cress et al. 1999). In one of the few studies examining the effects of EMFs on ODC in vivo, Mevissen (Mevissen et al. 1995) reported that exposure of rats to a 50 Hz, 50 uT field for 6 weeks doubled ODC activity in mammary tissue. However, the results of these studies have been weakened by the inability to replicate the findings and questions about the purity of the mammary tissue in which the ODC measurements were made. Other studies that have suggested that EMFs increase ODC activity in a variety of rat tissues did not include investigations of the series of harmonics and transients superimposed on the underlying 50–60 Hz environmental magnetic-field waveform. The study reported here was designed to provide a careful examination of the possible biological activity of complex EMF exposure metrics, employing the tumor promotion-relevant enzyme ODC as an experimental endpoint. Additionally, in order to overcome or reduce any laboratory-related methodological issues, enzyme activity was measured in four laboratories that had previously studied the enzyme system in cancer research models.

MATERIALS AND METHODS

Animals and Exposure Conditions

The exposure system used in this study has been previously described in detail (Gauger et al. 1999). Briefly, the exposure system consists of a specially designed facility constructed for long-term exposure of rodents to magnetic fields; it contains a separate room for each exposure group. The facility itself as well as the cages and racks were all constructed of nonmetallic materials so as to provide uniform exposure conditions. Horizontal, linearly polarized fields were generated by magnetic field coil sets with animal racks located between the coils. Identical coil sets, including "steering coils" to reduce stray fields and cross-talk between rooms, were located in each of five animal exposure rooms. The fields in each room were continuously monitored by computer to verify actual field levels throughout the study. Field uniformity in the animal exposure rooms was better than $\pm 10\%$. Ambient 60 Hz fields in all exposure rooms were less than 0.1 μT (1 mG), and static magnetic fields were carefully mapped. In addition to field strength and waveform, temperature, relative humidity, light intensity, noise level, vibration, and air flow were also regulated and continuously monitored during exposures.

Male Fischer 344 rats were obtained from the National Toxicology Program (NTP) colonies maintained at Taconic Farms, Germantown, NY, and were approximately 7 weeks old at the initiation of exposure to a magnetic field (MF). After a minimum 2-week quarantine, rats were exposed to an MF for 18.5 h per day for 4 weeks. In all studies, after 4 weeks of MF exposure, eight animals per group were euthanized by decapitation within 1 h of MF deactivation, and tissues were collected and prepared for analysis.

Three independent studies were performed: one study was designed to evaluate the influence of MF intermittency on ODC activity; the second study was designed to evaluate the influence of MF harmonics on ODC activity; and the third study was designed to evaluate the effects of time-varying fields and MF transients. Rats were divided into five exposure groups in all three studies. Each study included both a sham control group (ambient field strength < 0.1 μT, 1.0 mG) and a group that was exposed to a continuous 60 Hz MF at a flux density of 0.2 mT (2 G).

To investigate the effects of MF intermittency on ODC activity in rat tissues, the experiment's design included the sham control group that was exposed to ambient fields only and the group that was exposed to a continuous 60 Hz (0.2 mT) MF. Additional groups of rats were exposed to: 0.2 mT 60 Hz MF with a 5 s on-off cycle; 0.2 mT 60 Hz MF with a 15 s on-off cycle; and 0.2 mT 60 Hz MF with 300 s on-off cycle. The range of intermittency cycle times was selected to maximize changes in MF amplitude (5 s on-off cycle) and to investigate the possibility that some minimum duration of MF exposure may be required to induce biological effects (15 and 300 s on-off cycles).

The experiment to investigate the effects of MF harmonics on ODC activity included the sham control group and the group exposed to a pure 60 Hz MF at a total field strength of 0.2 mT. Additional exposure groups included those exposed to: pure 180 Hz MF (third harmonic) at 0.2 mT; 60 Hz MF with superimposed 180 Hz MF (10% third harmonic, total field strength $= 0.2$ mT); and 60 Hz MF with superimposed $180 + 300 + 420$ Hz magnetic fields (third + fifth + seventh harmonics; 35% total harmonic content, total field strength $= 0.2$ mT). Harmonic components and their relative magnitudes were selected to simulate the possible harmonic composition of an environmental MF.

