

Effect of Magnetic Field Exposure on Anchorage-independent Growth of a Promoter-sensitive Mouse Epidermal Cell Line (JB6)

John E. Snawder, Richard M. Edwards, David L. Conover, and W. Gregory Lotz

Division of Biomedical and Behavioral Sciences, National Institute for Occupational Safety and Health, Cincinnati, OH 45226 USA

The anchorage-independent growth of mouse epidermal cells (JB6) exposed to 60-Hz magnetic fields (MF) was investigated. Promotion-responsive JB6 cells were suspended in agar (10^4 cells/plate) and exposed continuously to 0.10 or 0.96 mT, 60-Hz magnetic fields for 10–14 days, with or without concurrent treatment with the tumor promoter tetradecanoylphorbol acetate (TPA). Exposures to MF were conducted in a manner such that the experimenter was blind to the treatment group of the cells. At the end of the exposure period, the anchorage-independent growth of JB6 cells on soft agar was examined by counting the number of colonies larger than 60 μ m (minimum of 60 cells). The use of a combined treatment of the cells with both MF and TPA was to provide an internal positive control to estimate the success of the assay and to allow evaluation of co-promotion. Statistical analysis was performed by a randomized block design analysis of variance to examine both the effect of TPA treatment (alone and in combination with MF exposure) and the effect of intra-assay variability. Transformation frequency of JB6 cells displayed a dose-dependent response to increasing concentrations of TPA. Coexposure of cells to both TPA and 0.10 or 0.96 mT, 60-Hz MF did not result in any differences in transformation frequency for any TPA concentrations tested (0–1 ng/ml). These data indicate that exposure to a 0.10 or 0.96 mT, 60-Hz MF does not act as a promoter or co-promoter in promotion-sensitive JB6 cell anchorage-independent growth. **Key words:** cancer promotion, JB6 cell proliferation, low frequency magnetic fields. *Environ Health Perspect* 107:195–198 (1999). [Online 29 January 1999] <http://ehpnet1.niehs.nih.gov/docs/1999/107p195-198snawder/abstract.html>

The Electric and Magnetic Fields Research and Public Information Dissemination (EMF-RAPID) program established under the Energy Policy Act (Section 7118) of 1992 has the central goal of determining if electric and magnetic fields associated with the generation, transmission, and use of electricity pose a risk to human health. The National Institute for Occupational Safety and Health (NIOSH), as part of an interagency agreement, maintains one of four Regional extremely low frequency magnetic field (MF) exposure systems. In addition to providing access and support for researchers outside of NIOSH desiring to use the regional exposure system, NIOSH is also responsible for performing replication studies to investigate the effect of 60-Hz MF on biological processes.

Epidemiologic evidence has demonstrated a putative link between exposure to MF and an increased risk for certain types of cancer (1,2). Because epidemiologic studies in the literature are often contradictory and at best can only demonstrate associations between cause and effect (3), *in vivo* and *in vitro* studies are often used to examine whether MF encountered in homes or the work place may exert biological effects (4–7).

Promotion-sensitive subclones of the mouse epidermal cell line JB6 (clone 41) respond to treatment with tumor-promoting agents by increasing colony growth under anchorage-independent conditions. Increased colony growth has been observed in JB6 cells

treated with phorbol esters, epidermal growth factor, lanthanides, and phthalate esters (8). West et al. (9) reported that 60-Hz sinusoidal MF exposure altered the growth of promotion-sensitive JB6 cells under anchorage-independent conditions. In their research, exposure of cells to a continuous 1.1-mT MF (11 G) led to significant increases in the soft agar colony-forming efficiency, i.e., 40–70% greater numbers compared with unexposed cells (>95% confidence level), at 10–14 days of MF exposure. A second study by this group investigated anchorage-independent growth of JB6 cells exposed to MF of 100, 10, and 1 μ T (1, 0.1, and 0.01 G, respectively). Although increases in anchorage-independent growth were observed in nearly all cell cultures exposed to MFs, there appeared to be no consistent response correlating increasing field strength with increased colony numbers (10). The present report describes efforts by NIOSH to investigate the effect of 0.10 and 0.96 mT MFs (1 and 9.6 G) on the growth of JB6 cells on soft agar. Promotion-responsive JB6 cells, obtained from Robert West at the National Center for Toxicological Research (NCTR; clone 41 passage 83), and also from the American Tissue Culture Collection (Rockville, MD), were suspended in agar and exposed continuously to a 0.10 or 0.96 mT, 60-Hz MF for 10–14 days, with or without concurrent treatment with the promoter tetradecanoylphorbol acetate (TPA). The exposures

were conducted in the RAPID program's regional MF exposure facility at NIOSH. The facility consists of twin exposure chambers in which the MF is produced by a stacked Merritt coil design, and controlled by a computer system that randomizes which chamber serves as the exposure chamber.

