Effects of 50- or 60-Hertz, 100 µT Magnetic Field Exposure in the DMBA Mammary Cancer Model in Sprague-Dawley Rats: Possible Explanations for Different Results from Two Laboratories

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In line with the possible relationship between electric power and breast cancer risk and the underlying melatonin hypothesis, 50-Hz magnetic field (MF) exposure at microtesla flux densities for either 13 or 27 weeks significantly increased the development and growth of mammary tumors in a series of experiments from Löscher's group in Germany. Löscher's group used the 7,12-dimethylbenz[*a*]anthracene (DMBA) model of breast cancer in Sprague-Dawley rats. The finding could not be replicated when a similar experimental protocol was used in a study conducted by Battelle in the United States. In the present paper, investigators from the two groups discuss differences between their studies that might explain the apparent discrepancies between the results. These differences include the use of different substrains of Sprague-Dawley rats (the U.S. rats were more susceptible to DMBA than the European rats), different sources for diet and DMBA, differences in environmental conditions, and differences in MF exposure metrics. Furthermore, the effects of MF exposure reported by Löscher's group, albeit significant, were weak. We also discuss the general problem of replicating such weak effects. *Key words*: breast cancer, electric power, electromagnetic fields, melatonin. *Environ Health Perspect* 108:797–802 (2000). [Online 21 July 2000]
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Electric and magnetic fields (MF) associated with the production, transmission, and use of electricity are ubiquitous in industrialized societies. There is an ongoing controversy about whether exposure to power-line frequency (50- or 60-Hz) MF is a risk factor for cancer (*1–4*). Interest in this question has been triggered primarily by epidemiologic studies that have suggested an association between 50- or 60-Hz MF exposure and increased risk of childhood leukemia (*2*). Furthermore, on the basis of earlier work reporting the effects of 60-Hz electric fields on melatonin levels, a hypothesis was developed stating that exposure to power line-frequency MF could be a risk factor for breast cancer (*5*). Because of the universal exposure to power-line-frequency MF and the increasing breast cancer rates in industrialized countries, this possible relationship between electric power and breast cancer risk as well as the underlying melatonin hypothesis has attracted much interest. Several epidemiologic and experimental studies have been conducted to study the effects of MF on breast cancer (*6*). So far the epidemiologic data are equivocal, but several occupational studies found a significantly increased relative risk of breast cancer in women by MF exposure in the low microtesla range (*7*). To assess the potential of MF to influence the process of mammary carcinogenesis, epidemiologic studies have been supplemented with controlled laboratory studies. The first experimental study using a rat mammary carcinoma model was published by Beniashvili et al. (*8*), who reported an increased incidence of mammary adenocarcinomas in nitrosomethylurea-treated rats (strain unspecified) exposed for 3 hr/day for 2 years to a 50-Hz MF of 20 µT. Most experimental work on the electric power/breast cancer hypothesis is from Löscher et al. (*9,10*) in Hannover, Germany, using 50-Hz MF exposure in the well-established 7,12-dimethylbenz[*a*]anthracene (DMBA) rat model of mammary carcinogenesis (*9,10*). In a series of experiments in female Sprague-Dawley (SD) rats, the authors found that, consistent with the melatonin hypothesis, prolonged exposure to 50-Hz MF at flux densities in the microtesla range decreases nocturnal melatonin plasma levels, increases the activity of ornithine decarboxylase (ODC) in breast tissue, impairs immune surveillance, and enhances mammary tumor development and growth in response to the chemical carcinogen DMBA in a flux-density dependent manner (*9,10*). These data prompted the U.S. National Toxicology Program (NTP) to initiate MF studies that were an attempt to replicate the results obtained by the Hannover group using the DMBA initiation/promotion mammary gland tumor model. The NTP studies were supported under the Electric and Magnetic Fields Research and Public Information Dissemination Program (EMF RAPID) and conducted by Anderson et al. (*11*) at Battelle. In contrast to the data from Löscher's group, the Battelle studies found no evidence for a cocarcinogenic or tumor-promoting effect of MF exposure (*12,13*). In the present paper, the investigators from the two groups discuss differences between their studies that might explain the apparent discrepancies between the results of MF exposure. The present discussion is not only relevant for the studies on the DMBA model; it may be important for other MF bioeffect studies because most reported bioeffects of low-level MF are weak and thus difficult to replicate (*3,4,14–18*).

Comparison of Experimental Procedures Used in the Studies

Table 1 compares the experimental parameters between the two laboratories.

Animal strain and source. In the Hannover experiments, female SD outbred rats were obtained from Charles River (Hagemann, Extertal, Germany) and acclimatized for at least 1 week before use in the experiments. At onset of exposure, rats were approximately 50 days of age (body weight about 170–180 g).

In the Battelle experiments, female SD outbred rats were obtained from Charles River Laboratory (Raleigh, NC) and acclimatized for 13–15 days before use for the experiments. At onset of exposure, rats were

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Table 1. Comparison of experimental parameters utilized in the Hanover studies and the Battelle studies.