To investigate the effects of MF transients and time-varying fields on ODC activity, the experiment's design included sham control and continuous 60 Hz (0.2 mT) groups, as well as groups exposed to 0.2 mT 60 Hz MF with superimposed transients; 0.02 to 0.2 mT randomly time-varying MF (pure 60 Hz); and 0.02 to 0.2 mT time-varying MF with superimposed transients. Superimposed transients were randomly selected from a library of 20 transients that were recorded under transmission and distribution lines, in close proximity to electrical panels, or adjacent to electrical equipment during startup. Transients were superimposed on the underlying 60 Hz signal only at points on the waveform where such transients would be encountered in occupational or residential environments. Transients were randomly selected by the system's computer, the number and sequence of transient events were recorded to permit replication of exposure conditions should positive effects be identified.

Tissue Collection, Homogenization and Supernatant Preparation, and Sample Distribution

After euthanasia, the animals' livers, both kidneys, spleens, and brains were collected, weighed, and placed in tubes on ice. Ice-cold homogenization buffer (50 mM Tris-HCl, 1.25 mM EDTA, 2.5 mM DTT, 0.1 mM pyridoxal phosphate, and 0.1 mM PMSF; 5 mL/g wet weight) was added to each tissue, and tissues were homogenized on ice with a polytron homogenizer. A supernatant fraction was prepared by centrifugation at approximately 30,000*g* for 30 min. Replicate aliquots of each supernatant sample were transferred to prelabeled, coded tubes, quick-frozen by immersion into a dry ice and ethanol bath, and stored at −70°C. Complete sets of blinded samples were then shipped by overnight delivery on dry ice to the participating investigators.

ODC Assay

The total protein content and the ODC activity in the homogenate supernatant samples were determined at each laboratory using methods previously established and validated at that laboratory. Although minor differences were present, the analytical method used in each laboratory to determine ODC activity was similar, the method involves measurement of $^{14}CO_2$ production from the substrate [carboxyl-^{14}C]-l-ornithine. Experimental procedures used at (IIT Research Institute) are described in detail; these procedures are representative of those used in all participating laboratories.

Frozen samples were thawed on ice immediately prior to use. A mixture of reaction components was prepared as a two fold concentrate on the day of use, and was kept on ice until preparation of the individual assay tubes. Duplicate glass test tubes were prepared for each sample to be assayed. A micro centrifuge tube containing 0.10 mL benzethonium hydroxide solution was suspended from a clip mounted on a rubber stopper inserted into each tube. Then 0.20 mL of the reaction mixture was added to each tube, and the tubes were placed in a 37°C water bath. In timed additions, 0.20 mL volumes of supernatant samples were added to the tubes, which were then tightly stoppered and returned to the water bath. After either 60 or 120 min, 0.20 mL of 6 M HCl was added to each tube, and the stopper was immediately replaced. All tubes were then removed to room temperature for overnight incubation. After the incubation, micro centrifuge tubes containing the CO_2 trapping medium were transferred to scintillation vials, scintillation fluid was added, and the contents were thoroughly mixed. The ^{14}C radioactivity content was quantitated by liquid scintillation spectrometry. Resulting disintegrations per minute (dpm) values were converted to pmol CO_2, using the known amounts of total radioactivity and total l-ornithine present in the reaction mixture. Protein was determined for all samples, and ODC activity was expressed as activity per unit protein.

Statistical Analysis

Coded enzyme activity data from each laboratory were forwarded to IITRI for statistical analysis; samples were decoded only after completion of the data set. ODC activity data were analyzed by one-way analysis of variance (ANOVA). Separate comparisons were made for each tissue from each laboratory. This ANOVA was used to evaluate the hypothesis that magnetic field exposure would alter ODC activity, that is, it was a 4-degrees-of-freedom test of the equality of means for all five exposure groups. In instances in which statistically significant differences were suggested by the ANOVA, Tukey's honestly significant difference (HSD) test (with the family error rate set to 0.05) was used for intergroup comparisons.

Because ODC activity demonstrated significant differences in baseline activity in the four tissues examined, no attempts were made to analyze pooled data from multiple tissues. Similarly, because interlaboratory differences in measured baseline ODC activities could mask a weak but significant EMF effect within a laboratory, no analyses were performed on pooled data from laboratories. As such, the statistical analyses reflect parallel comparisons of four independent assays of ODC activity on a common tissue set.

To simplify interlaboratory comparisons in the data tables presented, descriptive statistics of the grouped data (means and standard deviations) were normalized to the sham control treatment (set to be 100%). ANOVA was performed on data prior to normalization.