Materials and Methods

Plasticware, media, serum, and all other reagents were obtained from identical commercial sources as used previously by West et al. (9). Prior to beginning experiments at NIOSH, one of the authors (J.E.S.) visited NCTR, and 50 vials of JB6 cells (passage 83) were cryopreserved to provide sufficient quantities of similar passage cells to last the entire experiment. Because serum source and lot may affect *in vitro* transformation experiments with JB6 cells (11), the original serum lot (3M) from JRH Biosciences (Lenexa, KS), as used by West, was used in these experiments. Experiments with another lot of JRH serum (4M) that met specific requirements for JB6 cell growth were also performed to examine the effect of serum on JB6 anchorage-independent transformation. In addition, promotion-sensitive cells (clone 41) were also obtained from ATCC to examine differences in transformation frequency between cells from different sources.

Cell culture. Cells were maintained in minimum essential medium with Earle's salts and supplemented with 5% heat-inactivated (30 min at 56°C) fetal bovine serum, 2 mM glutamine, and penicillin-streptomycin (50 U/ml, 50 μ g/ml). Previously frozen cells were maintained in logarithmic growth for 1 week before use. Incubator conditions were maintained at 37°C, 5% CO₂ + 95% air, and 90% humidity. Temperature and CO₂ were monitored by the incubator's internal controls. In addition, daily measurements were made with a recording thermometer and Fyrite analyzer for CO₂ (Bacharach Inc., Pittsburgh, PA).

Soft-agar proliferation of JB6 cells in response to MF and/or TPA. JB6 cells (10^4 /dish) were suspended in 0.33% Bacto-agar (DifCo Laboratories, Detroit, MI) dissolved in complete Eagle's basal medium

Address correspondence to J.E. Snawder, Taft Laboratories, MS-C23, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226 USA.

Received 5 August 1998; accepted 26 October 1998.

with appropriate concentrations of TPA. For these assays, three sets (A, B, C) of three or more replications per treatment were used in each assay. The cell-containing layer was applied over a similar layer of 0.5% agar in 60-mm dishes. Dishes were sealed with Parafilm (American National Can, Greenwich, CN) and the plates from group A and B were placed in their respective exposure chambers of the Department of Energy (DOE) regional MF exposure facility at NIOSH. The exposure conditions of both chambers were randomized by the system. Cell plates marked "C" were maintained in the incubator used for normal cell culture. At days 10 or 14, plates were examined and scored for colony growth. Using an inverted microscope, colonies >60 μm (>60 cells) were counted and results were expressed as mean number of colonies \pm standard deviation/treatment.

Double-blind trials were conducted to examine the effect of 60-Hz magnetic fields on JB6 cells with or without TPA. This was a slight deviation from the work of West et al. (9) in that study cells were exposed without TPA. The combined treatment of the

cells (TPA and/or MF) resulted in an internal positive control that provided an estimate for the success of the assay and allowed the evaluation of co-promotion.

DOE regional MF exposure facility at NIOSH. The DOE regional MF exposure facility is located at the Taft Laboratories and is under the custodial administration of the Division for Biomedical and Behavioral Sciences, NIOSH. The facility at NIOSH is one of four nationally that were manufactured by Columbia Magnetics Inc. of Kennewick, WA, for the DOE and provided to NIOSH, the U.S. Food and Drug Administration, Oak Ridge National Laboratory, and Battelle Pacific Northwest Laboratory. The exposure device, designated the Model 2xc Exposure System, consists of two coil systems energized by a function generator/power amplifier combination under computer control. The software is custom programmed in LabVIEW (National Instruments, Austin, TX) to control field configuration and monitor temperature and MF flux density. The available MF flux density range is 0.1 μT –1 mT. Inside each of the two coil systems are double-walled,

