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Effects of Weak Alternating Magnetic Fields on Nocturnal Melatonin Production and Mammary Carcinogenesis in Rats

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Cancer
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Dimethylbenz(*a*)anthracene

Abstract
Since extremely low frequency (i.e., 50- or 60-Hz) magnetic fields (MFs) from overhead power lines and other electromagnetic sources are ubiquitous in modern societies, the possible carcinogenic effect of such fields recently suggested by epidemiological studies has engendered much concern. However, in view of various unknown and uncontrolled variables which may bias epidemiological studies on MF interactions, a causal relationship between MFs and tumorigenesis can only be determined precisely in animal experiments. The goal of the study reported here was to determine if low frequency MFs at the low flux densities which are relevant for human populations induce tumor-promoting or copromoting effects in a model of breast cancer. Furthermore, since reduction in pineal production of melatonin has been implicated as a cause of tumor promotion by electromagnetic fields, determinations of nocturnal melatonin peak levels in serum were performed during MF exposure. Mammary tumors were induced by intragastric administration of 20 mg (5 mg/week) 7,12-dimethylbenz(*a*)anthracene (DMBA) in female Sprague-Dawley rats. Groups of 36 rats were either sham-exposed or exposed for 91 days at a 50-Hz gradient MF of 0.3–1 μT, which is a relevant range for elevated domestic MF exposure as arising from neighboring power lines. Nocturnal melatonin levels were significantly reduced by exposure to this weak alternating MF. However, histopathological evaluation of mammary lesions did not disclose any significant difference between MF- and sham-exposed animals. Incidence of mammary tumors was 61% in controls versus 67% in MF-exposed rats. The predominant tumor type was the invasive adenocarcinoma, which was found in 21 rats of both groups. Examination of tumor size did not indicate significant differences in tumor burden between both groups. Furthermore, the incidence of preneoplastic lesions was not altered by MF exposure. Thus, the data of this study indicate that alternating MF do not exert signficant tumor promoting or copromoting effects at environmentally relevant flux densities in the rat mammary cancer system.

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Introduction

Concern with health effects of extremely low frequency (i.e., 50- or 60-Hz) magnetic fields (MFs) has been raised by epidemiological studies of childhood and adult cancers in relation to proximity to electric power distribution lines [1, 2]. While studies of cancer in laboratory animals exposed to MFs would be helpful in estimating risks of cancer, at the present time there are no published studies which have investigated if long-term MF exposure at the low flux densities relevant for residential exposures can increase the occurrence of cancer in laboratory animals [1]. Various in vitro studies on biological effects of MF indicated that low frequency MFs do not cause cytogenetic damage that can result in mutation or transformation, i.e. tumor initiation [1, 3, 4]. However, there is accumulating evidence from in vitro and in vivo experiments that 50- or 60-Hz MFs might induce tumor-promoting effects [1–4]. In several reports, it has been argued that suppression of pineal melatonin production by electric or magnetic fields might produce an increased risk of cancers of hormone-dependent tissues, such as breast and prostate [4–7]. An association between environmental exposure to MFs and breast cancer has also been suggested from epidemiological data [5, 6, 8]. However, most experimental studies on the effects of MF exposure on tumorigenesis or melatonin production used MFs at flux densities in the mT-range, i.e. far above the flux densities of some 100 nT that had been measured in residences [8–10]. Interestingly, there is some experimental evidence that MF exposure in this low flux density range might induce stronger pathophysiological effects than fields with higher flux densities [11]. This prompted us to study the effects of long-term exposure to a 50-Hz gradient MF of low flux density (0.3–1 μT) on tumor development and melatonin production in a 7,12-dimethylbenz(*a*)anthracene (DMBA) model of breast cancer in rats. This model has previously been used to evaluate the effect of electric fields on cancer risk in chemically initiated animals [12].

Materials and Methods

Animals

Female Sprague-Dawley albino rats were obtained from the Institute of Laboratory Breeding (Hagemann, Extertal, FRG) at the age of 40 days and were adapted for about 1 week in an animal room of the department in Hannover in which all experiments were done. The care of the animals was in accordance with institutional guidelines. After adaptation, the rats were randomly subdivided into two groups, i.e. a control group of 36 animals for sham exposure and a group of 36 animals for MF exposure. The animals were brought into the room with the MF exposure system (see below) and were adapted for at least 5 days to this room before the experiments were begun.

