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] http://ehpnet1.niehs.nih.gov/docs/2000/108p797-802anderson/abstract.html

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 increased breast cancer rates in industrialized countries, this possible relationship between electric power and breast cancer risk as well as the underlying melanin 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 Benashvili 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, Extetal, 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, N.C.) and acclimatized for 13–15 days before use for the experiments. At onset of exposure, rats were...
Table 1. Comparison of experimental parameters utilized in the Hanover studies and the Battelle studies.

<table>
<thead>
<tr>
<th>Experimental parameters</th>
<th>Hanover</th>
<th>Battelle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>Sprague Dawley</td>
<td>Sprague Dawley</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Source</td>
<td>Charles River, Extertal, Germany</td>
<td>Charles River, Raleigh, NC</td>
</tr>
<tr>
<td>Body weight at onset of exposure</td>
<td>170–180 gm</td>
<td>175–185 gm</td>
</tr>
<tr>
<td>Housing/maintenance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarantine/acclimation period</td>
<td>1 Week</td>
<td>15 Days</td>
</tr>
<tr>
<td>Rats/cage (n)</td>
<td>9–10</td>
<td>4–5</td>
</tr>
<tr>
<td>Cage size (cm)</td>
<td>59 × 39 × 22</td>
<td>48 × 26 × 20 (four rats)</td>
</tr>
<tr>
<td>Diet</td>
<td>Altromin 1324</td>
<td>NIH 07</td>
</tr>
<tr>
<td>Bedding</td>
<td>Corn cob</td>
<td>Sani chips</td>
</tr>
<tr>
<td>Room temperature</td>
<td>23–24°C</td>
<td>22.2 ± 1.5°C</td>
</tr>
<tr>
<td>Humidity</td>
<td>Approximately 50%</td>
<td>50 ± 15%</td>
</tr>
<tr>
<td>Lights (fluorescent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle (day/night)</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Intensity</td>
<td>47–85 lux</td>
<td>47–85 lux</td>
</tr>
<tr>
<td>Red light intensity</td>
<td>&lt;0.1 lux</td>
<td>&lt;0.1 lux</td>
</tr>
<tr>
<td>DMBA treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Source/purity                                | TCI America (98.6%)          | Ninety-five percent pure DMBA was obtained from Sigma (95%). | Nickoloff & Dreyer (11) 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-H z or 100 µT 60-H z 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 administration of DMBA. In the Battelle studies, the MF exposure system started 1 week before the first DMBA administration.

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 Hanover 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 (Portland, OR) for 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 Hanover, 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 (H annover) 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 Hanover 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 H annover study used 99 rats in all experiments discussed here. The Battelle study had groups of 100 rats per treatment group.

Exposure conditions. The H annover group studies used identical exposure chambers with four square coils each located in the same room (three at each side) by (Baum et al. (19) and Mewis 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 M F exposure and the other three for sham exposure, i.e., both sham and M F-exposed rats were in the same room during the experiment; therefore, the environmental conditions were the same for both groups except for the M F. The investigators were blinded with respect to sham and M F exposure. Field characteristics in the experiments with 100 µT were 50-H z, horizontal linear polarization, 100 µT root-mean-square. The 50-H z 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 M F was approximately 40 µT, with the generated 50-H z M F being horizontal and parallel to the horizontal component of the earth’s north/south M F (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 M F exposure system produced a stable flux density of 100 µT and stable frequency of 50-H z with negligible harmonics and no power spikes. M F exposure was 24 hr/day during the experiments (minus time for weight, 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, M F exposure was started 1 week before D M B A administration.

In the Battelle studies, 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-H z or 100 µT 60-H z 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 M F 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 H annover 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 H annover group studies.
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.

<table>
<thead>
<tr>
<th>Dosing protocol (DMBA), MF exposure</th>
<th>Incidence of carcinomas Rats/group MF/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 x 5 mg/rat, 13 weeks Controls</td>
<td>92/100</td>
</tr>
<tr>
<td>100 µT, 50-Hz</td>
<td>86/100</td>
</tr>
<tr>
<td>500 µT, 50-Hz</td>
<td>96/100</td>
</tr>
<tr>
<td>1000 µT, 50-Hz</td>
<td>96/100</td>
</tr>
<tr>
<td>4 x 2 mg/rat, 13 weeks Controls</td>
<td>43/100</td>
</tr>
<tr>
<td>100 µT, 50-Hz</td>
<td>48/100</td>
</tr>
<tr>
<td>500 µT, 50-Hz</td>
<td>38/100</td>
</tr>
<tr>
<td>1 x 10 mg/rat, 26 weeks Controls</td>
<td>96/100</td>
</tr>
<tr>
<td>100 µT, 50-Hz</td>
<td>96/100</td>
</tr>
<tr>
<td>500 µT, 50-Hz</td>
<td>96/100</td>
</tr>
<tr>
<td>1000 µT, 50-Hz</td>
<td>96/100</td>
</tr>
</tbody>
</table>

Data from the National Toxicology Program[11], Anderson et al.[20], and Boorman et al.[13].

*Significantly different from control (p < 0.05).

Discussion

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]

### 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 DMB model, although a trend to increased tumor incidence in response to MF exposure (at 0.3–1 µT or 10 µT did not significantly increase the incidence of grossly recorded mammary tumors after 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 × 1 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.

### 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 DMB model, although a trend 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.[12,13] 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 × 1 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.

### Discussion

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]
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, the authors 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 observed at 13 weeks; this was generally more comparable to the incidences observed in the Hannover studies. 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.** The Battelle study used SD rats obtained from a U.S. supplier, whereas the Battelle study used SD rats obtained from a U.S. supplier, whereas the Battelle study used SD rats obtained from a U.S. supplier, whereas the Battelle study used SD rats obtained from a U.S. supplier.
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 strain differences in the Hannover and Battelle experiments prompted the Hannover group to search for strains of SD rats that are insensitive to MF exposure under the conditions of the Hannover studies. Last year, a strain of SD rats was found that significantly differs in sensitivity to both DMBA and 50-Hz MF from the strain 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 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 include 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...
The M F 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 H annover experiments could be strengthened through the identification of mechanisms of action for the increased growth rate of D M BA-initiated tumors. In this respect, the effect of M F exposure on O D C activity in mammary tissue (30,44) should be noted, suggesting that it might be advantageous to expand and study in vivo studies to include biochemical parameters relevant to possible carcinogenic mechanisms of M F to increase the potential for reproducing positive MF effects.

REFERENCES AND NOTES

34. Löscher W, Fedrowitz M. Unpublished data.