

Do Cocarcinogenic Effects of ELF Electromagnetic Fields Require Repeated Long-Term Interaction With Carcinogens? Characteristics of Positive Studies Using the DMBA Breast Cancer Model in Rats

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The carcinogenic or cocarcinogenic potential of extremely low frequency (ELF; 50 or 60 Hz) magnetic fields (MFs) has been evaluated worldwide in diverse animal model systems. Though most results have been negative, weakly positive or equivocal results have been reported in several cancer models, including the rat DMBA (7,12-dimethylbenz[*a*]anthracene) model of mammary cancer. Based on the experimental conditions used in studies in which cocarcinogenic effects of ELF MF were found, it was recently proposed that MF exposure may potentiate the effects of known carcinogens only when the animals are exposed to both MF and carcinogen during an extended period of tumor development, i.e., when the carcinogen is given repeatedly during MF exposure. This review summarizes a series of experiments from our group, showing cocarcinogenic MF effects in the DMBA breast cancer model in rats, to test whether the above proposal is confirmed by existing data. Flux densities of 50 or 100 μ T significantly increased the growth of mammary tumors, independent of whether DMBA was given in a single administration or repeatedly over a prolonged period. Thus, these data do not substantiate the hypothesis requiring repeated doses of DMBA during MF exposure. Instead, several other aspects of study design and experimental factors are identified that seem to be critical for the detection of cocarcinogenic effects of MF exposure in the rat DMBA mammary cancer model. These include the rat subline used, the dose of DMBA, the duration of MF exposure, the flux density, the background (sham control) tumor incidence, and the location of mammary tumors in the mammary gland complex. These and other experimental aspects may explain why some laboratories did not detect cocarcinogenic MF effects in the DMBA model. We hope that direct comparison of MF bioeffects in different rat sublines and further evaluation of other experimental differences between studies on MF exposure in the DMBA model will eventually determine which genetic and environmental factors are critical for potential carcinogenic or cocarcinogenic effects of ELF MF exposure. Bioelectromagnetics 22:603–614, 2001. © 2001 Wiley-Liss, Inc.

Key words: magnetic fields; carcinogenesis; mammary cancer; melatonin hypothesis

INTRODUCTION

The generation, transmission, and use of electric energy is associated with the production of weak electromagnetic fields (EMFs) of extremely low frequency (ELF; 50 or 60 Hz). There is considerable debate about whether exposure to ELF EMFs, particularly the magnetic field (MF), is a risk factor for cancer [Portier and Wolfe, 1998]. Interest in this question has been triggered primarily by epidemiological studies that have suggested an association between 50 or 60 Hz MF exposure and increased risk of childhood leukemia and other types of cancer [Bates, 1991; Savitz and Ahlbom,

Contract grant sponsor: Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (Bonn, Germany); Contract grant number: StSch. 4095; Contract grant sponsor: U.S. Department of Energy, Office of Utility Technologies, through OAK Ridge National Laboratory (TN); Contract grant number: 19X-SU446; Contract grant sponsor: Deutsche Forschungsgemeinschaft (Bonn, Germany); Contract grant number: 027416-1.

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Received for review 5 May 2000; Final revision received 27 November 2000

1994; Portier and Wolfe, 1998]. Because direct DNA damage leading to mutations is generally not believed to result from ELF MF exposure [McCann et al., 1993, 1998; Murphy et al., 1993], the increased cancer risk observed during occupational or residential ELF EMF exposures, if causally related to MF, is thought to be the consequence of a cocarcinogenic or cancer promoting MF action [Löscher and Liburdy, 1998].

Because all known human carcinogens, including cocarcinogenic and cancer promoting agents, are also carcinogenic in experimental animals [Huff, 1993; Vainio and Wilbourn, 1993], various ELF MF cancer studies have been performed in animals [Holmberg, 1995; McCann et al., 1997; Löscher and Liburdy, 1998; Portier and Wolfe, 1998]. Long-term bioassays in rodents demonstrated no unequivocal carcinogenic response [Portier and Wolfe, 1998]. Similarly, experimental models of multistage carcinogenesis failed to provide convincing evidence for a promoting effect of MF on chemically induced cancers [McCann et al., 1997; Löscher and Liburdy, 1998; Portier and Wolfe, 1998]. However, when the multistep mammary cancer DMBA (7,12-dimethylbenz[*a*]anthracene) model in rats was used with a different approach than that used traditionally (i.e., repeated administrations of DMBA over a period of 4 weeks versus one administration of DMBA), our group previously found consistent and dose-dependent cocarcinogenic effects of ELF MF exposure [for review see Löscher and Mevissen, 1997, and Löscher, 2001], indicating that alternative approaches are needed for testing the possible cocarcinogenic effects of MF.

Based on the conditions used in experimental studies in which cocarcinogenic effects of ELF MF were found, Juutilainen et al. [2000] recently proposed that MF exposure may potentiate the effects of known carcinogens only when the effect of MF exposure and the known carcinogen interact repeatedly during a long-term experiment, i.e., when the carcinogen treatment is given as several small doses over a longer period of time. This, of course, would be relevant to human exposure, because exposures of humans to known carcinogens are typically long-term [Huff, 1993; Vainio and Wilbourn, 1993]. In the present paper, we summarize our previous series of experiments in the DMBA breast cancer model in rats to test whether the proposal of Juutilainen et al. [2000] is confirmed by existing data. Furthermore, the data from the Hannover experiments were used to identify other aspects of study design or model parameters which may affect the outcome. For comparison with the Hannover studies, data from other groups using chemical breast cancer models for MF exposure will be briefly discussed.