All animals were housed in groups of 3 in plastic (Makrolon) cages (26 × 42 cm) and received water and a standard rat diet ad libitum. In order to prevent distortion of the MF during MF exposure, the feeding dishes, water bottles and cage lids were made out of plastic, i.e. nonmagnetic material. The windowless animal rooms (with or without the MF exposure system) were automatically controlled for constant air temperature (23–24 °C), humidity (about 50%) and a 12-hour light/dark cycle with artificial white light from 7 a.m. to 7 p.m. and darkness from 7 p.m. to 7 a.m. The temperature in the MF coils was not different from that of the room (see below).

Treatment with DMBA and MF Exposure

At the onset of the experiments, all female rats were at the age of 52 days which is within the sensitive age of DMBA mammary carcinogenesis [13]. Based on previous dose-effect experiments with DMBA in rats of the same strain and age [14], repeated oral administration of low doses (5 mg) of DMBA was chosen for the present experiments in order to induce mammary tumors in about 50% of rats within 3 months after DMBA application. All groups of rats received the first administration of DMBA (5 mg dissolved in sesame oil and administered by gastric intubation) at the age of 52 days and were then placed in the MF coils (MF-exposed groups) or dummy coils (sham-exposed groups) for exposure or sham exposure. Oral treatment with DMBA (5 mg) was repeated at weekly intervals up to a total of 4 applications per animal. Animals were exposed for 24 h/day over 91 days to a gradient AC (alternating current) sine wave (50 Hz) field of 0.3–1 μT. Sham-exposed rats were maintained in dummy coils in the same room. Rats were palpated weekly to assess the development of mammary tumors. The intake of food and water and the increase in body weight were controlled once to twice per week. At the end of the exposure period of 91 days, all rats were sacrificed, and the number and size of tumors was determined after necropsy as described below. Some rats died or had to be sacrificed before 91 days of exposure (see results) because of open and bleeding mammary tumors or marked weight loss and vaginal bleeding. These rats were processed through histopathological examination in the same way as all the other animals (see below).

Exposure System

The exposure system consisted of six identical cylindrical coils with an inner diameter of 0.4 m and a length of 0.66 m and six sham coils with the same dimensions. Exposure coils were built by Nicke Elektroapparatebau (Berlin, FRG) and had 207 turns of copper wire (9.5 × 5.5 mm) using three layers and PVC water pipes for cooling between the layers. A detailed description of the exposure system and the positioning of the MF coils and dummy coils in the room has been given elsewhere [14].

The MFs in the volume of the exposure coils were not homogeneous, but ranged from 0.3 to 1 μT. Several epidemiological studies of residential exposure have shown that exposed humans do not stay in a uniform MF but rather in nonuniform conditions as produced by our protocol. The position of the plexiglas cages in the coils and the gradient of the field are shown in figure 1. The MF in the exposure coils was calculated using Biot-Savart's law and was measured with an EMDEXC meter (Electric Field Measurements Co., West Stockbridge, MA, USA). The rats (both MF-exposed and controls) spent most of the time at the center of the cages, i.e. the area with the 1-μT field (fig. 1).

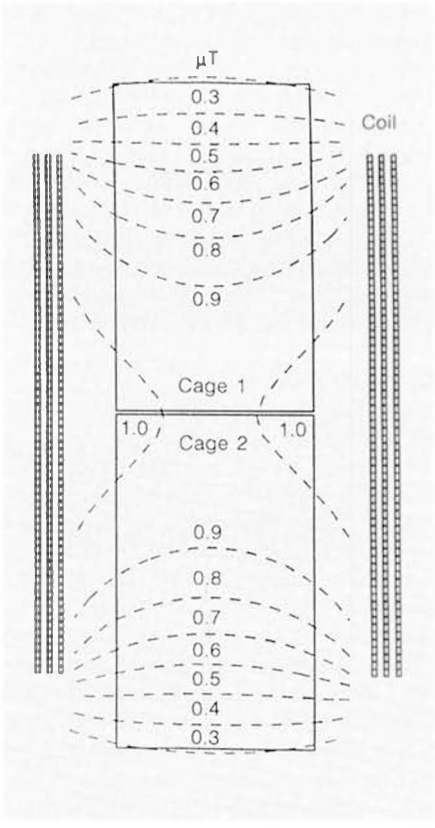

Fig. 1. Characteristics of the 50-Hz MF in the exposure coils. Two cages with 3 rats each were used per coil. MF values expressed in microteslas.