Experimental parameters	Study protocol	
	Hanover	Battelle
Experimental animals (rats)		
Stock	Sprague Dawley	Sprague Dawley
Sex	Female	Female
Source	Charles River, Extertal, Germany	Charles River, Raleigh, NC
Body weight at onset of exposure	170–180 gm	175–185 gm
Housing/maintenance		
Quarantine/acclimation period	1 Week	15 Days
Rats/cage (*n*)	9–10	4–5
Cage size (cm)	59 × 39 × 22	48 × 26 × 20 (four rats) 58 × 38 × 20 (five rats)
Diet	Altromin 1324	NIH 07
Bedding	Corn cob	Sani chips
Room temperature	23–24°C	22.2 ± 1.5°C
Humidity	Approximately 50%	50 ± 15%
Lights (fluorescent)		
Cycle (day/night)	12/12	12/12
Intensity	30–38 lux	47–85 lux
Red light intensity	< 0.1 lux	< 0.1 lux
DMBA treatment		
Source/purity	Sigma (95%)	TCI America (98.6%)
Age of rats at first DMBA treatment	52 ± 2 days	50 ± 2 days
Frequency/amount of DMBA (13-week studies)	4 × 5 mg/rat	4 × (5 and 2) mg/rat
Frequency/amount of DMBA (26-/27-week studies)	1 × 10 mg/rat	1 × 10 mg/rat
Magnetic field exposure		
Frequency	50 Hz	50 and 60 Hz
Magnetic field intensity	1, 10, 50, and 100 μT	100 and 500 μT
Days/week	7	7
Hours/day	23–24	18.5
Time of exposure	Continuous	1100–1400 hr, 1530–0700 hr
Study length	13/27 Weeks	13/26 Weeks

50 ± 2 days of age (body weight approximately 175–185 g).

Animal housing and diet. Animal caging configurations were somewhat different between the two laboratories: 9–10 animals per cage (cage size 55 cm × 39 cm × 22 cm) in the Hannover studies and 4 animals (cage size 48 cm × 26.7 cm × 20.3 cm) or 5 animals (cage size 58.4 cm × 38 cm × 20.3 cm) per cage in the Battelle studies. Diet also differed between the studies: Altromin 1324 was used in the Hannover studies and NIH 07 was used in the Battelle studies. In both cases, food and water were available for the rats *ad libitum*.

Animal room environment. Room temperature (22.2 ± 1.5°C and 23–24°C; Battelle and Hannover, respectively) and humidity (50 ± 15% relative humidity) were well-controlled and comparable between the studies. With respect to light, 12 hr fluorescent light per day with a range of 47–85 lux at cage level (Battelle) or 30–38 lux at cage level (Hannover) was followed by 12 hr dim red light (< 0.1 lux) per day with comparable light intensities.

Carcinogen dosage and administration schedule. Each group used two protocols. In the first protocol, rats were administered 20 mg DMBA (four weekly gavage doses of 5 mg/rat in sesame oil). In the Battelle study, an additional experiment was done with 4 weekly gavage doses of 2 mg DMBA in sesame oil. In the second protocol, rats were treated once with 10 mg DMBA (in sesame oil) by gavage. Ninety-five percent pure DMBA was obtained from Sigma (Deisenhofen, Germany) for the Hannover and 98.6% pure DMBA was obtained from TCI America (Portland, OR) for the Battelle studies.

Group size. Except for one experiment (*n* = 36 rats), the Hannover study used 99 rats in all experiments discussed here. The Battelle study had groups of 100 rats per treatment group

Exposure conditions. The Hannover group studies used six identical exposure chambers with four square coils each located in the same room (three at each side) [Baum et al. (*19*) and Mevissen et al. (*20*) provide details]. Each chamber had room for four cages (two levels with two cages each) with 9–10 animals per cage. Three of the chambers were used for MF exposure and the other three for sham exposure, i.e., both sham and MF-exposed rats were in the same room during the experiment; therefore, the environmental conditions were the same for both groups except for the MF. The investigators were blinded with respect to sham and MF exposure. Field characteristics in the experiments with 100 μT were 50-Hz, horizontal linear polarization, 100 μT root-mean-square. The 50-Hz stray fields in the sham-exposure coils were approximately 0.1 μT. In experiments with 50, 10, or 0.3–1 μT, stray fields for sham controls were correspondingly lower. The earth static MF was approximately 40 μT, with the generated 50-Hz MF being horizontal and parallel to the horizontal component of the earth's north/south MF (*20*). Measurement of the electric field in the exposure and sham-exposure chambers did not indicate any significant differences between exposed and sham-exposed locations. Twenty-four-hour measurements showed that under the conditions of the experiment the MF exposure system produced a stable flux density of 100 μT and stable frequency of 50-Hz with negligible harmonics and no power spikes. MF exposure was 24 hr/day during the experiments (minus time for weighing, tumor palpation, cage cleaning, and cage rotation) 7 days/week for a total duration of either 13 or 27 weeks. In experiments with 20 mg DMBA (4 weekly doses of 5 mg), the exposure was started immediately after the first administration of DMBA. In the experiment with one administration of 10 mg DMBA, MF exposure was started 1 week before DMBA administration.

In the Battelle studies [the NTP (*11*), Anderson et al. (*12*), and Boorman et al. (*13*) provide details], the MF exposure system consisted of four identical field-generating coil sets, each associated with three animal exposure racks in a single exposure room. The sham control rats were housed in an adjoining room in equivalent exposure racks. Exposures were not conducted in a blinded fashion, although tumor palpation, necropsies, and histopathology were blinded. Rats were exposed to < 0.06 μT (sham control), 100 μT, or 500 μT 50-Hz or 100 μT 60-Hz horizontal linear MF. Exposure occurred for 18.5 hr/day, 7 days/week during the studies. Each day the fields were turned off twice (between 0700 and 1100 hr and from 1500 to 1630 hr) to provide access to animals for husbandry and observation. In all experiments, the MF exposure was started immediately after the first DMBA application.

