

The Effect of 60-Hz Magnetic Fields on Co-promotion of Chemically Induced Skin Tumors on SENCAR Mice: A Discussion of Three Studies

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Three independent experiments involving a total of 288 SENCAR mice were used to study the effects of 60-Hz magnetic fields on the growth and development of skin tumors. Given the constraints imposed by the experimental design, the results did not support a role for magnetic fields as a tumor co-promoter. This negative finding could also be interpreted to mean that the SENCAR mouse skin tumor model was not sensitive enough to detect the action of a weak co-promoter. The two-stage (initiation/promotion) model was used to assess the genotoxic potential of magnetic fields because it had been widely used to evaluate chemical carcinogens. This model, however, lacks the sensitivity to detect all but the most potent direct-acting carcinogens, and the tumor response to the action of low doses of promoter results in large random fluctuations in tumor incidence, yield, and multiplicity. The need to limit tumor incidence in the sham is a necessary condition to ensure that a magnetic field-induced effect on tumorigenesis would have a reasonable chance of being detected. This requirement, and the variability in tumor development between and within experiments, increases the level of uncertainty in the system and makes a weak response to the magnetic field difficult to detect and interpret. *Key words:* 60-Hz magnetic fields, mouse skin tumor co-promotion. *Environ Health Perspect* 105:94-96 (1997)

In spite of numerous studies, epidemiology has failed to provide unequivocal evidence of an association between 60-Hz magnetic fields (MF) and cancer (1-3). Partly in response to this continuing ambiguity, laboratory experiments were begun at Health Canada in 1989 to assess the effect of MF on tumorigenesis in mice. At that time, there were indications that MF alone could not directly initiate cancer (4,5). Accordingly, an experiment was designed to test the effect of MF on tumor promotion using the well-known SENCAR mouse two-stage (initiation/promotion) skin tumor model (6,7). In this previously published preliminary experiment (7), the dorsal skin of mice was exposed to a single dose of 7,12-dimethylbenz[*a*]anthracene (DMBA) and then mice were randomly distributed to two treatment groups. One group received sham exposures and the other received MF exposures (2 mT, 60-Hz); both groups received topical applications of phorbol 12-myristate 13-acetate (PMA) at a dose rate of 1 µg/week (7). The proportion of mice with one or more tumors (tumor incidence) in this preliminary experiment was well above 80% halfway through the promotion period and exceeded 90% at the end of 20 weeks. The experimental design, as previously discussed (7), considered tumor incidence the most important measure of a putative tumor co-promoter, while tumor multiplicity (the average number of tumors per mouse) and tumor yield (the distribution of the total number of tumors in the two groups) were considered secondary. Therefore, to detect an increase in tumor incidence that could be reasonably attrib-

uted to MF, the proportion of mice developing tumors in the sham group had to be kept well below 0.80 (e.g., at 0.2-0.3). To attain this low tumor incidence over three independently replicated experiments, the dose rate of PMA was reduced to 0.3 µg/week after appropriate preliminary trials [described by Stuchly et al. (8)]. Results have been published for the preliminary experiment (7), which used a saturating dose rate of PMA (1 µg/week), and for the first of three replicate experiments involving PMA at a dose rate of 0.3 µg/week (8). The data discussed here were derived only from the three replicate experiments all using the same reduced dose rate of PMA.

Materials and Methods

Experimental design and statistical analysis. Three measures were used to assess the ability of MF to act as a co-promoter: the proportion of mice that developed one or more tumors (tumor incidence), the distribution of tumor counts between groups (tumor yield), and the number of tumors per mouse (tumor multiplicity). Tumor incidence could be kept relatively low, in the area of 0.2-0.3, by reducing the dose rate of PMA from 1 to 0.3 µg/week (8). To consider MF as a tumor co-promoter under these experimental conditions, the tumor incidence in the sham- and MF-exposed groups had to differ by no less than a factor of 2. For a group of 48 mice (with no allowance for possible mouse-to-mouse variability), the probability of rejecting the null hypothesis, if true, was set at 5% ($\alpha = 0.05$). This would allow the alternative hypothesis to be accepted 80%

of the time when the groups differed by a factor of 2 (i.e., $\beta = 0.20$). Differences in tumor incidence between the two groups in the three experiments were tested using either Fisher's exact method for a 2×2 table or Zelen's method for the analysis of several 2×2 tables (9). The number of tumors per mouse did not follow a Poisson distribution, and specialized methods were required to take into account the observed variation (10).