The ambient field for the controls (sham-exposed in the same room) varied between 0.02 and 0.04 μT over a 24-hour period as measured with the EMDEXC. The earth magnetic DC (direct current) field in the room with the exposure system was 42 μT (with vertical position to the generated AC field). This was determined with a Bell 610 Gaussmeter (F.W. Bell Inc., Orlando, FL, USA).

Melatonin Determinations

Serum levels of melatonin were determined 8–9 weeks after the first DMBA application in both sham- and MF-exposed animals. Blood (about 1–2 ml) was obtained by puncture of the retro-orbital plexus (after local anesthesia) either at 10.45–11.00 a.m. or 10.45–11.00 p.m. Each animal was only used for one blood sampling. Twelve rats of each group were used for nocturnal blood sampling and 24 animals of each group for morning sampling. Nocturnal sampling was carried out in dim red lighting to avoid any suppression of melatonin production. Light intensity in the room produced by the red light was between 0.5 and 0.8 lx (measured by a luxmeter) compared with 80–100 lx produced by the artificial white light in the same room at daytime. The rats were adapted for 2 weeks prior to blood sampling to the red light. Blood was stored for some hours at 4°C, then centrifuged, and the serum thus obtained was stored at −30°C until analysis. Serum melatonin was determined in a single-blind fashion by radioimmunoassay as described recently [15].

Histopathology

Thirteen weeks after the first DMBA application, the rats were sacrificed by an overdose of chloral hydrate and opened by a midline incision from the pubis to the submaxillary area. The skin of each half was dissected to expose the six mammary glands extending subcutaneously into the mammary fat pad, dorsolaterally to the medially located nipples. The presence of macroscopically visible tumors was recorded. Tumors with a size equal to a hazelnut or larger were cut by two vertical midline incisions for better fixation and subsequent microscopical determination of the diameter. Skin and tumor tissue were fixed by immersion in 4% phosphate-buffered formaldehyde (pH 7.3). The fixative was changed after 6 h. Skin of both sides with subcutaneous mammary glands was cut every 2 mm vertical to the surface and to the midline. These tissue samples and tumor quarters (see above) were dehydrated, embedded in paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin. Per animal, about 50 tissue samples were processed in this way. Tumors and serially sectioned mammary glands were examined microscopically, and neoplastic lesions were classified according to Russo et al. [16]. Hyperplastic alterations were classified according to Boorman et al. [17]. The diameter of each tumor was determined in the section plane of maximal tumor area standardized from the minimal and maximal diameter (since most tumors were oval).

The histopathological evaluation of mammary tissues from MF- and sham-exposed rats was done in a 'blind' fashion. In addition to mammary tumors and hyperplasias, all rats were examined macroscopically for other types of tumors induced by DMBA. Furthermore, the spleen and liver weights were recorded in all animals. With respect to mammary tumors, it has to be considered that spontaneous occurrence of these tumors in the strain and age of rats used for the present experiments is almost zero [13].

Statistical Evaluation

The statistical significance of differences in the incidence of tumors or hyperplasias was calculated by the χ^2 test. The significance of differences in tumor latency (median time to first appearance of palpable tumors in rats which developed palpable tumors), the number of tumors per tumor-bearing animal, the diameter of tumors, and the total tumor burden per animal with tumors was calculated by the Mann-Whitney U test. The signifciance of differences between melatonin levels was evaluated by one-way analysis of variance (Anova) followed by the Newman-Keuls test. Differences between body weight and weight of spleen and liver in sham and MF-exposed rats were statistically evaluated by Student's t-test. Since our initial hypothesis was that MF exposure would induce tumor-promoting effects, all statistical tests (except Anova and the Newman-Keuls test) were used one-sided.