Quantification of mammary tumors. During MF or sham exposure, rats were palpated once a week for the detection of mammary gland tumors in both studies. At the end of the exposure period, a necropsy was performed in all rats. In the Hannover group studies, grossly recorded (macroscopically visible) mammary tumors at time of necropsy were used for calculation of tumor incidence, multiplicity (number of tumors per rat), tumor size (volume or weight), and site of tumor development (location of tumors within each rat's six pairs of mammary glands). In the six Hannover group studies

discussed here, a microscopic examination of all grossly recorded tumors was done in three studies (*19,21,22*); in the other three studies only small tumors were microscopically examined to confirm the diagnosis (most mammary tumors were so large that they could be confidently diagnosed without histology). In two of the six studies (*19,21*), serial sections of all mammary glands were histologically examined (after staining with hematoxylin and eosin) to also detect very small tumors (only detectable at the microscopic level) and hyperplasias that were not grossly recorded during necropsy.

In the Battelle studies, masses were identified by specific lesion in each rat during the weekly palpations. Two individuals each palpated half of the rats each week, alternating by group of rats. Sizes were determined by comparison with wooden spheres of defined size. At the time of necropsy the clinical observations were available to the pathologist for each animal. The mammary glands and associated skin were transilluminated to identify all potential tumors and a few additional masses were found. Masses were measured in two directions and collected in formalin, stained by hematoxylin and eosin, and examined histologically. Each gross lesion was uniquely identified and the diagnosis verified by microscopic examination.

Comparison of Study Results

Hannover studies. MF exposure for 13 weeks at flux densities of 0.3–1 μT or 10 μT did not exert any significant facilitatory effect on mammary carcinogenesis in the DMBA model, although a trend to increased tumor incidence was seen in the 10 μT experiment (*23*). In the four experiments with 50 or 100 μT, MF exposure significantly increased the number of mammary tumors detected by palpation during exposure (*19,20,22,24,25*), but only the necropsy data will be discussed here. As shown in Table 2, MF exposure at flux densities of 50 and 100 μT exerted a significant effect on the incidence of grossly recorded mammary tumors after both 13 and 27 weeks of exposure, independent of the DMBA dosing and administration protocol used. Tumor multiplicity was not significantly affected by MF exposure. Tumor size was significantly increased in only one experiment with 100 μT (*19*), but not significantly altered by MF in the other experiments. In the study with 27 weeks of MF exposure (*22*), the most pronounced MF effect on tumor incidence in site of tumor development (location in which of each rat's six pairs of mammary glands) was determined in the cranial thoracic complex (L/R 1). Tumor incidence in this complex was 30 of 99 MF-exposed rats compared to 18 of 99 sham-exposed animals ($p < 0.05$). After reexamination of one of the previous studies with 100 μT MF exposure (*20*), a similar enhanced susceptibility of L/R 1 to increased tumor incidence in response to MF exposure was found; 50 of 99 exposed rats had tumors in L/R 1 compared to 36 of 99 controls ($p < 0.05$).

One of the experiments with 100 μT exposure for 13 weeks included a complete histologic examination of the mammary gland using serial sections of all mammary complexes (including those without grossly recorded mammary tumors) (*19*). This resulted in the histologic diagnosis of 65 tumors (compared to 51 grossly recorded tumors) in the MF-exposed group and 57 tumors (compared to 34 grossly recorded tumors) in the sham group; the difference between groups was not significant. The interpretation of this finding, i.e., no significant intergroup difference in incidence of all tumors (including those only detectable at the microscopic level) but a significant intergroup difference in incidence of grossly recorded tumors, was that MF exposure increased tumor growth (so that more tumors were at the macroscopic level at time of necropsy) but not the overall incidence of tumors. Furthermore, MF exposure enhanced tumor progression because the incidence of malignant tumors (adenocarcinoma) was significantly higher in the MF-exposed group (*19*).

Battelle study. In the eight Battelle experiments with 100 or 500 μT, MF exposure did not significantly increase the number of mammary tumors detected by palpation during exposure (*11–13*). We further discuss only the data from necropsy. As shown in Table 3, MF exposure at flux densities of 100 or 500 μT did not significantly increase the incidence of grossly recorded mammary tumors after either 13 or 26 weeks of exposure. Furthermore, no significant effects on tumor multiplicity or tumor size were observed. In general, the U.S. SD rats used in the Battelle studies (Table 3) appeared to be more sensitive to DMBA than the European SD rats used in the Hannover experiments (Table 2), as indicated by the higher mammary tumor incidence in control groups of the Battelle study. Although considered unlikely, the differences could also result from variations in the purity, concentration, or activity of the DMBA. Alternatively, some differences in tumor yield might result from differences in dosing regimes or technique. Clearly, when the lower dose of DMBA was used in the Battelle studies (i.e., 4 × 2 vs. 4 × 5 mg/dose; Table 3), the tumor incidence and multiplicity (at 13 weeks) was more comparable with the values observed at the higher doses in the Hannover studies. In the Battelle study, an independent laboratory analyzed the DMBA dose solutions and found that they were 99.8–101.4% of target concentration.