Hannover Studies of Possible Cocarcinogenic Effects of ELF MF

In the following, the data from six MF experiments (50-Hz, horizontally polarized) with flux densities ranging from 0.3–1 to 100 μT will be shortly summarized. The experiments are presented in order of increasing flux densities, not dates. Flux densities and exposure duration were as follows: *Experiment 1*, 0.3–1 μT (gradient field) for 13 weeks [Mevissen et al., 1993; Löscher et al., 1994]; *Experiment 2*, 10 μT for 13 weeks [Mevissen et al., 1996a]; *Experiment 3*, 50 μT for 13 weeks [Mevissen et al., 1996b]; *Experiment 4*, 100 μT for 13 weeks [Löscher et al., 1993; Baum et al., 1995]; *Experiment 5*, a replication study of Experiment 4, again using 100 μT for 13 weeks [Mevissen et al., 1998b]; *Experiment 6*, 100 μT for 27 weeks [Thun-Battersby et al., 1999]. In addition, we also studied much higher flux densities (30 mT, 50 Hz; 15 mT, d.c.), but sample sizes were small (18–36 rats per group), so that these experiments [Mevissen et al., 1993] are not discussed in any detail here (but see data for 13 weeks exposure at 30 mT, 50 Hz in Fig. 1).

The experimental procedures used in experiments 1–6 are summarized in Tables 1 and 2. Except for Experiment 1, the same exposure system was used for all experiments (for detailed description of exposure systems and experimental procedures see original publications). Two different DMBA application

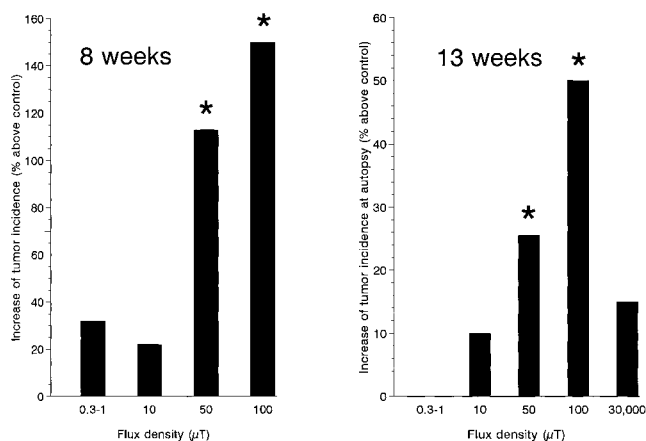


Fig. 1. Comparison of the data from MF experiments 1–4 of the Hannover series. For comparison, data are also shown from an experiment with a flux density of 30 mT [from Mevissen et al., 1993]. Two sets of data are shown. On the left side of the figure, data from palpation of mammary tumors at 8 weeks of exposure are illustrated, while the right part of the figure shows data from necropsy, i.e., incidence of grossly recorded mammary tumors after 13 weeks of exposure. Data are based on a total of 369 DMBA-treated sham-exposed controls and 366 DMBA-treated MF-exposed rats. (**P* at least < 0.05).

TABLE 1. Experimental Parameters Utilized in the Hanover Studies

Experimental animals (rats)	
Stock	Sprague-Dawley
Sex	Female
Source	Charles River, Exertal, Germany
Body weight at onset of exposure	170–180 g
Housing/maintenance	
Acclimation period	6–10 days
Rats/cage (n)	9–10*
Case size (cm)	59 × 39 × 22*
Diet	Altromin 1324
Bedding	Corn cob
Room temperature	23–24 °C
Humidity	Approximately 50%
Lights (fluorescent)	
Cycle (day/night)	12/12
Intensity	30–38 lux
Red light intensity (at night)	< 0.1 lux
DMBA treatment	
Source/purity	Sigma (95%)
Age of rats at first DMBA treatment	52 ± 2 days
Frequency/dose of DMBA (13-week studies)	4 × 5 mg/rat
Frequency/dose of DMBA (27-week studies)	1 × 10 mg/rat
Magnetic field exposure	
Frequency	50 Hz
Polarity	Horizontal
Magnetic field intensity	0.3–1, 10, 50, and 100 μT rms
Days/week	7
Hours/day	23–24
Time of exposure	Continuous
Study length	13/27 weeks

*Except Experiment 1 in which rats/cage were three and case size was 42 × 26 × 15 cm.

protocols were used: In the first protocol (experiments 1–5), rats were administered 20 mg DMBA in four weekly gavage doses of 5 mg/rat. MF exposure was started at the time of the first DMBA application. In the second protocol (Experiment 6), rats were treated once with 10 mg DMBA by gavage; MF exposure was started 1 week before DMBA application. The scientists and technicians involved in handling and treatment of animals and subsequent necropsy and pathological examination of rats were not aware of which group of animals was exposed or sham-exposed, to ensure “blind” conditions until all results were in.

The statistics used for evaluation of MF effects are described in detail in the original publications. Except for tumor incidence calculations, all statistical tests were used as two-sided tests; $P < 0.05$ was considered significant. Tests for tumor incidence calculations were one-sided, because our hypothesis was that MF exposure would increase tumor incidence. Because we have shown previously that the season affects the sensitivity of the mammary gland to DMBA [Löscher et al., 1997], explaining the variation in tumor incidences in sham controls between different experiments performed during different seasons, each

MF exposed groups was compared to the concurrent sham control group.