electrically and mechanically isolated exposure chambers (referred to as chamber A and chamber B). Uniform temperature, humidity, and CO_2 are maintained by a modified commercial incubator. Each chamber is foam insulated and foil backed to shield for electric fields and is equipped with a thermocouple, a three-axis magnetic field sensor, and a port for CO_2 concentration measurement. Inner dimensions of the chambers are 30 cm per side. Each coil system consists of two sets of concentric Merritt coils. The side dimensions of the inner and outer coils are 56 cm and 80 cm, respectively. The inner coils provide exposure and the outer coils limit stray fields. In addition, each coil system has two pairs of Helmholtz coils, one for each horizontally directed axis, measuring 90 cm per side. Coils were used to provide a continuous exposure to a predetermined vertical alternating field. For a sham exposure, the double wound coils were energized with opposing current flow so that the net applied magnetic field was zero. Although the coils could have been used to adjust for the ambient static field, this feature was not used in this study. Computer-controlled random determination of which side was exposed, or for sham/sham exposure was used to provide experimental blinding of exposures. All data (time, flux density, and temperature) were saved to disk. Field levels inside the exposure volume were within 5% of the absolute value defined by National Institute of Standards and Technology measurements. All measured values were within 1.4% of the chamber's central value, and along the coil axis, all measurements were within 0.6% of the central value. Temperature stability in the remote chambers was $\pm 0.1^\circ\text{C}$. The prototype for this system was developed at Battelle's Pacific Northwest Laboratories (12).

Statistical analysis. Statistical analysis was performed by a randomized block design analysis of variance to examine the effect of TPA treatment (alone and in combination with MF exposure) as well as the effect of intra-assay variability.

Table 1. Colony formation of JB6 cells obtained from the National Center for Toxicological Research in response to treatment with tetradecanoylphorbol acetate (TPA) and sham/sham^a exposure.

Experiment no. (exposure A/B)	TPA (ng/ml)	Incubator ^b	Chamber A ^c	Chamber B ^d
1 (Sham/sham)	0.00	45.0 \pm 7.5	40.0 \pm 5.6	49.3 \pm 7.2
	0.01	74.1 \pm 10.4	81.7 \pm 4.4	64.1 \pm 4.0
	0.03	67.3 \pm 4.4	60.6 \pm 22.9	83.6 \pm 12.8
	0.10	113.8 \pm 27.1	134.1 \pm 29.9	122.0 \pm 5.3
	1.00	400.3 \pm 11.3	369.3 \pm 6.6	374.7 \pm 8.7
2 (Sham/sham)	0.00	20.0 \pm 2.1	18.7 \pm 1.3	16.0 \pm 1.0
	0.01	76.0 \pm 14.1	63.1 \pm 5.2	75.0 \pm 6.9
	0.03	141.7 \pm 10.4	129.0 \pm 9.6	134.0 \pm 3.1
	0.10	281.0 \pm 11.3	210.0 \pm 15.1	204.7 \pm 14.4
	1.00	547.6 \pm 17.3	533.0 \pm 34.8	597.3 \pm 21.6
3 (Sham/sham)	0.00	12.7 \pm 1.2	13.0 \pm 1.1	13.7 \pm 4.3
	0.01	18.3 \pm 1.7	20.3 \pm 0.3	17.7 \pm 4.4
	0.03	43.7 \pm 2.4	43.7 \pm 3.2	36.4 \pm 5.6
	0.10	139.7 \pm 7.1	135.7 \pm 7.6	153.3 \pm 59.5
	1.00	306.0 \pm 23.1	312.7 \pm 16.8	356.3 \pm 8.9

Abbreviations: DOE, Department of Energy; NIOSH, National Institute of Safety and Health. Cells were treated as described in "Methods" and allowed to grow in the incubator used for normal cell culture or the DOE regional exposure facility. The number of colonies per plate was determined at day 14. Data are mean number of colonies/plate \pm standard deviation; $n = 3$ or 4 plates/treatment.

^aIn sham/sham exposure, double-wound coils were energized with opposing current flow so that the net applied magnetic field was 0.

^bIncubator used for normal cell culture.

^cChamber A of the DOE regional exposure facility at NIOSH.

^dChamber B of the DOE regional exposure facility at NIOSH.