Table 2. Incidences of neoplasms of the mammary gland observed grossly at necropsy in female SD rats in the experiments of Löscher's group.

Dosing protocol (DMBA), MF exposure	Incidence of mammary tumors: Rats/group (*n*)	%	MF/ control
4 × 5 mg/rat, 13 weeks			
Sham exposure	21/36	58	
0.3-1 μT, 50-Hz	21/36	58	1.0
4 × 5 mg/rat, 13 weeks			
Sham exposure	60/99	61	
10 μT, 50-Hz	66/99	67	1.1
4 × 5 mg/rat, 13 weeks			
Sham exposure	55/99	56	
50 μT, 50-Hz	69/99*	70*	1.25*
4 × 5 mg/rat, 13 weeks			
Sham exposure	34/99	34	
100 μT, 50-Hz	51/99*	52*	1.53*
4 × 5 mg/rat, 13 weeks			
Sham exposure	61/99	62	
100 μT, 50-Hz	82/99*	83*	1.34*
1 × 10 mg/rat, 27 weeks			
Sham exposure	50/99	51	
100 μT/ 50-Hz	64/99*	65*	1.27*

Data from Baum et al. (*19*), Mevissen et al. (*20,23,25*), Löscher et al. (*21,24*), and Thun-Battersby et al. (*22*).
*Significantly different from control (p at least < 0.05).

Discussion of the Differences between the Studies

In view of the Hannover findings indicating significant effects of 50-Hz MF exposure on mammary carcinogenesis (thereby supporting the melatonin hypothesis), it was important to examine whether these findings could be reproduced by other laboratories using the same or similar experimental protocols as in the Hannover experiments. Respective studies conducted at Battelle demonstrated no significant increases in mammary cancer incidence, multiplicity, or growth in rat groups exposed to either 50- or 60-Hz MF (*11*).

Table 3. Incidences of neoplasms of the mammary gland observed grossly at necropsy in female SD rats in the Battelle study.

Dosing protocol (DMBA), MF exposure	Incidence of carcinomas: Rats/group (*n*)	%	MF/ control
4 × 5 mg/rat, 13 weeks			
Controls	92/100	92	
100 μT, 50-Hz	86/100	86	0.93
500 μT, 50-Hz	96/100	96	1.04
100 μT, 50-Hz	96/100	96	1.04
4 × 2 mg/rat, 13 weeks			
Controls	43/100	43	
100 μT, 50-Hz	48/100	48	1.12
500 μT, 50-Hz	38/100	38	0.88
1 × 10 mg/rat, 26 weeks			
Controls	96/100	96	
100 μT, 50-Hz	90/100	90	0.94
500 μT, 50-Hz	95/100	95	0.99
100 μT, 60-Hz	85/100*	85*	0.86*

Data from the National Toxicology Program (*11*), Anderson et al. (*12*), and Boorman et al. (*13*).
*Significantly different from control ($p < 0.05$).

During the design of the Battelle study, investigators from the Hannover group were asked to review the study protocol to ensure a faithful replication between the two laboratories. In addition to using protocols similar to Löscher's initial experiments (*19–21,24,25*) with four weekly gavage doses of 5 mg DMBA and MF exposure for 13 weeks at 100 µT and a frequency of 50-Hz (European power-line frequency), the Battelle study also included experiments with 60-Hz (U.S. power-line frequency), higher flux density (500 µT), and a more traditional DMBA protocol (one administration of 10 mg/rat and necropsy after 26 weeks). The Hannover study using one administration of 10 mg DMBA and necropsy after 27 weeks of MF exposure was finished after the Battelle study and we included it here for comparison and discussion.

Despite comparable experimental designs and an attempt to conduct the Battelle study as similarly as possible to the initial experiments of the Hannover group, there are several differences between the studies that may have contributed to the differences in outcome. Furthermore, we discuss some factors that might be important for detectability of MF effects.

Variability of tumor incidence in sham control groups. Significant effects on the incidence of grossly recorded mammary tumors were obtained in all of the Hannover experiments with 50 or 100 µT MF exposure (Table 2). The first experiment with four weekly DMBA administrations and 100 µT exposure for 13 weeks (*19,24*) was repeated once to ensure the reproducibility of the MF effect, again resulting in a significant increase in mammary tumor incidence (*20*). In sham controls of the six experiments shown in Table 2, there was considerable variability in tumor incidence rates, which has been suggested to reflect seasonal variation in the sensitivity of the mammary gland to DMBA (*26*). This was one reason to include sham controls together with each MF study. Because tumor incidence in MF-exposed rats was greater than concurrent control in five of six experiments and was never less than control incidence, it is unlikely that the significant differences between sham and MF-exposed groups were the results of uncontrolled variability.

Variability in tumor incidence in control groups was not studied in the Battelle experiments for the different DMBA dosing protocols.