Exposure chambers. The construction and characteristics of the exposure chambers have been described in detail elsewhere (6). Briefly, the MF in the exposure volume was generated by a double set of square coils, each consisting of four windings on a wooden frame. This configuration was used to minimize stray MF outside the exposure system and to provide a uniform field within the volume occupied by 16 mouse cages. The coils were water cooled and consisted of plastic-insulated copper tubing. Current to the coils was provided by a transformer (Hammond model EP4EA120/240-12/24-1500 VA; Hammond Manufacturing, Guelph, Ontario) connected to a standard 110 V, 15 A AC outlet. The MF had a mean flux density of 2 mT that varied $< \pm 10\%$ within the volume occupied by the cages. The electric field was minimized by shielding the windings with thin copper tape. The current in the coils, the magnetic flux density in the exposure chambers, and the light level and temperature in the exposure room were recorded every 20 min by an on-site computer and routinely monitored off-site by a system that had been developed jointly with Ottawa Instrumentation Inc., Ottawa, Ontario. The exposure cages were made from acrylic (0.1 m \times 0.25 m \times 0.18 m) and contained a series of 7-mm diameter holes to facilitate air flow to the mice.

Chemicals and the treatment of mice. DMBA was obtained from Aldrich Chemical Company (St. Louis, MO) at a purity of $>98\%$, and PMA from L.C. Laboratories, (Woburn, MA). DMBA was dissolved in acetone, protected from light, used within 2 hr of preparation, and applied

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to the dorsal skin of mice under subdued light. PMA was prepared as a 1 mg/ml stock solution in acetone, stored at -30°C , and diluted with acetone immediately before use. SENCAR mice were acquired from Harlan Sprague-Dawley (Indianapolis, IN) at 5 weeks of age and allowed to acclimatize at 3 mice/cage. Tumorigenesis was initiated on the shaved backs of mice by the topical application of 10 nmol DMBA at the estimated start of the third resting phase of the hair cycle (7). Five days after DMBA treatment, mice with visible signs of hair regrowth were eliminated from the study; the rest were randomly distributed into two groups of 48 mice each. Mice were assigned to specific home and exposure cages and identified within cages by ear punch.

One week following initiation, both groups were treated with PMA for 23 weeks at a dose rate of 0.3 $\mu\text{g}/\text{week}$ (8). During this period of tumor promotion, one group of mice was exposed to a 2 mT, 60-Hz MF for 6 hr/day, 5 days/week, and the other group was exposed to sham conditions. All mice were exposed during the light cycle from 8:00 A.M. to 2:00 P.M. (dark cycle, 6:00 P.M.–6:00 A.M.). The exposure duration and the magnetic flux density were selected to simulate intense occupational exposures. In scaling from man to mouse, the maximum current loop radius was assumed to be proportional to the cube root of the body weight. Under this condition, the scaling factor was estimated at 12–14 for an average 70 kg man and for the range of body weights for mice of 25–40 g. Thus, 2 mT for mice corresponds to approximately 0.15 mT for adult human males, which represents an intense occupational exposure. Mice had free access to Purina rodent chow (PMI Feeds, Richmond, IN) and water in their home cages and only to food in the exposure cages. They were housed according to Canadian standards (11). In the first (8) and third replicate experiments, PMA was applied at mid-week, 1 hr into the light phase and 0.5–1 hr before the start of MF or sham exposures. In the second experiment, PMA was applied at mid-week 1 hr after the cessation of the MF and sham treatments (i.e., between 2:00 and 3:00 P.M.). Dorsal hair was removed from each mouse with electric clippers 1 day before PMA application. Tumor counts were recorded every week and body weights every second week. To be considered part of the data set, a tumor had to attain a diameter of >1 mm and remain at the original site for 3 consecutive weeks. The location, size, and morphology of all tumors were recorded weekly for each mouse by a computer-based imaging/archiving system, which was developed in collaboration with Ottawa Instrumentation.

Results

The results for tumor incidence, multiplicity, and yield at week 23 are shown in Table 1. Of the 284 mice that survived 23 weeks, 62 (21.8%) responded to tumor promotion, with 34 tumor-bearing mice in the sham and 28 in the group exposed to MF. There were 146 tumors in the 141 mice exposed to MF and 184 tumors in the 143 mice exposed to sham conditions. From pooled data to week 23, the tumor incidence was 23.8% for sham-exposed mice and 19.8% for those exposed to MF (Table 1). The mean number of tumors in mice with one or more tumors was 5.2 for those exposed to MF and 5.4 for sham. A test for heterogeneity of the differences in incidences between MF and sham groups across the three experiments yielded $p = 0.07$. While not significant, this value was sufficiently close to the traditional $p < 0.05$ level to rule out further statistical tests on pooled data.

When individual experiments are considered at week 23, the differences in tumor incidences (Table 1) between the MF and sham groups were not statistically significant for the first and second experiments ($p = 0.34$ and $p = 1.0$, respectively). However, for the last experiment, the tumor incidence was significantly higher for mice exposed under sham conditions ($p = 0.04$). The distribution of tumor counts (tumor yield) among sham and MF-exposed mice of each experiment at week 23 is shown in Table 1. The variation in the counts was greater than that expected for a Poisson distribution. As a result, the differences in tumor yield between the MF and sham groups were tested using statistical methods that allowed for this additional variation (10).