Table 2. Anchorage-independent growth of JB6 cells (no tetradecanoylphorbol acetate) exposed to a 0.10-mT magnetic field (MF)

Control (7 days)	MF (7 days)	GR	Control (14 days)	MF (14 days)	GR
16.0 \pm 1.0	14.3 \pm 3.1	0.89	49.0 \pm 7.2	40.0 \pm 5.6	0.82
16.3 \pm 3.5	16.7 \pm 3.7	1.02	6.3 \pm 0.8	7.3 \pm 1.45	1.16
9.7 \pm 1.3	13.6 \pm 3.7	1.40	12.4 \pm 0.7 ^a	11.8 \pm 4.5	0.95
19.3 \pm 2.7	19.2 \pm 4.0	0.99	16.7 \pm 1.7 ^a	17.8 \pm 3.2	1.06

GR, growth ratio (MF colonies/control colonies). Values shown are number of colonies per dish (mean \pm standard deviation).

^aExperiments halted at day 10 instead of day 14.

Table 3. Anchorage-independent growth of JB6 cells (no tetradecanoylphorbol acetate) exposed to a 0.96-mT magnetic field (MF)

Control (14 days)	MF (14 days)	GR
22.7 \pm 1.8	12.7 \pm 2.4	0.56
13.3 \pm 1.9	14.3 \pm 1.4	1.08
16.0 \pm 1.0	18.7 \pm 1.3	1.17
17.7 \pm 4.4	13.0 \pm 4.0	0.73
20.0 \pm 2.0	23.7 \pm 1.8	1.18
34.0 \pm 4.0	45.7 \pm 7.9	1.34

GR, growth ratio (number of MF colonies per number of control colonies). Values shown are number of colonies per dish (mean \pm standard deviation).

Results

The DOE regional MF exposure facility at NIOSH maintained identical physical conditions in either chamber. Temperature, atmospheric CO₂, and relative humidity never differed by more than 1% between chambers; only the presence or absence of an applied MF differed between the two chambers (data not shown). Although our goal was to expose cells to magnetic fields of 0, 0.1, and 1.0 mT, a MF strength of 0.96 ± 0.01 mT was the greatest field that was consistently maintained by the facility over the duration of the exposure period. The background static (DC) field for the chambers was determined with a MultiWave II magnetic field exposure monitor (ERM, Inc., State College, PA). The magnitude of the static field in chamber A was 40.0 μT; field inclination (elevation) was 76° above horizontal, and field declination (azimuthal angle) was 90° (rotated clockwise from north). For chamber B, static field magnitude was 40.5 μT; field inclination (elevation) was 58° above horizontal, and field declination (azimuthal angle) was 122° (rotated clockwise from north). Magnetic fields measured in inactive chambers and during sham exposures were below 2 mG in

either chamber of the facility. Magnetic fields measured in the incubator used for cell culture, C, were 0.36 ± 0.03 μT when the heat source was inactive, and 4.08 ± 0.89 μT when the heating element was on.

Transformation frequency of JB6 cells displayed a similar dose-dependent response to increasing concentrations of TPA, whether grown in the incubator used for cell culture or the DOE regional MF exposure facility (Table 1). Exposure to MF of 0.10 mT (Table 2) or 0.96 mT (Table 3) did not alter colony formation of JB6 cells. Co-exposure of cells to both TPA and the 0.10 mT (Table 4) or 0.96 mT (Table 5), 60-Hz field also did not result in any differences in transformation frequency for any TPA concentrations tested (0–1 ng/ml). In tests comparing cells obtained from ATCC or West and the same lot of serum, cells obtained from West produced significantly greater numbers of colonies without TPA treatment or the presence of MF (Fig. 1).

In tests to determine reasons for the discrepancy between our results and those reported by West et al. (9,10), factors such as serum lot, cell-plating technique, the effect of increasing or decreasing the time the cells are held at 42°C prior to plating, and other experimenter-dependent factors were examined. Of the factors above, serum

lot and cell plating order had the greatest effect on colony formation. Two serum lots obtained from the same supplier (JRH Biosciences) were examined. Initial growth tests determined that the two serum lots were both capable of supporting the JB6 cell line (JRH 3M and JRH 4M). JRH 3M was provided by West as a lot that gave consistent positive results of MF exposure in his laboratory. Samples of JRH 4M were provided to West and failed to show a positive effect of MF exposure on JB6 cell-proliferation in his laboratory (personal communication). It was observed in our laboratory that JRH 3M caused a noticeable aggregation of cells despite repeated titration. Because the aggregated cells tended to settle to the bottom of the pipet during the plating procedure, and based on West's technique of plating six plates from a single 10-ml pipette, in our normal replication studies every effort was made to ensure that each treatment group had at least one dish from the first 4.5 ml of suspended cells. In tests comparing the effect of plating order on cell colony formation, significant differences were observed between JRH serum lots 3M and 4M. As seen in Table 6, with JRH 3M serum there was a significant difference between colony counts of plates that were plated with the initial 4.5 ml of cell