Effect of background tumor incidence on detectability of MF effects. The effects of 100 µT MF exposure in the Hannover experiments with four weekly gavage doses of 5 mg DMBA per rat, albeit significant, were not marked (Table 2). This was a reason to undertake a more recent study in which MF exposure was started 1 week before DMBA application. The DMBA dose was decreased to one intragastric dosing with 10 mg/rat, and the duration of MF exposure was increased to a total of 27 weeks because it was thought that these protocol modifications could enhance the effect of MF exposure on mammary carcinogenesis (*22*). Thirteen weeks after DMBA application (i.e., 14 weeks after the initiation of MF exposure), tumor incidence was 8% in controls but 23% in MF-exposed rats (data based on palpation), thus indicating that tumor incidence in MF-exposed rats was increased 3-fold ($p = 0.003$). Because tumor incidence in sham controls 13 weeks after application of DMBA with 20 mg DMBA was substantially higher compared to tumor incidence 13 weeks after 10 mg DMBA, this might indicate that the magnitude of the MF effect at the same duration of exposure depends on the background (control) tumor incidence in this model; i.e., the lower the control tumor incidence the higher the increase in tumor incidence by MF exposure. When we plot the data from the three experiments with 100 µT MF exposure as shown in Figure 1, there appears to be an inverse relationship between control incidence and the magnitude of the MF effect on tumor incidence 13 weeks after DMBA application. The marked difference in incidence of palpable tumors between MF-exposed and sham-exposed groups 13 weeks after administration of 10 mg DMBA was reduced during further exposure (*22*), suggesting that the MF effect was due to a tumor growth-enhancing action rather than to a cocarcinogenic effect.

In the first Battelle 13-week study, background (control) tumor incidence was 92% when a DMBA dose comparable to that in the Hannover studies was used (4 × 5 mg DMBA per rat). In the Battelle 26-week study, the control tumor incidence was 96% at the end of the 26-week period using 1 × 10 mg/rat. Because of the high incidence of tumors in both cases, the sensitivity of these experiments to detect cocarcinogenic effects of MF exposure at the end of the study was low. In the study using 4 × 2 mg DMBA per rat, a lower background tumor incidence (43%) was obtained at 13 weeks; this was generally more comparable to the incidences observed in the Hannover studies. None of the Battelle studies, using either palpation data throughout the course of the experiments or the confirmatory data at necropsy, showed significant MF effects on grossly recorded mammary tumors.

Effect of location of mammary tumors on detectability of MF effects. In the Hannover experiment with 10 mg/rat and 27 weeks of MF exposure, the development of mammary tumors was affected unequally across the six mammary complexes of the female rat (the cranial thoracic complex is particularly sensitive to MF exposure). A similar enhanced susceptibility of this mammary complex to MF exposure was also found by reevaluating one of the previous Hannover experiments with 100 µT (*20*). Previous studies showed that not all of the mammary glands respond to the administration of DMBA in the same fashion; tumor incidence in thoracic mammary glands is higher than in the abdominal glands (*27–29*). It is thought that this different carcinogenic response is due to the asynchronous development of mammary glands in different topographic areas; thoracic glands lag behind in development and retain a higher concentration of terminal end buds (i.e., the site of origin of mammary carcinomas) (*28*). Recent experiments from the Hannover laboratory (*30*) indicate that the L/R 1s are particularly sensitive to increased proliferation in response to 50-Hz, 100 µT MF exposure, which might explain the higher susceptibility of these complexes to tumor-promoting effects of MF exposure. These data thus strongly indicate that not only the background (control) tumor incidence but also the site of origin of mammary carcinomas may possibly influence the extent to which MF exposure increases mammary tumorigenesis in the DMBA model.

In the Battelle studies, no data on the location of mammary tumors were reported for the different MF exposure experiments.

Differences in substrains of rats used in the studies. The Battelle study used SD rats obtained from a U.S. supplier, whereas

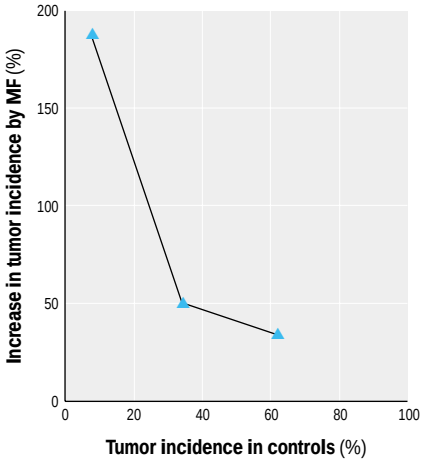

Figure 1. Relationship between control incidence of mammary tumors and increase in tumor incidence by MF exposure. Data from three separate experiments with 50-Hz MF exposure at 100 µT. In each experiment, a sham control group of 99 rats was exposed together with an MF group of 99 rats. The sham control mammary tumor incidence is plotted against the MF-induced increase in mammary tumor incidence determined 13 weeks after application of DMBA in the same experiment. Data adapted from Thun-Battersby et al. (*22*).

Hannover studies used animals procured from a German supplier. Using the same dosing level, 4 × 5 mg/rat DMBA, a much higher control incidence of mammary tumors (92%) was observed in the experiments at Battelle than in the experiments in Hannover (34–62% control incidence; Table 2). The same was true for the Battelle experiment with 10 mg DMBA and 26 weeks of exposure (control tumor incidence was 96% at the end of the study compared to 51% in a similar experiment using a comparable dosing level and duration as the Hannover group). The high percentage of animals with tumors at the end of the two Battelle studies, although not precluding differentiation between groups in rate of tumor development or tumor multiplicity, would generally mask any discrimination of tumor incidence in exposed versus control groups. The high control incidence led Battelle investigators to perform the additional study using four weekly doses of 2 mg DMBA. The latter experiment is presumably the most important of the Battelle studies because the control tumor incidence was comparable (43%) to that of the Hannover experiments, thus allowing the discrimination of any incidence-enhancing MF effect. However, no MF effect was observed in this study as well.