RESULTS

ODC activity in replicate sets of homogenate samples prepared from rat brain, kidney, liver, and spleen was measured independently by four laboratories. Scientists at all four laboratories were blinded to the exposure status of each group; the identities of the samples were decoded only after the results had been received from all laboratories. ANOVA was used to determine whether differences in ODC activity in tissue samples from rats in different exposure groups (for each laboratory analysis) were significant at a level of $p < .05$. The data from each laboratory were then normalized to the sham control group data

TABLE 1
ODC activity in rat brain

Exposure group	Laboratory A	Laboratory B	Laboratory C	Laboratory D
Intermittency study				
Sham	100.0 ± 95.5	100.0 ± 12.1	100.0 ± 34.0	100.0 ± 28.1
2 G, continuous	70.4 ± 41.6	82.1 ± 13.2	86.7 ± 55.6	106.5 ± 30.7
On-off, 5 s	76.3 ± 80.2	65.8 ± 14.9	53.1 ± 11.2	93.3 ± 25.5
On-off, 15 s	143.4 ± 95.8	55.3 ± 23.0	55.2 ± 13.9	96.6 ± 25.8
On-off, 300 s	151.8 ± 115.6	53.8 ± 12.6	53.9 ± 7.3	91.5 ± 22.3
Transients study				
Sham	100.0 ± 54.7	100.0 ± 9.3	100.0 ± 24.3	100.0 ± 25.4
60 Hz	121.3 ± 56.2	103.8 ± 24.3	84.1 ± 17.3	104.3 ± 31.0
60 Hz + transients	87.6 ± 54.1	84.9 ± 27.9	101.9 ± 27.6	96.3 ± 31.1
Time-varying	189.3 ± 119.7	74.1 ± 18.8	96.7 ± 7.9	64.9 ± 19.7
Time-varying + transients	52.8 ± 30.6	53.9 ± 20.1	73.4 ± 23.1	79.6 ± 25.0
Harmonics study				
Sham	100.0 ± 57.0	100.0 ± 65.0	100.0 ± 29.0	100.0 ± 23.0
60 Hz	61.0 ± 45.0	266.0 ± 504.0	83.0 ± 19.0	76.0 ± 14.0
180 Hz	86.0 ± 29.0	126.0 ± 102.0	74.0 ± 17.0	75.0 ± 12.0
60 Hz + 180 Hz	109.0 ± 87.0	106.0 ± 37.0	87.0 ± 27.0	66.0 ± 9.0
60 Hz + 180 Hz + 300 Hz + 400 Hz	108.0 ± 65.0	121.0 ± 131.0	74.0 ± 31.0	62.0 ± 14.0

Note: ODC activity is normalized to the sham control group in each exposure group (set to 100%).

(100%) for comparison across laboratories. Once normalized, the results were compared across laboratories.

In all of the tissues investigated, there were few examples of robust differences in ODC activity between the groups exposed to the EMFs and the sham control groups. Table 1 presents the results of assays of ODC activity in brain as a representative example of the data generated in this study. Given the large volume of data generated for each tissue and the lack of consistent differences across tissues, we have chosen not to present separate data tables for the other three tissues investigated.

A summary of the statistical analyses is presented in Table 2. On the basis of the very large number of comparisons made, it was anticipated that a finite number of instances of statistical significance at the 5% level would be encountered on the basis of chance alone. There were four labs times three studies times four organs; therefore, in the 48 ANOVA tests, it would be expected that there would be 2.4 (5% × 48) cases of statistical significance due to chance. Considered by exposure group rather than by study, 16 ANOVA tests were performed for each group, each consisting of 10 intergroup comparisons. In that situation, 8 instances of statistical significance would be expected in each exposure set on the basis of chance (5% × 160). As presented in Table 2, the number of statistically significant intergroup differences exceeded the number expected by chance. Nine statistically significant intergroup differences in ODC activity were identified in analysis of data from the intermittency study; 15 statistically significant differences were identified in the analysis of data from the transients study; and 5 statistically significant differences were identified in the analysis of data from the harmonics study. Essentially all statistically significant differences were tissue-specific and were generally laboratory-specific. In no case were statistically significant findings replicated in more than two of the four laboratories participating in the study.