By these methods, the significance of these differences were 0.15, 0.26, and 0.01 for the first, second, and third experiments, respectively. Again, the lowest significance was associated with the third experiment in which the tumor yield in the sham group exceeded that in the mice exposed to MF.

Discussion

In a previous experiment (7), an MF alone was unable to promote the growth of skin tumors on DMBA-initiated SENCAR mice or to enhance the action of high doses of the tumor promoter PMA (7,12). The data compiled from the three replicate experiments are further evidence in support of the contention that MF is not a skin tumor co-promoter under these experimental conditions. For the experimental design to detect a moderate effect of MF on tumor incidence, the proportion of mice with tumors in the three replicate shams had to be kept low, preferably in the range of 0.2–0.3, and relatively stable from experiment to experiment. The large variation in tumor counts that existed between the three sham groups and the need to keep tumor incidences low in the shams combined to severely limit the ability of the experimental design to detect small differences between the sham and MF exposed groups.

The lack of any significant difference in tumor incidence for these data underscores the importance of not attaching too much weight to any one experiment. For example, at weeks 16 and 17 of the first experiment (8), the tumor incidence was significantly higher for mice exposed to MF, tentatively suggesting a role for MF as a tumor co-promoter. However, by week 23 this difference had disappeared and, in retrospect, it was

Table 1. Results for tumor incidence, multiplicity, and yield at week 23

	Experiment		
	1	2	3
Number of mice			
Magnetic field	47	47	47
Sham	48	47	48
Number of mice with one or more tumors (% tumor incidence)			
Magnetic field	13 (27.7%)	7 (14.9%)	8 (17.0%)
Sham	9 (18.8%)	7 (14.9%)	18 (37.5%)
Significance level	0.34	1	0.04*
Conservative confidence limits for tumor incidence			
Magnetic field	(15.6, 42.6)	(6.2, 28.3)	(7.7, 30.8)
Sham	(9.0, 32.6)	(6.2, 28.3)	(24.0, 52.7)
Total numbers of tumors across all mice in each group			
Magnetic field	86	33	27
Sham	48	50	86
Significance level	0.15	0.26	0.01*
Average number of tumors per mouse			
Magnetic field	2.15	0.7	0.5
Sham	1.19	1.04	1.64

*Denotes statistical significance ($p < 0.05$).

most likely due to a temporary rise in tumor incidence among MF-exposed mice, coupled with a static response in the sham. In the third experiment in this series, tumor incidence and yield at all times through week 23 were significantly higher in mice exposed to sham conditions. These differences were statistically significant for tumor incidence ($p = 0.04$), as well as for the distribution of tumors between groups ($p = 0.01$), and suggest MF may have exerted a protective effect. No explanation can be offered for this observation except that the experimental design accepts the probability of making a type 1 error 5% of the time. The results of a recent supplementary experiment (data not shown) suggests the observed differences in the third experiment could also be due to chance. In this supplementary 30-week experiment, two identically treated DMBA-initiated, PMA-promoted groups (i.e., no sham or MF exposures) were monitored weekly for changes in tumor incidence, multiplicity, and yield. These parameters varied by a factor of 2 between the groups during the first 10 weeks of tumor promotion and, while the tumor incidences converged at 23 weeks, the difference in tumor yields remained significant (unpublished data). Speculating further, if this supplementary experiment had been one of the three replicates discussed here, MF would likely have been classified either as a co-promoter or an antipromoter depending on which group had been selected as the sham.

In a retrospective assessment of variation among the three replicate experiments, tumor incidence and yield in the shams varied by factors of 2 and 2.5, respectively. In a previous discussion of experimental design, as published in the preliminary study (7), the random variation in tumor yield and incidence had been considered to be no more than 1.4 and 1.5, respectively. Clearly, these latter predictions underestimate the variability in the response of SENCAR mice to the initiation/promotion protocol. When this added variability is considered, the smallest relative risk that can be declared significant (when the background tumor incidence in the sham is 0.2–0.3) was judged to be 2 for a sample size of 48 mice. Such a difference would have an 80% chance of being detected at the 5% level. The variability inherent in the SENCAR mouse skin model is also evident from the work of Bull et al. (13), who found that for multiple experiments with benzo[*a*]pyrene/PMA as initiator/promoter, the tumor yield varied by a factor of 6. In addition, these authors found that the SENCAR mouse skin tumor model gave a false negative rate of 60% for 20 known carcinogens (14). The variability in

the tumor data, apparently due to chance, and the potentially high false negative rate make it difficult to accept a marginal response in tumor incidence or yield as statistically significant and underscore the problems associated with trying to assign a co-promotional effect to MF using the SENCAR mouse skin tumor model.