A summary presentation of data from the 6 experiments is given in Table 2. Tumor latency was only calculated in some experiments. The last row of Table 2 indicates whether a complete histopathology of all grossly recorded tumors was done. In experiments without complete histopathology (experiments 2, 3, and 5), only small tumors were microscopically examined to confirm the diagnosis (most mammary tumors were so large that they could be diagnosed undoubtedly without histology). “Serial sections” in the last row of Table 2 means that, in addition to histopathology of all grossly recorded tumors, complete mammary complexes of each rat were serially sectioned to find lesions which are too small to be grossly recorded, i.e., seen without a microscope.

With respect to grossly recorded tumors at time of necropsy, a significant increase in *tumor incidence* was seen after exposure with 50 μT (Experiment 3) and 100 μT (Experiments 4–6), irrespective of whether DMBA was given repeatedly or once (Table 2). The data on grossly recorded tumors of experiments 1–4 were used by Löscher and Mevissen [1995] to

TABLE 2. Summary Presentation of Data From the Six MF/DMBA Experiments of the Hanover Series

	1		2		3		4		5		6	
	Sham	MF	Sham	MF	Sham	MF	Sham	MF	Sham	MF	Sham	MF
Flux density [μT]		0.3–1		10		50		100		100		100
Experimental period												
Initiation:	23–25 July 1991		5–9 Apr 1993		4–8 April 1994		14–18 Sep 1992		12–16 Feb 1996		12–16 May 1997	
Termination:	21–23 Oct 1991		5–9 July 1993		4–8 July 1994		14–18 Dec 1992		13–17 May 1996		17–21 Nov 1997	
Duration of exposure	13 weeks		13 weeks		13 weeks		13 weeks		13 weeks		27 weeks (total); 26 weeks following DMBA	
Total dose of DMBA	20 mg		20 mg		20 mg		20 mg		20 mg		20 mg	
Dose regime	4 × 5 mg (weekly)		4 × 5 mg (weekly)		4 × 5 mg (weekly)		4 × 5 mg (weekly)		4 × 5 mg (weekly)		1 × 10 mg	
Group size	36		99		99		99		99		99	
No. of rats with tumors												
(palpated)	21	21	55	60	51	55	34	51*	60	77*	46	56
No. of rats with macroscopic tumors ^c	21	21	60	66	55	69*	34	51*	61	82*	50	64*
Total no. of tumors ^c	60	47	147	168	139	193	53	79	230	297	116	166
Tumor incidence % ^c	58	58	60.6	66.6	55.6	69.7*	34.3	51.5*	61.6	82.8*	50.5	64.7*
Tumor latency ^c (1st tumor) days	75	65	—	—	—	—	—	—	—	—	131 ± 32	125 ± 42
No. of tumors/tumor bearing rat ^c	2.9 ± 0.45	2.2 ± 0.3	2.5 ± 1.99	2.6 ± 1.89	2.53 ± 1.9	2.7 ± 2.0	1.8 ± 1.55	1.6 ± 0.98	3.8 ± 2.75	3.7 ± 3.13	2.3 ± 1.8	2.6 ± 2.2
Tumor volume/size ^c (autopsy) mm ³	4.0 g ^a (0.5–22)	4.0 g ^a (0.5–15.5)	817 (209–2587) ^b	910 (189–3098) ^b	656 (253–2310) ^b	518 (170–1602) ^b	367 (101–1178) ^b	733* (183–2994) ^b	203 (64–1024) ^b	189 (58–902) ^b	105 (26–361) ^b	118 (32–566) ^b
Complete histopathology	Yes (serial sections)	Yes (serial sections)	No	No	No	No	Yes (serial sections)	Yes (serial sections)	No	No	No	Yes

For further experimental details see text.

^aSignificantly different from sham controls (P at least < 0.05).

^bMedian and range of tumor weight [g].

^cMedian and 25%/75% quantile.

^dGrossly recorded tumors.

determine whether flux density and effect on tumor growth are related, indicating a highly significant linear relation between flux density and effect (see also Fig. 1, data for 13 weeks). However, it should be noted that a flux density in the mT-range failed to cause significant effects in the DMBA model (Fig. 1), indicating the existence of a *flux density window* in the μ T-range within which MF exposure acts to affect tumor growth in this model. It is interesting to note that during MF exposure, e.g., after 8 weeks as illustrated in Fig. 1, percent increases in the incidence of (palpable) tumors in response to MF were more marked than at the end of MF exposure, i.e., after 13 weeks (Fig. 1), indicating that MF exposure enhances *tumor growth* rather than *tumor incidence*.

This is supported by the data of Experiment 4. In this experiment, the incidence of grossly recorded (and histologically confirmed) tumors was 50% higher in MF exposed rats compared to controls (Table 2). Furthermore, the size of these tumors was increased by MF exposure (Table 2), while tumor multiplicity was not affected. In Experiment 4, a complete histopathology of the mammary gland with serial sections was done [Baum et al., 1995]. The number of lesions thus detected was considerably higher than the number of grossly recorded tumors (Fig. 2), because many tumors were so small that they only could be detected at the microscopic level. Tumor incidence based on these serial sections was 65.7% in MF and 57.6% in sham exposed rats, the difference being not significant (Fig. 2). However, significantly more MF exposed

than sham exposed rats had malignant adenocarcinomas [Baum et al., 1995].