The baseline hypothesis investigated in the studies was that continuous exposure to pure 60 Hz EMFs would increase ODC activity in one or more tissues. This hypothesis was not substantiated by the data generated in the program. In 48 comparisons of ODC activity in the tissues of groups receiving exposure to a pure 60 Hz EMF at 0.2 mT versus the sham exposures, only three statistically significant differences were seen. These differences included one increase and two decreases in ODC activity, which were seen in three different tissues.

The hypotheses that exposure to intermittent or to time-varying 60 Hz fields alters ODC activity in tissues were also investigated. Again, the data provided no support for either of these hypotheses. In comparisons of ODC activity in tissues from the groups receiving intermittent exposure to pure 60 Hz fields, the only significant differences from the sham controls were decreases in ODC activity in the brain, which was seen at two of four laboratories. Similarly, no significant differences in ODC activity in tissue were seen in 16 comparisons of groups receiving sham exposure versus exposure to time-varying fields.

Additional studies were included to determine whether superimposition of transients onto continuous or time-varying 60 Hz EMFs would increase ODC activity. When compared to ODC activity in tissues from animals exposed to pure 60 Hz fields, the superimposition of transients onto continuous 60 Hz fields resulted in no increases in ODC activity in tissues; statistically significant reductions in ODC activity were seen in 3 of the 16 comparisons made. By contrast, superimposition of transients

TABLE 2
ANOVA study of ODC activity data

Exposure group	Laboratory A	Laboratory B	Laboratory C	Laboratory D
Intermittency study				
Brain	NSD*	Sham > each on-off group Continuous exposure > 15 s and 300 s on-off	Sham > each on-off group	NSD
Kidney	NSD	NSD	NSD	NSD
Liver	NSD	NSD	NSD	NSD
Spleen	NSD	NSD	NSD	NSD
Transients study				
Brain	Time-varying group > time-varying + transients continuous + transients	Time-varying + transients sham, continuous and continuous + transients	NSD	Continuous > time-varying group
Kidney	NSD	Continuous group > continuous + transients	Continuous group > continuous + transients, time-varying + transients	NSD
Liver	NSD	NSD	Sham > continuous, time-varying groups	NSD
Spleen	NSD	NSD	Continuous > continuous + transients groups	Continuous > sham, time-varying + transients > sham, continuous groups
Harmonics study				
Brain	NSD	NSD	NSD	Sham > each EMF exposure group
Kidney	NSD	NSD	NSD	NSD
Liver	NSD	NSD	NSD	NSD
Spleen	NSD	NSD	Sham > 60 + 180, 60 + 180 + 300 + 400	NSD

*No statistically significant differences.

onto time-varying 60 Hz fields resulted in one reduction and one increase in ODC activity from that seen in 16 comparisons of animals exposed to 60 Hz fields only. Similarly, when compared to the activity in the sham controls, ODC activity in the tissues of animals exposed to 60 Hz EMFs + transients differed in only 2 of 16 comparisons (one increase, one decrease).

Similar studies were performed to investigate the hypothesis that superimposition of third, fifth, or seventh harmonics onto an underlying 60 Hz signal would increase ODC activity in tissue. In 32 comparisons of ODC activity in animals exposed to 60 Hz fields versus 60 Hz fields plus harmonics, no statistically significant differences were found. When compared to sham controls, significant reductions in tissue ODC activity in groups exposed to 60 Hz fields plus harmonics were seen in 4 of 32 comparisons; no increases were observed.