Table 4. Effect of tetradecanoylphorbol acetate (TPA) and/or a 60-Hz magnetic field (MF; 0.10 mT) on anchorage-independent growth of JB6 mouse epidermal cells

Experiment	TPA (ng/ml)	Chamber A (colonies/plate)	Chamber B (colonies/plate)
1	0.00	11.0 ± 3.5	13.7 ± 2.5
Chamber A ^a	0.01	21.3 ± 7.9	25.3 ± 6.5
(14 days)	0.03	50.0 ± 2.5	89.3 ± 4.7
	0.10	118.3 ± 19.1	98.3 ± 5.8
	1.00	239.6 ± 21.2	214.7 ± 17.4
2	0.00	19.3 ± 2.8	19.2 ± 3.8
Chamber B ^a	0.01	24.7 ± 3.9	16.5 ± 4.9
(14 days)	0.03	67.2 ± 6.3	74.5 ± 4.8
	0.10	175.5 ± 16.4	183.4 ± 9.2
	1.00	302.3 ± 9.2	286.7 ± 19.6
3	0.00	6.3 ± 0.9	7.3 ± 1.4
Chamber A ^a	0.01	17.3 ± 4.7	23.0 ± 3.2
(10 days) ^b	0.03	39.1 ± 8.7	44.7 ± 1.8
	0.10	111.7 ± 8.1	133.3 ± 6.3
	1.00	175.7 ± 14.2	162.7 ± 11.2
4	0.00	14.7 ± 2.7	16.1 ± 1.2
Chamber B ^a	0.01	31.0 ± 2.9	36.3 ± 6.1
(10 days) ^b	0.03	49.3 ± 2.2	51.2 ± 3.6
	0.10	86.0 ± 4.3	87.7 ± 3.8
	1.00	173.7 ± 9.9	176.7 ± 7.7

Cells were treated with TPA as described in "Methods" and exposed to either no applied MF or 0.10-mT MF in chamber A or chamber B of the Department of Energy (DOE) regional exposure system. The number of colonies per plate was determined at day 10 or 14. Data shown are mean number of colonies per plate ± standard deviation; *n* = 3 or 4 plates/treatment.

^aMF applied.

^bExperiment halted on day 10 because of power failure to DOE exposure facility.

Table 5. Effect of tetradecanoylphorbol acetate (TPA) and/or a 60-Hz magnetic field (MF; 0.96 mT) on anchorage-independent growth of JB6 mouse epidermal cells on day 14

Experiment	TPA (ng/ml)	Chamber A (colonies/plate)	Chamber B (colonies/plate)
1	0.00	7.7 ± 0.9	12.3 ± 2.4
Chamber A ^a	0.01	30.7 ± 3.2	29.3 ± 4.7
(14 days)	0.03	38.7 ± 2.9	40.7 ± 2.0
	0.10	76.0 ± 4.0	79.3 ± 3.2
	1.00	231.0 ± 12.4	259.5 ± 12.2
2	0.00	13.3 ± 1.8	14.3 ± 1.4
Chamber A ^a	0.01	18.3 ± 4.2	23.7 ± 1.8
(14 days)	0.03	131.3 ± 7.9	123.3 ± 11.7
	0.10	273.3 ± 12.3	276.0 ± 18.1
	1.00	868.0 ± 10.6	866.7 ± 17.6
3	0.00	18.7 ± 1.3	16.0 ± 1.0
Chamber B ^a	0.01	63.0 ± 5.2	75.0 ± 6.9
(14 days)	0.03	129.0 ± 9.6	134.0 ± 3.1
	0.10	210.0 ± 15.1	204.7 ± 14.4
	1.00	533.0 ± 34.8	597.3 ± 21.6
4	0.00	13.0 ± 4.0	17.7 ± 4.4
Chamber B ^a	0.01	44.0 ± 15.7	35.0 ± 5.6
(14 days)	0.03	70.0 ± 11.1	71.0 ± 3.6
	0.10	325.0 ± 43.6	207.3 ± 19.7
	1.00	490.0 ± 38.5	497.0 ± 19.1

Cells were treated with TPA as described in "Methods" and exposed to either no applied MF or 0.96-mT MF in chamber A or chamber B of the Department of Energy regional exposure system. The number of colonies per plate was determined at day 14. Data shown are mean number of colonies per plate ± standard deviation; *n* = 3 or 4 plates/treatment.