The finding that MF exposure significantly increased the incidence of *grossly recorded* mammary tumors, but not the incidence of *all* mammary tumors, including the microscopic ones only detected by serial sections (Fig. 2) is important, because it strongly supports the notion that MF exposure increased tumor growth but not tumor incidence during exposure. In other words, the number of tumors which can be grossly recorded and must have a certain size to be detectable at time of necropsy was higher in MF exposed rats, whereas the total number of tumors was not affected. Such an effect can only be detected when the dose of DMBA and the exposure period after DMBA is submaximal, allowing a second agent (in this case MF) to increase the effect of DMBA. Thus, the experimental protocol and the sensitivity of the rats to DMBA will determine whether an effect of MF exposure on tumor growth can be detected. This is important when comparing the results from the Hannover experiments with those of other groups (see below). In contrast to the experiment with 100 μ T (Experiment 4), no difference between MF effects on grossly versus microscopically recorded tumors was seen in the experiment with 0.3–1 μ T (Fig. 2), substantiating the dose-dependence of MF effects on tumor growth.

The fact that the percentage of rats with palpable and macroscopically visible mammary tumors in the sham-exposed group in our first study on 100 μ T MF exposure in the DMBA model (Experiment 4) was well below the baseline of 50–60% obtained by us in other experiments (see Table 2), whereas the MF-exposed group of Experiment 4 appeared to be within the “normal range,” was previously used as an argument to criticize our conclusions on potential carcinogenic effects of MF exposure. It is therefore important that the data of Experiment 5, a replicate of Experiment 4 carried out during another season (Table 2), demonstrated that a significant effect of MF exposure of similar magnitude is obtained under our experimental protocol when the concurrent sham control group is within the “normal” range of 50–60%. Indeed, the percentage of animals with grossly recorded mammary tumors in the MF exposed group of Experiment 5 (82.8%) was significantly above sham control values of experiments 1–4, indicating that the difference from control was not just due to accidental variation of tumor incidence between groups during the period of the experiment. In fact, in all of our previous experiments with MF exposures in the flux density range of 10–100 μ T tumor, incidence in MF-exposed groups was above that for the concurrent sham control

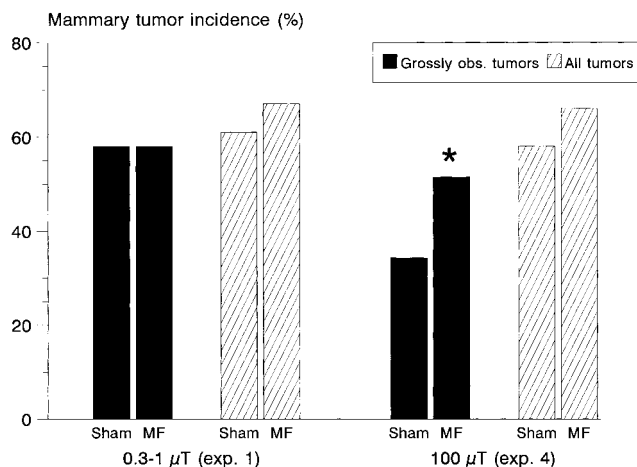


Fig. 2. Comparison of the two experiments (#1 and #4) in which a complete histopathology with serial sections of the mammary glands was performed. In the experiment with 100 μ T, EMF exposure significantly enhanced the incidence of grossly recorded tumors (* $P = 0.0107$, $n = 99$ animals) but not the incidence of all tumors. In the experiment with 0.3–1 μ T, no significant effects on tumor incidence or growth were seen ($n = 36$).

(Fig. 1). The only study in which no increase above concurrent control was seen after 13 weeks of exposure was the experiment using 0.3–1 μ T (Fig. 1).

Experiment 6 (27 weeks MF exposure at 100 μ T) differed in several aspects from experiments 1–5. First, it used a more conventional DMBA dosing protocol, i.e., one intragastric dosing with 10 mg per rat instead the 4×5 mg used in the previous experiments. Second, in an attempt to enhance the potential of MF exposure to interact with DMBA induced tumorigenesis, rats were MF exposed for 1 week prior to DMBA application. Previous experiments had shown that MF increases ornithine decarboxylase [ODC] in the mammary gland after 1 week of exposure, indicating enhanced cell proliferation [Mevisen et al., 1999], thus possibly increasing the sensitivity of the mammary gland to DMBA. Third, the exposure period was increased to 26 weeks after DMBA application. Fourth, in addition to evaluating the total number of mammary tumors per animal, we examined whether MF exposure differentially affects the carcinogenic response of glands in different topographic areas. This was prompted by the known differences in susceptibility of different parts of the rat mammary complex to the carcinogenic effect of DMBA [Russo and Russo, 1996] and the recent observation that MF exposure of female rats increases ODC primarily in the thoracic glands [Mevisen et al., 1999].

In Experiment 6, the incidence of grossly recorded tumors was roughly increased by 30% after 27 weeks of MF exposure, which was significantly different from controls (Table 2, Fig. 3). However, 13 weeks after DMBA application, i.e., the exposure period used in the previous experiments, tumor incidence in the MF group was 190% higher than in the sham group ($P = 0.003$; Fig. 3). This suggests that the maximal effect of MF exposure had taken place midway through the 27 week exposure period, resulting in an almost threefold increase in tumor incidence after 14 weeks, but that part of this effect was lost during subsequent exposure due to tumor growth in controls. In other words, MF accelerated tumor growth.