Results

General Behavior, Mortality, and Body and Organ Weights

No differences in general behavior of sham- or MF-exposed rats were observed. The body weight of the MF-exposed rats at the end of exposure was not significantly different from that of sham-exposed controls. Furthermore, no significant differences were found in spleen and

Table 1. Latency and final incidence of hyperplasias and tumors, and tumor burden induced by DMBA in the mammary gland of MF and sham-exposed rats

	Sham-exposed controls (n = 36)	MF-exposed animals (n = 36)
Latency[1], days	75	64.5
Animals with hyperplasias	15	18
Total number of hyperplasias	29	27
Animals with tumors	22	24
Total number of tumors	95	77
Animals with hyperplasias and/or tumors	26	32
Tumor incidence, %	61	67
Incidence of hyperplasias and tumors[2]	72	89
Tumors per tumor-bearing animal	4.3 ± 0.83	3.2 ± 0.54
Mean diameter of tumors[3], cm	1.02 ± 0.061	0.98 ± 0.065
Mean diameter of tumors per tumor-bearing animal[3], cm	0.996 ± 0.084	1.09 ± 0.091
Cumulative tumor diameter per tumor-bearing animal[3], cm	3.02 ± 0.64	2.15 ± 0.34

Average data are means ± SE, except tumor latency which is shown as median. χ^2 was used to calculate significance of differences between incidences, whereas the Mann-Whitney U test was used for all other data. None of the data differed significantly between sham-exposed and MF-exposed rats.

[1] Median time between first DMBA administration and detection of palpable tumors.

[2] Incidence of hyperplasias and tumors was determined histologically (see table 2 for classification of hyperplasias and tumors).

[3] Tumor diameter was only measured in those tumors which were recorded at necropsy (n = 59 in controls, 46 in MF-exposed rats).

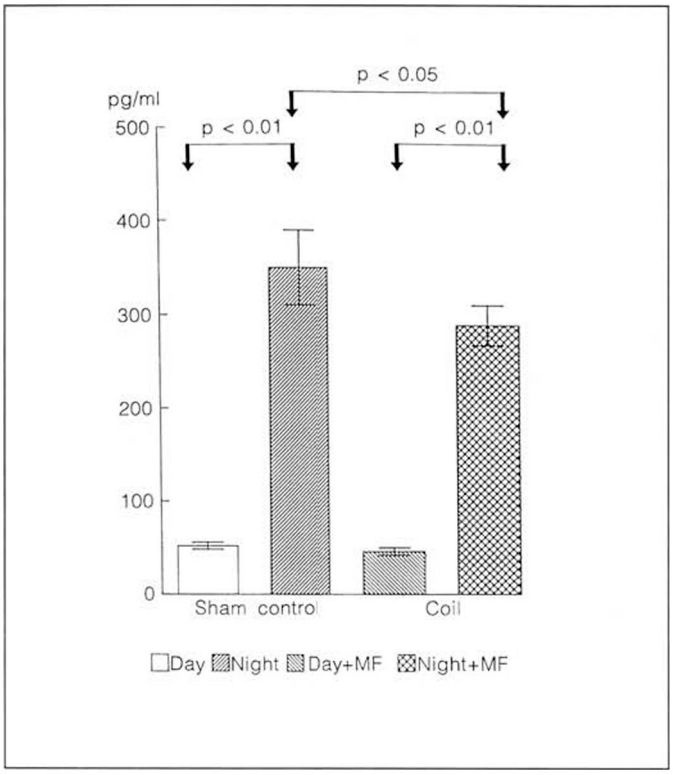

Fig. 2. Melatonin levels in serum 8–9 weeks after the first DMBA application. At the time of melatonin determinations shown in the figure, the MF group had been exposed at a gradient MF of 0.3–1 μT for 8–9 weeks. Melatonin levels were either determined between 10.45 and 11.00 a.m. ('day') or 10.45 and 11.00 p.m. ('night'). Data are means ± SE of 24 (day) or 12 (night) rats, respectively. Statistical evaluation of data by Anova indicated that means differed significantly ($F = 96.15$; $p < 0.01$). Results of subsequent data analysis by Newman-Keuls test are shown in the figure.

liver weights. In sham-exposed rats, the average weight of spleen and liver was 0.85 ± 0.07 and 10.9 ± 0.76 g compared with 0.92 ± 0.11 and 10.4 ± 0.53 g in MF-exposed rats. Three rats of the control group died before the end of the 91-day exposure period. Furthermore, 5 rats of the control group and 9 rats of the MF-exposed group had to be sacrificed some days before the end of the exposure period because of large bleeding mammary tumors or pronounced weight loss and vaginal bleeding. The histopathological alterations of these animals are included in the figures shown in table 1.