DISCUSSION

The induction of the enzyme ODC and the subsequent increases in polyamine synthesis have remained a focus of cancer research since studies first reported in 1972 that increases in both endpoints were correlated with tumor cell multiplication (Anderson and Heby 1972). ODC, the rate-limiting enzyme in polyamine synthesis, is induced by hormones, drugs, mitogens, many tumor promoters, and complete chemical carcinogens. Induction of the enzyme occurs prior to the stimulation of cell proliferation and tissue growth that characterizes the spectrum of the biological effects of the aforementioned compounds. It seems appropriate that researchers interested in investigating a potential link between EMFs and cancer would investigate this enzyme. Peer-reviewed reports from eight research groups showed that EMFs can alter the activity of ODC and the concentrations of polyamines. These reports include work with 11 different cell culture models (Byus et al. 1987, 1988; Cain et al. 1985; Litovitz et al. 1991, 1993, 1994, 1997a, 1997b; Mattsson et al. 1993; Mullins et al. 1993, 1998; Penafiel et al. 1997; Somgen et al. 1983; Valtersson et al. 1997, 1999) and six animal studies in which ODC activity was analyzed in a total of seven different tissues (Farrell et al. 1998; Kaicer and Mandeville 1999; Kumlin et al. 1998a, 1998b; Mevissen et al. 1995). Where positive results have been reported, most cell culture studies have shown approximately a 1.5- to 4.0-fold enhancement of ODC activity in response to exposure to EMFs, and the results of rodent studies have demonstrated increases in ODC activity 1.6 to 4.2 times those of matched controls. On a quantitative basis, these increases in ODC activity pale in comparison to the induction seen with classic tumor-promoting agents, particularly those employed in animal studies. For example, the tumor-promoting phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces ODC activity 45-fold above control levels in rat liver and 9-fold above control levels in rat brain (Weiner and Byus 1980). Even the weak tumor-promoting barbiturates barbital and phenobarbital induce rat liver ODC activity by 7.7- and 5.7-fold, respectively (Pereira et al. 1982). In vitro studies of TPA have resulted in stimulations more than 25-fold above controls (Kanitz et al. 1997). Thus, the enhancement of ODC activity by EMFs reported in previous studies is not of magnitude consistent with previously studied and well-established tumor-promoters or complete carcinogens.

Of equal importance in the consideration of EMFs and the role of ODC in carcinogenesis is the duration of the increased elevation in enzyme activity. O'Brien (1976) postulated that not only was the induction of the enzyme key for tumor promotion, but also that the activity must be sustained for a prolonged period of time. In order to determine this, previous studies involving many chemical and physical agents have followed the time course of induction of ODC activity after exposure. The design of the present study precluded the conduct of a time-course analysis. Regardless, it is logical to expect that were EMFs tumor promoters of even modest potency, some elevation of ODC activity should be detectable at the time point selected for these studies.

It is important to emphasize that the ODC assays performed by the four independent laboratories were conducted on homogenates prepared from the same tissues. These were not experiments in which animals were exposed at each of the four laboratory sites. Animals were exposed and euthanized in Chicago. Tissues were prepared, aliquoted, and shipped by overnight delivery to the laboratories; assays were conducted on individual aliquots of common samples. The most compelling and obvious conclusion to be drawn from the results of this interlaboratory study is that it provides no evidence that ODC activity in the kidney, spleen, liver, or brain is altered in male F344 rats exposed to pure 60 Hz magnetic fields, pure 180 Hz magnetic fields, 60 Hz magnetic fields with superimposed magnetic field harmonics, or 60 Hz magnetic fields with superimposed transients. Furthermore, varying periods of magnetic field intermittency or random time-related variations in magnetic field intensity had no effect on ODC activity in any tissue.

Although the number of instances in which statistically significant differences existed among various treatment groups in data from a single laboratory was greater than would be expected to occur on the basis of chance alone, we think these changes were not biologically significant, based on the magnitude of the changes as compared to the magnitude of the changes induced by documented tumor promoters. Additionally, in no case was any difference observed in more than two of the four participating laboratories. Also, there was no internal consistency in the pattern of these observed differences among laboratories or among exposure groups. As such, the magnitude of the effect and the inability of the effect to be replicated in a majority of the participating laboratories strongly suggests these observed differences were spurious associations and were not related to the exposure to EMFs.

Thus, we conclude that the results of these studies do not support the hypothesis that exposure to 60 Hz magnetic fields or a range of complex magnetic field metrics stimulates cell proliferation or neoplastic transformation through a mechanism involving alterations in ODC activity. No clear pattern of ODC induction by exposure to any metric in the EMFs was identified in samples analyzed in any of the four participating laboratories,

and comparisons of data between laboratories also failed to identify evidence of ODC induction in tissues collected from animals exposed to EMFs.

Several issues should be considered in an overall assessment of the literature concerning the effects of EMFs on ODC activity. First, measurement of ODC is a relatively complex procedure containing a number of steps in which artifacts may occur. The present study is unique in that it included parallel but independent analyses of ODC activity by four laboratories with established and validated methods for ODC measurement (Cress et al. 1999; Kaicer and Mandeville 1999; Kanitz et al. 1997). Second, in studies in which the effects of EMFs on ODC activity have been reported, the magnitude of enzyme induction has been modest (1.5- to 4-fold), and the kinetics of such induction have generally not been investigated. The magnitude of reported effects of EMFs on ODC activity is considerably smaller than that reported for tumor promoters such as TPA. Given the relatively small size of the effects and the lack of confirmation by additional studies, reported changes in ODC activity in response to exposure to EMFs appear unlikely to be of biological significance.