The data from the two labs suggest that the rats used in the Battelle study might be more sensitive to the carcinogenic effect of DMBA than the European rats but possibly less sensitive to any influence of MF exposure. It has previously been demonstrated that there are inherent differences between substrains of SD outbred rats obtained in the United States and those from Europe with regard to neoplastic response of mammary tissue to DMBA and their response to radiation (*32*). Outbred rats of the same strain obtained from different breeders may differ markedly in various genetic factors (*33*); therefore, different genetics of the SD rats used in the Battelle and Hannover studies present a reasonable candidate for the significantly differing results.

The likely involvement of substrain differences in the Hannover and Battelle experiments prompted the Hannover group to search for substrains of SD rats that are insensitive to MF exposure under the conditions of the Hannover studies. Last year, a substrain of SD rats was found that significantly differs in sensitivity to both DMBA and 50-Hz MF from the substrain used in the published reports from the Hannover group, thus substantiating that the genetic background plays a pivotal role in the cocarcinogenic effects of MF exposure (*34*).

Dietary differences. Diet may also explain the differing study results. Diet has a substantial impact on the sensitivity of rats to DMBA-induced cancer (*35–37*). Food was procured from different sources with slight differences in compositions of the diet. Furthermore, to avoid any metal in the exposed cages, rats in the Hannover studies were offered food in acrylic feeding dishes with perforated lids (*24*) so that access to food was somewhat more restricted for the rats compared to conventional feeding devices. Although body weight gain was normal in these rats, any restriction of calorie intake decreases the sensitivity of rats to chemically induced breast cancer (*38*).

Animal housing. An interesting and potentially important difference between studies in the two laboratories was the number of rats housed per cage. Caging in both systems was in compliance with the recommended housing space for laboratory animals (*39*). However, with fewer animals per cage in the Battelle studies (and correspondingly more floor space per animal), there may be some difference in animal stress between the studies. Currently no data are available to address the potential influence of these differences on any EMF effects in the DMBA mammary cancer model.

DMBA sources. Although slight differences in purity of the DMBA were recorded, the more significant potential for an effect on experimental outcome is in the preparation of the DMBA dose solution. If significant differences exist between the dosing effectiveness of the two labs it might provide some explanation for the marked difference in tumor level in control rats but it would not explain the differences observed with field exposure.

Location of controls. There were differences in control exposures (sham exposure in identical coils in the same room in the Hannover experiments vs. control rats in an adjoining room in the Battelle study). These differences would seem to provide a more relevant explanation, however, if the results were the opposite of those obtained (i.e., with MF effects in the Battelle studies and no effects in the Hannover studies).

Lighting conditions. Some experiments have suggested that MF effects can be affected by light level and light spectral composition, which were only partly characterized in the studies discussed here. However, the light–dark cycle was equivalent in both studies at 12/12 hr light/dark. A dim red light was used during the dark period.

MF exposure metrics. Although both studies used linearly polarized sine wave MF, the effects of other aspects of MF exposure were not considered in sufficient detail in the two studies. These aspects involve geomagnetic field, transients, and exposure duration [reviewed by Polk (*40*), Misakian et al. (*41*), and Valberg (*42*)].

Different physical models for MF–biosystem interaction have been proposed that suggest outcomes which depend on the magnitude of the static (geomagnetic) field and its direction relative to an MF in the microtesla range. The magnitude and direction of the geomagnetic field relative to the MF have been described for the exposure conditions of the Hannover experiments (*20*) and for the Battelle study (*43*). This argument, as a possible explanation for the differences in results, is weakened by the housing configuration that allows free and random movement of the animals during exposure.

Biologic effects have also been suggested as possibly arising from power system transients of increased intensity. Transients, as well as amplitude variations, could be caused by turning equipment on and off in the building complex where the experiments were performed. Measurements of transients were not performed and not corrected for in the Hannover studies. Investigators at Battelle took a slightly different approach: the exposure system was supplied power through line conditioners that were used to eliminate peak transients from the exposure system operation.

Another aspect of exposure that was different between the two labs was exposure duration. Daily exposure in the Hannover studies extended for ≥ 23 hr/day, whereas exposure in the Battelle studies was 18.5 hr/day. The differences in daily exposure duration resulted in > 400 hr less exposure in Hannover during a 13-week study.

Because of the importance of MF exposure metrics, a plan to characterize the fields in more detail in the two laboratories would contribute to a determination of whether the differing results might be ascribed to differences in exposure metrics.

Statistics. Another topic relevant to the present discussion is the statistical chance to reproduce weak effects as reported by the Hannover group even when all factors described here are dealt with in an independent replication study by another laboratory. For instance, taking the experiment from the Hannover group with the most marked effect, i.e., the 100 μT experiment with 51 of 99 exposed rats with mammary gland tumors versus 34 of 99 for the unexposed controls (Table 2), the chance of repeating the effect with 100 rats per group is only 75%. To increase the chance much above this would require a large increase in group size. This should be carefully considered in the discussion of replicate experiments and in the protocol design of future studies.