Serum Levels of Melatonin

The serum levels of melatonin at day and night are shown in figure 2. These levels were determined after 8–9 weeks of MF or sham exposure. As expected, nocturnal levels of melatonin were markedly higher than those measured at daytime. Whereas there was no difference between daytime levels of melatonin in MF- and sham-exposed rats, nocturnal melatonin levels were significantly lower in the MF-exposed group compared with sham-exposed controls.

Mammary Tumorigenesis

The first palpable mammary tumors were recorded 7 weeks after the first DMBA administration in 5 controls and 4 MF-exposed rats. In the weeks thereafter, the number of rats with palpable tumors increased rapidly. Twelve weeks after the first DMBA application, 15 controls and 20 MF-exposed rats had palpable tumors, indicating a reduced tumor latency in the MF-exposed group. Calculated tumor latencies were 64.5 days in the MF-exposed

Table 2. Classification of mammary gland tumors and hyperplasias induced by DMBA in sham and MF-exposed rats

	Sham-exposed controls (n = 36)	MF-exposed animals (n = 36)
Animals with lobular hyperplasias	14	15
Total number of lobular hyperplasias	19	20
Animals with atypical hyperplasias	6	5
Total number of atypical hyperplasias	10	7
Animals with benign tumors	5	4
Total number of benign tumors	5	4
Animals with carcinomas	22	23
Total number of carcinomas	90	73
Carcinomas per animal with carcinomas	4.09 ± 0.78	3.17 ± 0.52
Animals with noninvasive carcinomas	7	6
Total number of noninvasive carcinomas	12	8
Animals with invasive carcinomas	21	21
Total number of invasive carcinomas	78	65
Invasive carcinomas per animal with invasive carcinomas	3.71 ± 0.71	3.1 ± 0.58

Average data are means ± SE. χ^2 was used to calculate significance of differences between incidences, whereas the Mann-Whitney U test was used for all other data. None of the data differed significantly between sham- and MF-exposed rats.

rats and 75 days in controls, the difference being statistically nonsignificant (table 1).

At necropsy, i.e. 13 weeks after the first DMBA application, the only tumors which were recorded in the rats of both groups were mammary tumors. These tumors were macroscopically visible at necropsy in 21 animals of both groups. Histopathological examination of the mammary gland by serial sections of the whole mammary tissue revealed that the incidence of DMBA-induced mammary tumors was 61% in controls and 67% in MF-exposed rats (table 1). Furthermore, in about 40–50% of the animals, hyperplasias were found in addition to the tumors. The incidence of tumors *and* hyperplasias was 72% in controls but 89% in MF-exposed rats. Although this difference was not significant by the χ^2 test, the χ^2 value was 2.217, i.e. near the critical value (2.706) of the one-sided test. In contrast to tumor incidence, the total number of tumors was higher in the control group than in the MF-exposed group (table 1). Sham-exposed rats with tumors had an average of 4.3 tumors compared with 3.2 tumors per tumor-bearing rat in the MF-exposed group.

Tumor burden was examined by the mean diameter of those tumors which were excised at necropsy. No significant differences in the mean diameter of tumors were found between controls and MF-exposed animals (table 1).

The classification of neoplastic and preneoplastic lesions of the rat mammary gland is shown in table 2. Almost all tumors induced by DMBA in both groups were malignant epithelial neoplasms. The majority of these were invasive carcinomas. In the control group, the invasive carcinomas comprised 39 papillary, 27 cribriform and 12 tubular types, whereas the respective figures were 37, 14 and 14 in the MF-exposed group. Noninvasive carcinomas comprised 9 papillary and 3 cribriform types in the controls and 7 papillary and 1 cribriform types in the MF-exposed rats. The incidence of carcinomas or the number of carcinomas per rat with carcinomas was not significantly different between controls and MF-exposed rats (table 2).

Only few benign lesions were found (table 2). In controls, 1 tubular adenoma, 1 lactating adenoma and 3 fibroadenomas were recorded, whereas the MF-exposed group showed 3 lactating adenomas and 1 fibroadenoma.

With respect to hyperplasias, most were of the lobular type without cellular atypia (table 2). Atypical hyperplasias were of the intraductal type. Again, no significant differences were recorded in the incidence of lobular or atypical hyperplasias between sham- and MF-exposed animals.