REFERENCES

Anderson, G., and Heby, O. 1972. Polyamine and nucleic acid concentrations in Ehrlich ascites carcinoma cells and liver at various stages of tumor growth. *J. Natl. Cancer Inst.* 48:165–172.

Azadniv, M., Klinge, C. M., Gelein, R., Carstensen, E. L., Cox, C., Brayman, A. A., and Miller, M. W. 1995. A test of the hypothesis that a 60-Hz magnetic field affects ornithine decarboxylase activity in mouse L929 cells in vitro. *Biochem. Biophys. Res. Commun.* 214:627–631.

Byus, C. V., Kartun, K., Pieper, S., and Adey, W. R. 1988. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. *Cancer Res.* 48:4222–4226.

Byus, C. V., Pieper, S. E., and Adey, W. R. 1987. The effects of low-energy 60-Hz environmental fields upon the growth-related enzyme ornithine decarboxylase. *Carcinogenesis* 8:1385–1389.

Cain, C. D., Donato, N. J., Byus, C. V., Adey, W. R., and Luben, R. A. 1985. Pulsed electromagnetic field modifies cAMP metabolism and ornithine decarboxylase activity in primary bone cells. In *International Conference on Electric and Magnetic Fields in Medicine and Biology*, Conference Publication No. 257, 9–14. London and New York: Institute of Electrical Engineers.

Cress, L. W., Owen, R. D., and Desta, A. B. 1999. Ornithine decarboxylase activity in L929 cells following exposure to 60 Hz magnetic fields. *Carcinogenesis* 20:1025–1030.

Farrell, J. M., Barber, M., Krause, D., and Litovitz, T. A. 1998. The superposition of a temporally incoherent magnetic field inhibits 60 Hz–induced changes in the ODC activity of developing chick embryos. *Bioelectromagnetics* 19:53–56.

Gauger, J. R., Johnson, T. R., Stangel, J. E., Patterson, R. C., Williams, D. A., Harder, J. B., and McCormick, D. L. 1999. Design, construction, and validation of a large-capacity rodent magnetic field exposure laboratory. *Bioelectromagnetics* 20:13–23.

Kaicer, E.-K., and Mandeville, R. 1999. Increase in ODC activity in organs of animals exposed to 60-Hz linear, sinusoidal continuous-wave magnetic fields of different intensities. In *Electricity and Magnetism in Biology and Medicine, Proceedings of the 2nd World Congress for Electricity and Magnetism in Biology and Medicine*, ed. F. Bersani, 951–954. New York: Kluwer Academic/Plenum.

Kanitz, M. H., Li, E. I., Schulte, P. A., Anderson, N. L., Rothman, N., and Savage, R. E., Jr. 1997. Investigation of ornithine decarboxylase activity and two-dimensional electrophoretic protein profile following exposure of T24 bladder carcinoma cells to tumor promoter and carcinogen. *Toxicol. Methods* 7:27–41.

Kumlin, T., Alhonen, L., Janne, J., Lang, S., Kosma, V. M., and Juutilainen, J. 1998a. Epidermal ornithine decarboxylase and polyamines in mice exposed to 50 Hz magnetic fields and UV radiation. *Bioelectromagnetics* 19:388–391.

Kumlin, T., Kosma, V. M., Alhonen, L., Janne, J., Komulainen, H., Lang, S., Rytomaa, K., Servomaa, K., and Juutilainen, J. 1998b. Effects of 50 Hz magnetic fields on UV-induced skin tumourigenesis in ODC-transgenic and non-transgenic mice. *Int. J. Radiat. Biol.* 73:113–121.

Litovitz, T. A., Krause, D., Montrose, C. J., and Mullins, J. M. 1994. Temporally incoherent magnetic fields mitigate the response of biological systems to temporally coherent magnetic fields. *Bioelectromagnetics* 15:399–409.

Litovitz, T. A., Krause, D., and Mullins, J. M. 1991. Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity. *Biochem. Biophys. Res. Commun.* 178:862–865.