Conclusions

Two carefully conducted series of studies on MF effects in the rat DMBA mammary

cancer model resulted in different outcomes. The critical question is whether the results observed by the Hannover group are real or are due to chance or methodologic biases. The fact that tumor incidence in MF-exposed groups in the six experiments carried out by the Hannover group was never below sham controls but was above controls in five experiments argues against chance as a likely explanation for the findings. Furthermore, in view of the blinded conditions under which the Hannover experiments were conducted, methodologic biases are also unlikely to be determinant in the findings. Assuming that the positive findings in the Hannover experiments are real, the lack of replication by the Battelle experiments suggests that such a positive MF effect can be detected only under certain experimental conditions. In the present paper we have presented a number of conditions that could potentially explain the differing results between the Hannover and Battelle studies.

The issue of MF exposure and risk of breast cancer is an important, not yet completely resolved issue that requires further study to address apparent conflicts between carefully conducted comparable studies. The positive results from the Hannover experiments would be strengthened through an identification of mechanisms of action for the increased growth rate of DMBA-initiated tumors. In this respect, the effect of MF exposure on ODC activity in mammary tissue (*30,44*) should be noted, suggesting that it might be advantageous to expand end points in *in vivo* studies to include biochemical parameters relevant to possible carcinogenic mechanisms of MF to increase the potential for reproducing positive MF effects.