Compared to the significant 30% increase in the incidence of histologically verified mammary gland tumors observed grossly in female Sprague–Dawley (SD) rats after 27 weeks of MF exposure (Fig. 3), previous experiments of our group with 20 mg DMBA per rat (four weekly gavage doses of 5 mg) and 13 weeks of MF exposure at 100 μ T yielded similar differences to concurrent sham control of 50% (Experiment 4) and 34% (Experiment 5). Thus, the protocol of experiment 6 with one 10 mg DMBA application, MF exposure for 1 week prior to DMBA

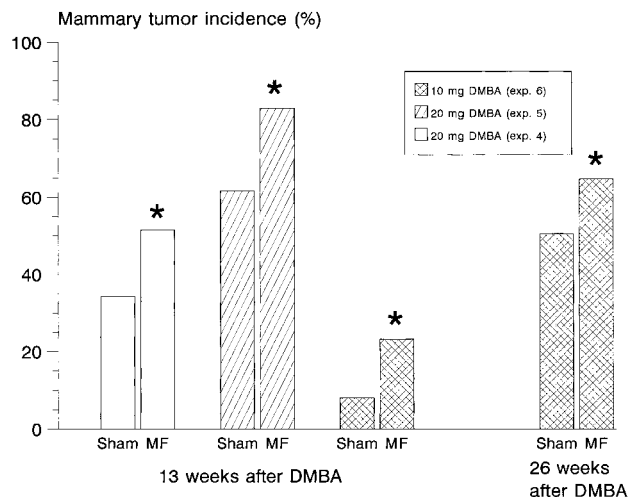


Fig. 3. Comparison of MF effects on incidence of grossly recorded tumors in the three experiments (# 4–6) of the Hannover series with 100 μ T. Data from 13 weeks after first DMBA application of experiments 4 and 5 are from grossly recorded tumors at time of necropsy, while data from 13 weeks after DMBA application of experiment 6 are from palpation of tumors, which were later verified histologically. Data from 26 weeks after DMBA of experiment 6 are from grossly recorded tumors at time of necropsy. (* $P < 0.05$, each $n = 99$ rats).

application, and prolongation of MF exposure to 27 weeks apparently did not increase the effect of MF exposure on breast cancer development and growth, but rather led to a similar effect as obtained with 20 mg DMBA but only 13 weeks of exposure, indicating that the magnitude of the MF effect depends on both DMBA dose and duration of exposure (Fig. 3). However, if Experiment 6 had been terminated 13 weeks after DMBA (14 weeks after initiation of MF exposure) as in the previous experiments, tumor incidence (based on palpation of subsequently verified mammary tumors) would have been 23.2% in MF exposed compared to 8.1% in sham exposed rats, indicating that tumor incidence in MF exposed rats was increased threefold (Fig. 3).

Since tumor incidence in sham controls 13 weeks after application of 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 same duration of exposure depends on the background (basal, control) tumor incidence in this model, i.e., the lower the control (background) tumor incidence the higher the effect of MF exposure on tumor growth. Indeed, a correlation analysis of background (sham control) tumor incidence versus MF effect on tumor incidence gave a correlation coefficient (r) of -0.904 , substantiating an inverse relationship between control incidence and the magnitude of the MF effect on tumor incidence 13 weeks after DMBA application [see also Thun-Battersby et al., 1999].

Another interesting finding of Experiment 6 was related to the location of mammary tumors in that MF exposure affected the development of mammary tumors unequally across the six mammary complexes of the female rat [Thun-Battersby et al., 1999]. The most pronounced MF effect on tumor incidence was seen in the cranial thoracic complex (L/R 1; Fig. 4). This prompted us to reexamine one of our previous studies with 100 μ T exposure (Experiment 4) resulting in a similar enhanced susceptibility of L/R1 to increased tumor growth in response to MF exposure as in Experiment 6. Thus, at necropsy (i.e., after 13 weeks

of MF exposure), tumor incidence in L/R1 of Experiment 4 was 36.4% in sham controls but 50.5% in MF exposed rats ($P < 0.05$; unpublished data). This demonstrates that the enhanced tumor growth in L/R 1 observed in response to MF in Experiment 6 (Fig. 4) also occurred in other experiments with 100 μ T performed in our laboratory.

It has been previously described that not all the rat 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 [Torgersen, 1975; Russo et al., 1977; Russo and Russo, 1996]. This was also found in Experiment 6, in which more than 70% of all grossly recorded tumors were found in the three thoracic complexes [Thun-Battersby et al., 1999]. Similar observations were made in our other experiments. This different carcinogenic response is thought to be 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 (TEBs), which are the sites of origin of mammary carcinomas [Russo and Russo, 1996]. We have recently found that the cranial thoracic mammary complexes (L/R1) are particularly sensitive to 50 Hz MF exposure at 100 μ T in terms of ODC increase [Mevisen et al., 1999], which might explain the higher susceptibility of these complexes to cocarcinogenic or tumor-promoting effects of MF exposure seen in our experiments. These data thus strongly indicate that not only the basal (control) tumor incidence (see above) but also the site of origin of mammary carcinoma determine to what extent MF exposure increases mammary tumorigenesis in the DMBA model. This could also be important for human studies, because mammary tumors are known to not occur homogeneously across the breast tissue of women.