When the experiments described here began in 1989, there was no plausible mechanism of action to guide experimental design, and the use of a traditional bioassay for genotoxicity was accepted as a starting point for the investigation. Since then, theoretical arguments (14) and some empirical evidence (15–19) suggest that magnetic fields may be able to indirectly amplify or modulate some biological output or function that cannot be detected by simply enumerating tumors. Based solely on the analysis of tumor count data, the present study does not support a role for MF as a strong co-promoter in the SENCAR mouse skin tumor model. However, the poor sensitivity associated with this mouse model means that any weak co-promotional effect associated with MF would, most likely, go undetected.

REFERENCES

1. Milham S. Mortality from leukemia in workers exposed to electrical and magnetic fields [letter]. *N Engl J Med* 307:249 (1982).
2. Holmberg B. Magnetic fields and cancer: animal and cellular evidence—an overview. *Environ Health Perspect* 103(suppl 2):63–67 (1995).
3. Savitz DA, Loomis DP. Magnetic field exposure in relation to leukemia and brain cancer mortality among electric utility workers. *Am J Epidemiol* 141:123–134 (1995).
4. Juutilainen J, Liimatainen A. Mutation frequency in *Salmonella* exposed to weak 100 Hz magnetic fields. *Hereditas* 104:145–148 (1986).
5. Cohen M, Kunska A, Astembarski JA, McCulloch D, Pakewitz D. Effects of low-level, 60-Hz electromagnetic fields on human lymphoid cells. I. Mitotic rate and chromosomal breakage in human peripheral lymphocytes. *Bioelectromagnetics* 7:415–423 (1986).
6. Stuchly MA, Lecuyer DW, McLean J. Cancer promotion in a mouse-skin model by 60 Hz magnetic field. I. Experimental design and exposure system. *Bioelectromagnetics* 12:261–271 (1991).
7. McLean JRN, Stuchly MA, Mitchel REJ, Wilkinson D, Yang H, Goddard M, Lecuyer DW, Schunk M, Callary E, Morrison D. Cancer promotion in a mouse-skin model by a 60 Hz magnetic field. II. Tumor development and immune response. *Bioelectromagnetics* 12:273–287 (1991).
8. Stuchly MA, McLean JRN, Burnett R, Goddard M, Lecuyer DW, Mitchel REJ. Modification of tumor promotion in the mouse skin by exposure to an alternating magnetic field. *Cancer Lett* 65:1–7 (1992).
9. Zelen M. The analysis of several 2×2 contingency tables. *Biometrika* 58:129–137 (1971).
10. Breslow N. Extra-Poisson variation in log-linear models. *Applied Statistics* 33:38–44 (1984).
11. Guide to the care and use of experimental animals, vol. 1 (Olfert ED, Cross B, McWilliam A, eds). 2nd Ed. Ottawa, Ontario:Canadian Council on Animal Care, 1993.
12. Rannug A, Holmberg B, Ekstrom T, Mild KH, Gimenez-Conti I, Slaga TJ. Intermittent 50-Hz magnetic field and skin tumour promotion in SENCAR mice. *Carcinogenesis* 15:153–157 (1994).
13. Bull RJ, Robinson M, Laurie RD. Association of carcinoma yield with early papilloma development in SENCAR mouse. *Environ Health Perspect* 68:11–17 (1986).
14. Scaiano JC, Cozens FL, McLean J. Model for the rationalization of magnetic field effects *in vivo*. Application of the radical-pair mechanism to biological systems. *Photochem Photobiol* 59:585–589 (1995).
15. Takatsuji T, Sasaki MS, Takekoshi H. Effect of static magnetic field on the induction of chromosome aberrations by 4.9 MeV protons and 23 MeV alpha particles. *J Radiat Res (Japan)* 30:238–246 (1989).
16. Mevissen M, Stamm A, Buntenkotter S, Zwingelberg R, Wahnschaffe U, Loescher W. Effects of magnetic fields on mammary tumor development induced by 7,12-dimethylbenz[*a*]anthracene in rats. *Bioelectromagnetics* 14:131–143 (1993).
17. McLean JRN, Thansandote A, Lecuyer D, Goddard M, Tryphonas L, Scaiano JC, Johnson F. Preliminary report: A 60-Hz magnetic field increases the incidence of squamous cell carcinomas in mice previously exposed to chemical carcinogens. *Cancer Lett* 92:121–125 (1995).
18. Harkins TT, Grissom CB. Magnetic field effects on B₁₂-ethanolamine ammonia lyase: evidence for a radical mechanism. *Science* 263:958–960 (1994).
19. Feinendegen LE, Muhlensiepen H. Effect of static magnetic field on cellular metabolism in the living mouse. *Endeavour* 12:119–123 (1988).