References and Notes

1. Sagan LA. Epidemiological and laboratory studies of power frequency electric and magnetic fields. JAMA 268:625–629 (1992).
2. Savitz DA, Ahlbom A. Epidemiologic Evidence on Cancer in Relation to Residential and Occupational Exposures, Biological Effects of Electric and Magnetic Fields (Carpenter DO, Ayrapetyan S, eds). San Diego, CA: Academic Press, 1994;233–261.
3. Portier CJ, Wolfe MS. Assessment of Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields. NIEHS Working Group Report. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1998.
4. Löscher W, Liburdy RP. Animal and cellular studies on carcinogenic effects of low frequency (50/60-Hz) magnetic fields. Mutat Res 410:185–220 (1998).
5. Stevens RG. Electric power use and breast cancer: a hypothesis. Am J Epidemiol 125:556–561 (1987).
6. Stevens RG, Wilson BW, Anderson LE. The Melatonin Hypothesis—Breast Cancer and Use of Electric Power. Columbus, OH:Battelle Press, 1997.
7. Erren TC. Epidemiologic Studies on EMF and Breast Cancer Risk, the Melatonin Hypothesis—Breast Cancer and Use of Electric Power (Stevens RG, Wilson BW, Anderson LE, eds). Columbus, OH:Battelle Press, 1997;701–738.
8. Beniashvili DS, Bilanishvili VG, Menabde MZ. Low-frequency electromagnetic radiation enhances the induction of rat mammary tumors by nitrosomethyl urea. Cancer Lett 61:75–79 (1991).
9. Löscher W, Mevissen M. Magnetic Fields and Breast Cancer: Experimental Studies on the Melatonin Hypothesis, The Melatonin Hypothesis—Breast Cancer and Use of Electric Power (Stevens RG, Wilson BW, Anderson LE, eds). Columbus, OH:Battelle Press, 1997;555–584.
10. Löscher W. Breast Cancer and Use of Electric Power: Experimental Studies on the Melatonin Hypothesis. In: The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy (Bartsch C, Bartsch H, Blask DE, Cardinali D, Hrushesky W, Mecke D, eds). Berlin:Springer, in press.
11. NTP. Technical Report on the Studies of Magnetic Field Promotion (DMBA Initiation) in Sprague-Dawley Rats (Gavage/Whole Body Exposure Studies). TR 489. Research Triangle Park, NC:National Toxicology Program, 1998.
12. Anderson LE, Boorman GA, Morris JE, Sasser LB, Mann PC, Grumbein SL, Hailey JR, McNally A, Sills RC, Haseman JK. Effect of 13 week magnetic field exposures on DMBA-initiated mammary gland carcinoma in female Sprague-Dawley rats. Carcinogenesis 20:1615–1620 (1999).
13. Boorman GA, Anderson LE, Morris JE, Sasser LB, Mann PC, Grumbein SL, Hailey JR, McNally A, Sills RC, Haseman JK. Effect of 26 week magnetic field exposures in a DMBA initiation-promotion mammary gland model in Sprague-Dawley rats. Carcinogenesis 20:899–904 (1999).
14. Adey WR. Biological effects of electromagnetic fields. J Cell Biochem 51:410–416 (1993).
15. Frey AH. Electromagnetic field interactions with biological systems. FASEB J 7:272–281 (1993).
16. Blank M. Biological effects of environmental electromagnetic fields: molecular mechanisms. Biosystems 35:175–178 (1995).
17. Hong FT. Magnetic field effects on biomolecules, cells, and living organisms. Biosystems 36:187–229 (1995).
18. Smith SD, Liboff AR, Mcleod BR. Effects of Magnetic Fields on Living Systems, Bioelectrochemistry of Cells and Tissues (Walz D, Berg H, Milazzo G, eds). Basel, Switzerland:Birkhäuser, 1995;245–282.
19. Baum A, Mevissen M, Kamino K, Mohr U, Löscher W. A histopathological study on alterations in DMBA-induced mammary carcinogenesis in rats with 50 Hz, 100 µT magnetic field exposure. Carcinogenesis 16:119–125 (1995).
20. Mevissen M, Häußler M, Lerchl A, Löscher W. Acceleration of mammary tumorigenesis by exposure of 7,12-dimethylbenz[*a*]anthracene-treated female rats in a 50-Hz, 100 µT magnetic field: replication study. J Toxicol Environ Health 53:401–418 (1998).
21. Löscher W, Wahnschaffe U, Mevissen M, Lerchl A, Stamm A. Effects of weak alternating magnetic fields on nocturnal melatonin production and mammary carcinogenesis in rats. Oncology 51:288–295 (1994).
22. Thun-Battersby S, Mevissen M, Löscher W. Exposure of Sprague-Dawley rats to a 50-Hertz, 100-µTesla magnetic field for 27 weeks facilitates mammary tumorigenesis in the 7,12-dimethylbenz[*a*]anthracene model of breast cancer. Cancer Res 59:3627–3633 (1999).
23. Mevissen M, Lerchl A, Löscher W. Study on pineal function and DMBA-induced breast cancer formation in rats during exposure to a 100-mG, 50-Hz magnetic field. J Toxicol Environ Health 48:101–117 (1996).
24. Löscher W, Mevissen M, Lehmacher W, Stamm A. Tumor promotion in a breast cancer model by exposure to a weak alternating magnetic field. Cancer Lett 71:75–81 (1993).
25. Mevissen M, Lerchl A, Szamel M, Löscher W. Exposure of DMBA-treated female rats in a 50-Hz, 50 µTesla magnetic field: effects on mammary tumor growth, melatonin levels, and T lymphocyte activation. Carcinogenesis 17:903–910 (1996).
26. Löscher W, Mevissen M, Häußler M. Seasonal influence on 7,12-dimethyl[*a*]anthracene-induced mammary carcinogenesis in Sprague-Dawley rats under controlled laboratory conditions. Pharmacol Toxicol 81:265–270 (1997).
27. Torgersen O. Regional distribution of DMBA-induced mammary tumours in the rat. Acta Pathol Microbiol Scand 83:639–644 (1975).
28. Russo J, Russo IH. Experimentally induced mammary tumors in rats. Breast Cancer Res Treat 39:7–20 (1996).
29. Russo J, Saby J, Isenberg WM, Russo IH. Pathogenesis of mammary carcinoma induced in rats by 7,12-dimethylbenz[*a*]anthracene. J Natl Cancer Inst 59:435–445 (1977).
30. Mevissen M, Häußler M, Löscher W. Alterations in ornithine decarboxylase activity in the rat mammary gland after different periods of 50 Hertz magnetic field exposure. Bioelectromagnetics 20:338–346 (1999).
31. 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).
32. van Zwieten MJ, Shellabarger CJ, Hollander CF, Cramer DV, Stone JP, Holtzman S, Broerse JJ. Differences in DMBA-induced mammary neoplastic responses in two lines of Sprague-Dawley rats. Eur J Cancer Clin Oncol 20:1199–1204 (1984).
33. Rapp KG, Kluge R, Burow K. Genetische Steuerung von Auszuchtstämmen am Beispiel der Han:NMRI-Mäusepopulationen, Qualitätskriterien der Versuchstierforschung (Gärtner K, ed). Weinheim:VCH, 1991;149–174.
34. Löscher W, Fedrowitz M. Unpublished data.
35. Clinton SK, Li PS, Mulloy AL, Imrey PB, Nandkumar S, Visek WJ. The combined effects of dietary fat and estrogen on survival, 7,12-dimethylbenz(*a*)anthracene-induced breast cancer and prolactin metabolism in rats. J Nutr 125:1192–1204 (1995).
36. Jackson CD, Weis C, Chen JJ, Bechtel DH, Poirier LA. Relative contribution of calories from dietary fat, carbohydrate and fiber in the promotion of DMBA-induced mammary tumors in Sprague-Dawley rats. Nutr Cancer 30:194–200 (1998).
37. Zile MH, Welsch CW, Welsch MA. Effect of wheat bran fiber on the development of mammary tumors in female intact and ovariectomized rats treated with 7,12-dimethylbenz(*a*)anthracene and in mice with spontaneously developing mammary tumors. Int J Cancer 75:439–443 (1998).
38. Zhu Z, Jiang W, Thompson HJ. Effect of energy restriction on tissue size regulation during chemically induced mammary carcinogenesis. Carcinogenesis 20:1721–1726 (1999).
39. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC:National Academy of Sciences, 1996;27.
40. Polk C. Dosimetry of extremely-low-frequency magnetic fields. Bioelectromagnetics (suppl 1):209–235 (1992).
41. Misakian M, Sheppard AR, Krause D, Frazier ME, Miller DL. Biological, physical, and electrical parameters for in vitro studies with ELF magnetic and electric fields—a primer. Bioelectromagnetics (suppl 2):1–73 (1993).
42. Valberg PA. Designing EMF experiments: What is required to characterize "exposure"? Bioelectromagnetics 16:396–401 (1995).
43. Sasser LB, Morris JE, Miller DL, Rafferty CN, Ebi KL, Anderson LE. Exposure to 60 Hz magnetic fields does not alter clinical progression of LGL leukemia in Fischer rats. Carcinogenesis 17:2681–2687 (1996).
44. Mevissen M, Kietzmann M, Löscher W. 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 (1995).