In conclusion, the data of our experiments in the DMBA model of breast cancer in SD rats clearly indicate that—at least under the experimental conditions of our studies—50 Hz MF exposure in the μ T range significantly enhances mammary tumor growth. At time of necropsy, the effect of MF exposure on the incidence of grossly recorded mammary tumors was not marked. However, it was much more pronounced at earlier time points of exposure (e.g., compare 8 weeks and 13 weeks of exposure in Fig. 1), when only few tumors could be palpated in controls, indicating that the effect of MF exposure on tumor growth is particularly high at early phases of carcinogenesis in the DMBA model. An inverse relationship between the effect of MF on tumor growth and the background (control) tumor incidence was seen when different

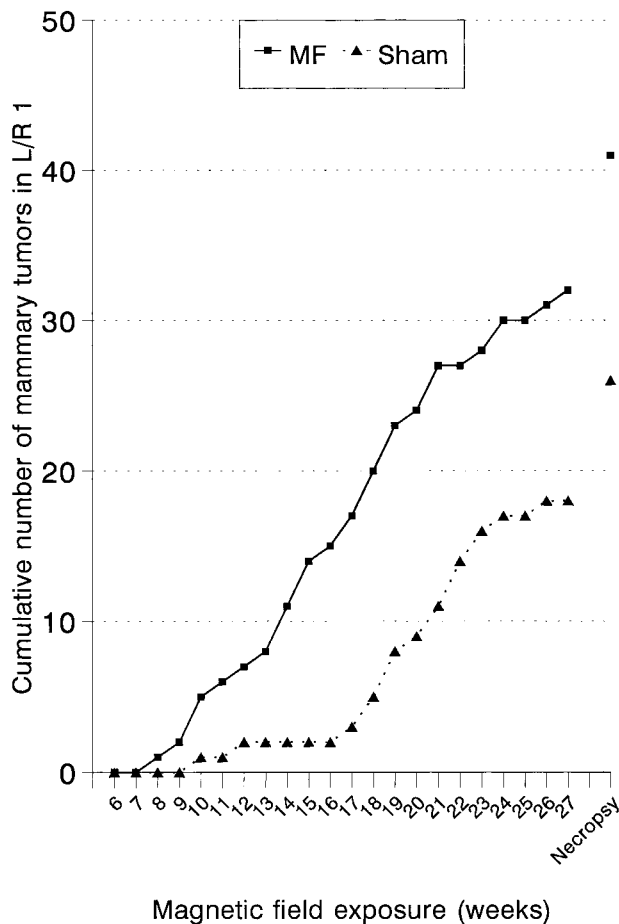


Fig. 4. Cumulative proportion of rats with mammary tumors in the cranial thoracic complexes (L/R1) as a function of duration of MF exposure (incidence curves). DMBA was administered per-orally at 10 mg/rat after 1 week of MF exposure ($n = 99$ rats). In addition to the data from palpation (weeks 6–27), the percentage of rats with macroscopically visible (and histologically verified) mammary tumors in L/R1 at necropsy (i.e., after 27 weeks of exposure) is shown. With respect to the tumors palpated before necropsy, only neoplasms which were subsequently histologically verified as mammary tumors are shown. Tumor incidences in the MF group were significantly higher ($P < 0.05$) at all time points except weeks 6–12.

experiments with 100 μT were compared, indicating that a high basal tumor growth counteracts any growth-promoting effect of MF exposure. At least in part, this may explain that experiments of two other groups failed to find any significant effects of MF exposure in the DMBA model (see below).

Studies by Other Groups

Three other groups have published MF data from chemical breast cancer models in peer-reviewed journals. A group from the Oncology Research Center in Tbilisi, Republic of Georgia, used the "complete" carcinogen nitrosomethylurea (NMU) to produce mammary tumors in female rats [Beniashvili et al., 1991]. MF exposure started 2 days after NMU administration. Groups of 50 rats were exposed at a 50 Hz, 20 μT field for 0.5 or 3 h daily over a period of up to 2 years after the carcinogen injection. The 3 h daily MF exposure significantly increased mammary tumor incidence and progression. These data were the first to indicate that MF exposure at relatively low flux density facilitates cancer development in a mammary tumor model.

More recently, Ekström et al. [1998] reported that intermittent 50 Hz MF exposure at flux densities of 250 or 500 μT with a 15 s on/15 s off schedule for 21 weeks did not significantly affect mammary tumor growth in response to intragastric application of 7 mg DMBA per SD rat. However, the MF exposure scheme used by Ekström et al. [1998] differed from that used in our experiments and was applied in a strict promotional scheme, i.e., MF exposure was started 1 week after DMBA administration. Furthermore, the subline of SD outbred rats used by Ekström et al. [1998] was much more sensitive to DMBA than the SD rats used in the Hannover studies, pointing to genetic differences between the two outbred sublimes of SD rats used.

Our data prompted the U.S. National Toxicology Program (NTP) to initiate a replication attempt, which was conducted by Anderson and colleagues at Battelle [NTP TR 498, 1998]. Data from these studies were recently published [Anderson et al., 1999; Boorman et al., 1999]. In the Battelle experiments, different DMBA dosing protocols were used, four times 5 mg/rat and 13 weeks exposure, four times 2 mg/rat and 13 weeks of exposure, and once 10 mg DMBA/rat and 26 weeks of exposure. Exposure conditions were 100 or 500 μT at 50 or 60 Hz. In none of the experiments were significant MF effects observed.