Discussion

The present experiments did not disclose any significant tumor-promoting or copromoting effects of long-term MF exposure at flux densities similar to residential exposures arising from neighboring power lines. In order to assess factors that modulate chemical carcinogenesis in the mammary gland of the female rat, changes in tumor latency and incidence probably indicate the most powerful effects of modulators [18]. Similar to recent data from experiments with DMBA-induced skin tumors in mice [19, 20], the time to appearance of palpable mammary tumors was somewhat shorter (but not statistically so) in the group of rats exposed to MFs. Furthermore, the incidence of mammary tumors and hyperplasias tended to be higher in the MF-exposed group, again without statistical

significance. However, subtle effects of modulating factors in initiation, promotion and progression of tumors may be detectable only in examination of tumor burden or histology [18]. The present data on histological classification of mammary gland lesions and tumor burden did not indicate that MF exposure at low flux densities altered the spectrum, incidence or size of proliferative lesions. MF-exposed rats did not exhibit more malignant tumors than sham-exposed rats. Furthermore, the number of hyperplasias, which may be a precursor of adenoma, fibroadenoma or adenocarcinoma [17], did not differ between MF- and sham exposed rats.

One might object that the fields (0.3–1 μT) applied in the present study were too low to be of interest relative to human exposures, because we did not consider scaling factors in selecting the exposure field flux densities. Indeed, if biological effects of MFs are not caused by the MF per se but by an induced current, the rat-to-human scaling factor would be between 5 an 7, i.e. the human induced current equivalent of a 1 μT field in rats would be approximately 0.15–0.2 μT [21]. However, even this weak MF would be well in the range of elevated exposure by the epidemiological scale, especially when the reduced field exposure at night, which is the important time period for melatonin production, is considered [22]. However, the induced current model is a matter of dispute. The difficulty with this model is that the normal physiological level of induced currents is two orders of magnitude greater than those that can be calculated to result from usual ambient MF levels [cf. 1]. Furthermore, there is recent experimental evidence that the mammalian pineal gland can respond *directly* to alternating MFs, i.e. independent of induced currents [23]. A direct effect of weak alternating MFs on melatonin production is also indicated by the results of the present study. The mechanisms involved in this effect of MF exposure on melatonin production remain to be resolved in future studies.

Another criticism with respect to the experimental protocol used in the present study could be that MF exposure was initiated too late to constitute a reasonable test of the hypothesis that MF exposure effects cancer risk by reducing melatonin production. To fairly test the hypothesis, the MF should be administered prior to treatment with the chemical initiator (DMBA). However, our protocol used 4 separate injections of 5 mg DMBA at intervals of 1 week, in other words the rats were MF exposed for 3 weeks before the final DMBA administration. As shown recently [14], 1 single injection with 10 mg (or lower doses) of DMBA was almost ineffective to induce cancers in rats of the same strain and age as used in the present study, whereas the repeated injections of 5 mg at weekly intervals induced cancers in about 50% of the animals. Thus, by using this protocol, the animals were MF-exposed prior to the accumulating effects of the repeated DMBA administrations. In a recent study with the same experimental protocol [14], significant tumor-promoting effects were found during exposure to high (15–30 mT) AC or DC MFs, thus demonstrating that the protocol is capable of detecting such effects of MF exposure.

There have been theoretical considerations that extremely low frequency MF in the low microtesla range might induce stronger pathophysiological effects than fields with higher flux densities [11, 24]. Such 'window effects', i.e. ranges in which the system exhibits enhanced sensitivity, have been reported for flux density, frequency, and duration of MF exposure [24]. However, there is considerable controversy about the 'window effect' interpretation on data from experimental studies on MF exposure [24, 25]. Furthermore, there is no evidence that 'window effects' play a crucial role in tumor promotion by MF. Mevissen et al. [14], who exposed DMBA-treated female rats to DC or AC (50-Hz) MFs at intensities in the micro- to millitesla range, found significant mammary tumor-promoting effects of DC or AC MFs only at high (15–30 mT) flux densities. Furthermore, the present study, which is the first that used extensive histopathological evaluation to detect modulating effects of MF at flux densities similar to those reported to induce 'window effects' [11] did not disclose any tumor-promoting effects in a DMBA model of breast cancer.