^aMF applied.

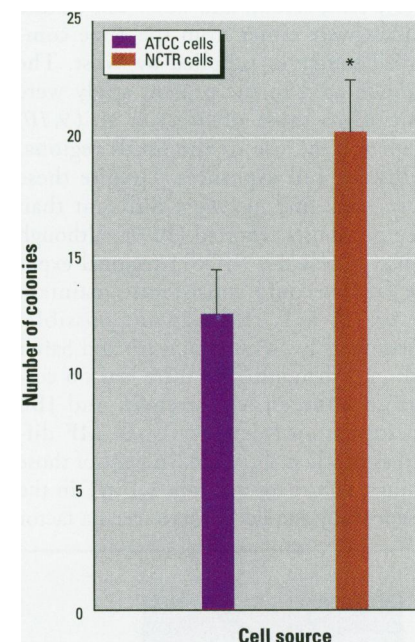


Figure 1. Anchorage-independent growth of JB6 cells from the National Center for Toxicological Research (NCTR) or the American Tissue Culture Collection (ATCC). Colony formation rates of cells from either source were examined for growth rates in the absence of tetradecanoylphorbol acetate or magnetic field exposure. Cells (1×10^4) were plated as described in "Methods." Colonies >60 μm were counted on day 14. Error bars indicate standard deviation.

*Statistically significant at *p* ≤ 0.05.

Table 6. Effect of serum lot (JRH 3M or JRH 4M) and plating order on anchorage-independent growth of JB6 cells treated with tetradecanoylphorbol acetate (0.01 ng/ml).

Plating order	Serum	No. of colonies
First 4.5 ml	JRH 3M	43.50 ± 3.44*
Second 4.5 ml	JRH 3M	23.00 ± 2.97
First 4.5 ml	JRH 4M	18.00 ± 5.71
Second 4.5 ml	JRH 4M	16.00 ± 5.00

Values shown are number of colonies per dish (mean ± standard deviation).

*Statistically significant at $p \leq 0.05$.

suspension versus the second 4.5 ml. This was not observed with JRH serum lot 4M (Table 6).

Discussion

The data obtained using the NIOSH regional exposure facility indicate that exposure of JB6 cells to a 0.10 or 0.96 mT, 60-Hz MF, does not increase the anchorage-independent proliferation of promotion-sensitive JB6 cells. In addition, the data support the conclusion that exposure to 0.10 or 0.96 mT MF does not enhance the effect of TPA on cellular transformation frequency.

Extensive efforts have been made to decrease intra-assay variability while duplicating the experimental design of West et al. (9); cells, serum, and other cell culture supplies were either from the same commercial source or supplied by West. The methods used in the present study were identical to those of West et al. (9,10) except for the use of the DOE regional facility for cell exposures. Despite these efforts, our findings were different than those previously reported (9,10). Although we had hoped the NIOSH regional exposure facility could consistently maintain MF of 1.1 mT, this was not possible. Experiments by West et al. (10) and Saffer et al. (11) demonstrated there was no correlation between MF strength and JB6 transformation frequency when MF differed as much as threefold. In light of those findings, failure to maintain 1.1 mT in the present study should not have been a factor

in the absence of an MF-dependent effect on anchorage-independent cell proliferation. Because of equipment malfunctions, two experiments were ended after 10 days. However, we chose to include these experiments as valid replications for two reasons. First, the TPA-treated cells showed a typical dose-response curve compared to 14-day exposed cells (with predictably lower cell numbers). Second, previous experiments by West et al. (9) reported that although MF-dependent differences were not evident at day 7 of exposure, differences were discernable after 10 days.

The possibility that serum lot contributed to differences between the data reported here and those reported by West et al. (9,10) cannot be discounted. As indicated in Table 6, plating order with JRH serum lot 3M (a serum lot previously reported to yield an increase in JB6 anchorage-independent growth) produced apparent increases in cell proliferation similar in magnitude to those reported by previous researchers (9,10). Our experimental design always included three groups (EMF-exposed, sham, and control) of five TPA concentrations and exposure assignment of these was random, decreasing the likelihood of plating order contributing to erroneous interpretation. In previous studies (9,10) only two groups of cells were included with no internal control (no TPA). Although not discounting the effect of MF exposure, if only two groups of cells are plated (as in the previous publications), the differences that could result from plating order may be inadvertently attributed to MF exposure.