Unfortunately, although the studies were an attempt to replicate our previous MF studies using the DMBA model, there were various differences from our experiments, including another diet, shorter exposure per day (500 h less total exposure over 13

weeks), use of different rooms for sham and MF exposure, differences in the exposure systems, and use of a subline of SD rats with markedly higher susceptibility to DMBA than our rats. Because of this higher sensitivity, two of the three DMBA protocols used in the Battelle study resulted in almost 100% tumor incidence in sham controls, thus not allowing any additional effect of MF exposure. Thus, because of these various differences [for a detailed discussion see Anderson et al., 2000], these experiments cannot be considered as replicate studies of the Hannover experiments.

It has previously been demonstrated that there are inherent differences between SD rats obtained in the U.S. and SD rats obtained in Europe in regard to their mammary neoplastic response to DMBA, as well as in their response to radiation [van Zwieten et al., 1984]. The use of different rat sublimes and other experimental differences between studies on MF exposure in the DMBA model may eventually allow evaluation of the environmental and genetic factors that are critical for effects of MF exposure in this model. In this respect, it is important to note that there is an ongoing cooperation between the Hannover and Battelle groups aimed at explaining the apparent discrepancies between their experiments [Anderson et al., 2000].

Conclusion: Cocarcinogenic Effects of ELF MF do not Require Repeated Long-Term Interaction With Carcinogens

Our data summarized above can be used to address the interesting recent hypothesis of Juutilainen et al. [2000]. Using the term "cocarcinogenic" to describe all possible types of tumor-enhancing effects of ELF MF, Juutilainen et al. [2000] proposed that such cocarcinogenic effects of ELF MF exposure only occur when the effects of MF exposure and the chemical carcinogen interact repeatedly during a long-term experiment, such as during our previous studies with DMBA, in which the carcinogen was repeatedly administered (4×5 mg/rat over 4 weeks) during MF exposure. However, our recent experiment (# 6) with one application of DMBA clearly argues against this proposal (Fig. 3). Instead, as discussed above, the background (control) tumor incidence appears to be much more important in determining the magnitude of cocarcinogenic effects of MF. Furthermore, the genetic background of the exposed animal seems to be critically involved in MF effects on cancer development and growth.

Possible Mechanisms of MF Effects on Carcinogenesis

Using the MF-sensitive subline of SD rats which we used in the DMBA mammary cancer studies

summarized in this paper, we examined various potential mechanisms of MF effects on carcinogenesis. Our studies from the DMBA mammary cancer model provided the first direct experimental evidence that the melatonin hypothesis originally proposed by Stevens [1987] for chronic 60 Hz electric fields may explain cocarcinogenic effects of 50/60 Hz MF, particularly facilitation of development and growth of breast cancer. One premise of this hypothesis is that chronic exposure to ELF MF suppresses the normal nocturnal synthesis of melatonin in the pineal gland, which in turn results in increased production of estrogen and prolactin and thereby induces increased turnover of the breast epithelial stem cells at risk for malignant transformation [Fig. 5; Stevens, 1987; Stevens et al., 1992, 1997]. With respect to this hypothesis, however, one should note that we failed to demonstrate a significant reduction of pineal or plasma melatonin in response to MF exposure in several of our studies, including those demonstrating a significant effect of MF exposure on mammary carcinogenesis [Mevisen et al., 1996b, 1998b; Löscher et al., 1998]. Thus, other explanations than mere decrease of melatonin *concentrations* are needed to explain our findings in the DMBA model.

One very likely explanation stems from the work of Liburdy et al. [1993] showing that MF exposure in the μT range inhibits the oncostatic effect of melatonin on breast cancer growth *in vitro*, a finding that has been replicated by several other laboratories [cf., Löscher and Liburdy, 1998]. If interaction between MF and melatonin at the cellular level also occurs *in vivo*, it would add to any impairment of melatonin production so that even small decreases in circulating melatonin levels could have pronounced consequences. Such an

effect of MF exposure on melatonin level and function could not only increase breast cancer growth but also other types of cancer [cf., Blask, 1993]. The data of Liburdy et al. [1993] seem to indicate that a decrease in cellular *function* rather than concentration of melatonin may be critically involved in the MF induced breast cancer growth in the DMBA model. This modified melatonin hypothesis is illustrated in Figure 5. In addition to MF effects on melatonin concentration or function, other actions of MF exposure may be involved in increased breast cancer formation, such as direct effects on Ca^{2+} mobilization, cell proliferation, immune surveillance, or oncogene function [cf., Löscher and Liburdy, 1998].

To directly address these possible mechanisms, we have performed various experiments using our MF-sensitive SD rat substrain. Consistent with an *in vivo* effect of MF exposure on cell proliferation, we found a consistent increase in ODC in mammary tissue after 50 or 100 μT , 50 Hz MF exposure [Mevisen et al., 1995, 1999]. Experiments with proliferation markers (BrdU, Ki67) are underway to characterize the presumed proliferative effect of MF exposure on mammary tissue in more detail. In these experiments, melatonin will also be determined in the mammary tissue, because it is conceivable that tissue melatonin levels are affected by MF exposure in the absence of detectable effects on melatonin in plasma or pineal gland. With respect to immune functions, we found impaired T lymphocyte proliferation in response to the mitogen concanavalin A (Con A) after long-term *in vivo* MF exposure at 50 or 100 μT [Mevisen et al., 1996b, 1998a], indicating that MF exposure impairs immune surveillance. With respect to calcium mobilization, we found a significant increase in number of blood lymphocytes not reacting with intracellular calcium increase in response to Con A after *in vivo* MF (100 μT , 50 Hz) exposure [Gollnick et al., 2000]. Experiments on induction, expression or function of oncogenes are underway.