Litovitz, T. A., Krause, D., Penafiel, M., Elson, E. C., and Mullins, J. M. 1993. The role of coherence time in the effect of microwaves on ornithine decarboxylase activity. *Bioelectromagnetics* 14:395–403.

Litovitz, T. A., Penafiel, L. M., Farrel, J. M., Krause, D., Meister, R., and Mullins, J. M. 1997a. Bioeffects induced by exposure to microwaves are mitigated by superposition of ELF noise. *Bioelectromagnetics* 18:422–430.

Litovitz, T. A., Penafiel, M., Krause, D., Zhang, D., and Mullins, J. M. 1997b. The role of temporal sensing in bioelectromagnetic effects. *Bioelectromagnetics* 18:388–395.

Mattsson, M. O., Mild, K. H., and Rehnholm, U. 1993. Gene expression in tumor cell lines after exposure to a 50 Hz sinusoidal magnetic field. In *Electricity and Magnetism in Biology and Medicine*, ed. M. Blank, 502–503. San Francisco: San Francisco Press.

Mevissen, M., Kietzmann, M., and Loscher, W. 1995. In vivo exposure of rats to a weak alternating magnetic field increases ornithine decarboxylase activity in the mammary gland by a similar extent as the carcinogen DMBA. *Cancer Lett.* 90:207–214.

Mullins, J. M., Krause, D., and Litovitz, T. A. 1993. Simultaneous application of a spatially coherent noise field blocks the response of cell cultures to a 60 Hz electromagnetic field. In *Electricity and Magnetism in Biology and Medicine*, ed. M. Blank, 345–346. San Francisco: San Francisco Press.

Mullins, J. M., Litovitz, T. A., Penafiel, M., Desta, A., and Krause, D. 1998. Intermittent noise affects EMF-induced ODC activity. *Bioelectrochem. Bioenerg.* 44:237–242.

O'Brien, T. G. 1976. The induction of ornithine decarboxylase as an early, possibly obligatory event in mouse skin carcinogenesis. *Cancer Res.* 36:2644–2653.

Penafiel, L. M., Litovitz, T., Krause, D., Desta, A., and Mullins, J. M. 1997. Role of modulation in the effect of microwaves on ornithine decarboxylase activity in L929 cells. *Bioelectromagnetics* 18:132–141.

Pereira, M. A., Savage, R. E., Jr., Herren, S. L., and Guion, C. W. 1982. Comparison of enhancement of GGTase-positive foci and induction of ornithine decarboxylase in rat liver by barbiturates. *Carcinogenesis* 3:147–150.

Russell, D. H. 1981. Ornithine decarboxylase: a key regulatory protein. *Med. Biol.* 59:286–295.

Somgen, D. M., Yariv, M., Kaye, A. M., Korenstein, R., Fischler, H., and Binderman, I. 1983. Ornithine decarboxylase activity in cultured bone cells is activated by bone-seeking hormones and physical stimulation. *Adv. Polyam. Res.* 4:713–718.

Takigawa, M., Verma, A. K., Simsiman, R. C., and Boutwell, R. K. 1983. Inhibition of mouse skin tumor promotion and of promoter-stimulated epidermal polyamine biosynthesis by alph-difluoromethylornithine. *Cancer Res.* 43:3732–3738.

Valtersson, U., Mild, K. H., and Mattsson, M. O. 1997. Ornithine decarboxylase activity and polyamine levels are different in jurkat and CEM-CM3 cells after exposure to a 50 Hz magnetic field. *Bioelectrochem. Bioenerg.* 43:169–172.

Valtersson, U., Mild, K. H., and Mattsson, M. O. 1999. Uncharacterized physical parameters can contribute more than magnetic field exposure to ODC activity in vitro. In *Electricity and Magnetism in Biology and Medicine, Proceedings of the 2nd World Congress for Electricity and Magnetism in Biology and Medicine*, ed. F. Bersani, 449–453. New York: Kluwer Academic/Plenum.

Weiner, R. A., and Byus, C. V. 1980. Induction of ornithine decarboxylase by 12-*O*-tetradecanoylphorbol-13-acetate in rat tissue. *Biochem. Biophys. Res. Commun.* 97:1575–1581.

Wertheimer, N., and Leeper, E. 1979. Electrical wiring configurations and childhood cancer. *Am. J. Epidemiol.* 109:273–284.