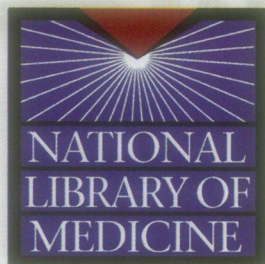
The NIOSH JB6 replication effort closely mirrors that of Saffer et al. (11). These researchers reported that 60-Hz MF of 0.01, 0.1, 1.0, or 1.1 mT flux density did not induce anchorage-independent growth. In addition, these authors reported that MF did not enhance TPA-induced transformation.

In conclusion, although we observed that JB6 cells displayed a dose-dependent response to increasing concentrations of

TPA, co-exposure of cells to both TPA and 0.10 or 0.96 mT, 60-Hz MF did not result in any differences in transformation frequency for any TPA concentrations tested (0–1 ng/ml). These data indicate that exposure to a 0.10 or 0.96 mT, 60-Hz MF does not act as a promoter or co-promoter in promotion-sensitive JB6 cell anchorage-independent growth.

REFERENCES AND NOTES

- Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. *Am J Epidemiol* 109:273–284 (1979).
- Washburn EP, Orza MJ, Berlin JA, Nicholson WJ, Todd AC, Frumkin H, Chalmers TC. Residential proximity to electricity transmission and distribution equipment and risk of childhood leukemia, childhood lymphoma and childhood nervous system tumors: systematic review, evaluation and meta-analysis. *Cancer Causes Control* 5:299–309 (1994).
- Savitz DA. Overview of epidemiologic research on electric and magnetic fields and cancer. *Am Ind Hyg Assoc J* 54:197–204 (1993).
- Holmberg B. Magnetic fields and cancer: animal and cellular evidence—an overview. *Environ Health Perspect* 103(suppl 2):63–67 (1995).
- Cridland NA, Cragg TA, Haylock RG, Saunders RD. Effects of 50 Hz magnetic field exposure on the rate of DNA synthesis by normal human fibroblasts. *Int J Radiat Biol* 69:503–511 (1996).
- McCann J, Kavet R, Rafferty CN. Testing electromagnetic fields for potential carcinogenic activity: a critical review of animal models. *Environ Health Perspect* 105(suppl 1):81–103 (1997).
- McClean JRN, Thansandote A, Lecuyer D, Goddard M. The effect of 60-Hz magnetic fields on co-promotion of chemically induced skin tumors on SENCAR mice: a discussion of three studies. *Environ Health Perspect* 105:94–96 (1997).
- Colburn NH, Smith BM, Wendel EJ, Nakamuro Y, Winterstein D. Comparison of mouse pro-1 and pro-2 transfectants for responses to tumor promoters and antipromoters. *Cancer Res* 48:6076–6080 (1988).
- West RW, Hinson WG, Lyle DB, Swicord ML. Enhancement of anchorage-independent growth in JB6 cells exposed to 60 hertz magnetic fields. *Bioelectrochem Bioenerg* 34:39–43 (1994).
- West RW, Hinson WG, Swicord ML. Anchorage-independent growth with JB6 cells exposed to magnetic fields at several flux densities. *Bioelectrochem Bioenerg* 39:175–179 (1996).
- Saffer JD, Chen G, Colburn NH, Thurston SJ. Power frequency magnetic fields do not contribute to transformation of JB6 cells. *Carcinogenesis* 18:1365–1370 (1997).
- Wilson BW, Caputa K, Stuchly MA, Saffer JD, Davis K, Washam CE, Washam LG, Washam GR, Wilson MA. Design and fabrication of well confined, uniform magnetic fields exposure systems. *Bioelectromagnetics* 15:563–577 (1994).



EHP puts even more environmental health information right at your fingertips!

EHP online articles contain convenient links to PubMed—the National Library of Medicine's free online search service of more than 9 million citations! Search MEDLINE and Pre-MEDLINE (including links to other online journals and databases) for information directly related to each EHP article's topic!

Subscribe to EHP today at <http://ehis.niehs.nih.gov>