Decrease of pineal melatonin production by AC electromagnetic fields has been implicated in the carcinogenesis of mammary tumors [3–6]. Melatonin secretion by the pineal gland exhibits a pronounced circadian rhythm with the highest levels occurring during the night [26]. Melatonin has been shown to suppress chemically induced mammary tumorigenesis in the rat [27, 28]. The exact mechanisms through which melatonin exerts its oncostatic effect are as yet unknown, but interactions with sex hormones, growth factors, lymphokines, cytokines, as well as with various signal transduction pathways, cytoskeletal elements and genomic components such as oncogenes might be involved [29]. Reduction or elimination of pineal melatonin production, by light exposure or pinealectomy, have been reported to encourage mammary tumorigenesis in DMBA-treated rats [5, 27]. Since melatonin secretion is also suppressed by exposure to 60-Hz electrical fields and DC or AC magnetic fields [7, 30], it has been argued that this action may possibly increase the potential for breast cancer [5, 6]. On the basis of these

considerations, it has recently been suggested that the higher risk of breast cancer in industrialized societies compared with nonindustrialized areas might be due, at least in part, to the use of electrical power accounts [6]. However, no reports have been published as yet which directly examined this hypothesis.

In the present experiments with DMBA-treated rats, a significant difference in nocturnal peak melatonin levels was found between MF- and sham-exposed animals. However, the magnitude of the difference was small (about 20%). Furthermore, the reduced melatonin levels in MF-exposed rats were not associated with marked alterations in DMBA-induced carcinogenesis. Nevertheless, the data demonstrate that 50-Hz MF at low flux densities in the range of elevated domestic MF exposures are capable of significantly reducing nocturnal melatonin levels. In a recent study by Kato et al. [31], a nocturnal melatonin reduction of similar magnitude was found in male rats subchronically exposed to a rotating 50-Hz MF at flux densities of 1, 5, 50 or 250 μT, while 0.1 or 0.02 μT were without significant effect on plasma and pineal melatonin levels.

With respect to the serum control levels of melatonin determined in the present study, it should be noted that these levels were higher than those reported by other groups [e.g., 30, 31]. This might be due to the fact that we used a retro-orbital puncture for blood sampling instead of decapitation as is commonly used for melatonin analysis in rat serum [32]. In this respect, one explanation for the differences in basal melatonin levels would be the different extent of stress associated with different procedures of blood sampling [33, 34]. Furthermore, it is conceivable that Harder's gland or other melatonin sources of the eye contributed to the serum levels determined in the present experiments. This interesting possibility will be investigated in separate experiments.

Other effects of MF that have been implicated as possible causes of tumor promotion are alterations in gene expression, disruption of calcium homeostasis, and suppression of immune system functions [1, 3, 19]. Most studies in this respect used high MF flux densities in the millitesla range so that the relevance of these findings is uncertain. For instance, McLean et al. [19] reported from a skin tumor model in mice that the spleen size and number of mononucleated spleen cells were significantly increased by prolonged exposure to a 60-Hz, 2-mT MF, possibly indicating development of leukemia due to suppression of the immune system, while no alterations in spleen size were found in the present study at environmentally relevant flux densities. However, in some industrial, scientific and medical applications, exposures to much stronger, both static and time-varying MFs occur [35]. Occupational exposure to such fields have been reported to increase the risk of cancers, such as breast cancer [36, 37]. Thus, tumor-promoting effects of DC or AC MF fields with high flux densities as recently reported from animal experiments [14, 20] might be relevant for risk assessment in humans.

In conclusion, the present study indicates that 50-Hz MF exposure at flux densities in the range of residential exposures does not induce significant tumor promoting or copromoting effects in a rat model of breast cancer. However, due to the restrictions of the exposure system, the sample number was relatively small so that slight differences in mammary carcinogenesis might have been missed. Furthermore, the strength of the carcinogenic (i.e., DMBA) stimulus, which far exceeds human exposure, may have masked subtle promoting or copromoting effects of MF exposure. Nevertheless, in comparison with previous studies [14, 19, 20], the present data do not indicate that 'window effects' play a crucial role in tumor promotion by MF exposure. Furthermore, modest changes in melatonin secretion in response to MF exposure are not associated with gross effects on DMBA mammary carcinogenesis.

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