Apart from the mechanisms described above and illustrated in Fig. 5, there are various other mechanisms that could be involved in cocarcinogenic effects of MF exposure, involving alterations in the lifetime of free radicals, impairment of DNA repair, and alterations in intercellular communication [Löscher and Liburdy, 1998; Juutilainen et al., 2000]. However, the existing data on such bioeffects of MF are controversial, which may at least in part be due to the fact that such effects were studied in diverse preparations from different species. Our approach to perform mechanistic studies using a SD rat subline which has proved sensitive to cocarcinogenic MF effects, may provide more consistent data.

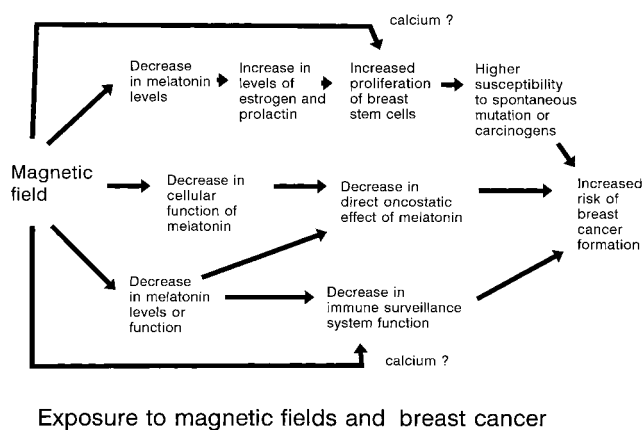


Fig. 5. Modified version of the melatonin hypothesis, including MF effects on melatonin function and MF effects unrelated to melatonin.

CONCLUSIONS

By reviewing the Hannover series of experimental studies on MF effects in the DMBA model, we have identified a number of factors that seem to be critical for the outcome of such laboratory studies (Table 3):

- (1) The rat subline used is possibly the most important factor in determining the results from EMF exposure. We have started to directly compare MF bioeffects in different SD substrains in our laboratory, seeking a subline which is insensitive to MF exposure and can therefore be directly compared to the MF-sensitive subline used in our previous experiments.
- (2) The dose of DMBA is critical because the cocarcinogenic effect of MF increases with decreasing background tumor incidence. Hence, a dose of DMBA which is submaximal in a given rat subline should be used to allow the EMF exposure to exert an enhancing action on tumor growth.
- (3) The duration of MF exposure is important, because our data strongly indicate that MF exposure affects tumor growth rather than tumor incidence. Hence, the maximal effects are seen relatively early after DMBA application, but are more difficult to detect later because of progressive tumor growth in controls.
- (4) The flux density used for MF exposure is important, because the cocarcinogenic effect of MF seems to be lost at high (mT) flux densities, indicating a flux density-window in the μ T range.

- (5) The location of tumors across the mammary gland complexes of the female rat is important, with the cranial thoracic complexes being most sensitive to EMF exposure.

Furthermore, previous lighting exposure (light history) of the rats before the MF experiments and the concentration of estrogen-like compounds and melatonin in the diet might affect MF experiments in mammary cancer models. In contrast to these factors, the dosing protocol of DMBA seems not to be critical for detecting cocarcinogenic effects of MF. Juutilainen et al. [2000] proposed that mammary tumor experiments show a stronger response to MF exposure if the DMBA treatment is given as several small doses over a longer period of time, a hypothesis that our data do not support. However, independent replication of the positive findings from the Hannover experiments is needed, preferably using the MF-sensitive SD rat subline of the Hannover studies.

Even though the effects of MF exposure seen in our experiments were small, MF effects of similar magnitude in human populations would represent a critical adverse health effect because of the high incidence of female breast cancer. A critical question is whether these results are real or are due to chance or methodological biases. That in our six experiments group tumor incidence in MF exposed groups was never below sham controls, but above controls in five experiments, argues against chance as an explanation for the findings. Furthermore, in view of our blinded

TABLE 3. Aspects of Study Design and Experimental Factors That May Affect the Cocarcinogenic Effect of MF Exposure in the Rat DMBA Mammary Cancer Model*

Study design/experimental factors	Presumably important	Presumably not important
Rat subline	+++	
Dose of DMBA	++	
Dosing protocol (acute vs. repeated dosing)		++
Duration of MF exposure	++	
Flux density	++	
Other MF exposure metrics	+	
Background tumor incidence	++	
Location of mammary tumors	++	
Diet	+	
DMBA source		+
Daily exposure duration	+	
Lighting conditions	+	
Previous lighting exposure (light history of rats)	+	
Statistical problems to verify weak positive effects	++	

*Based on experimental data from the Hannover studies summarized in this paper and a recent comparison of the Hannover and Battelle studies (Anderson et al., 2000).

experimental conditions, methodological biases are unlikely to be involved in the findings.

Assuming that the positive findings in the Hannover experiments are real, the lack of replication by the Battelle experiments indicate that such a positive MF effect can be detected only in certain experimental conditions. In the present paper we have outlined a number of factors that could be important for detection of cocarcinogenic EMF effects, but which are usually not dealt with in animal studies using classical toxicological designs. We hope that the use of different rat sublines and other experimental differences between studies on MF exposure in the DMBA model will eventually allow to determine which environmental and genetic factors are critical for effects of MF exposure in this model.

ACKNOWLEDGMENTS

The Hannover studies were supported by equipment from the Forschungsverbund Elektromagnetische Verträglichkeit Biologischer Systeme (Department of High Voltage Engineering, Technical University, Braunschweig, Germany).

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