Testing Electromagnetic Fields for Potential Carcinogenic Activity: A Critical Review of Animal Models

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In order to assess the potential of electromagnetic fields (EMF) to influence the process of carcinogenesis, it will be necessary to supplement epidemiological studies with controlled laboratory studies in animals. There are now a number of suitable assays available that focus on different histopathological forms of cancer and on different stages of carcinogenesis—induction, promotion, progression. In this review we discuss eight major systems in the context of this generalized carcinogenesis paradigm. Our aim is to bring together what is currently known about the biology of carcinogenesis in these systems in order to provide a context for evaluating EMF results as they become available. We also critically discuss EMF test results that have so far been obtained in the animal models reviewed. Most of the 19 completed studies identified were negative. However, suggestive positive results were reported in three promotion assays (in rat mammary gland, in rat liver, and in mouse skin), and in one multigeneration study in mice. Results in the rat liver assay and in the multigeneration study have only been reported in abstract form and cannot be adequately evaluated. Positive results reported in both the rat mammary gland and the mouse skin assays are of weak statistical significance and have not been independently replicated. However, it may be of interest that effects in both systems appear primarily to involve the progression stage of carcinogenesis. We suggest that more definitive conclusions as to the carcinogenic potential of EMF may require expanded test protocols that reinforce traditional carcinogenesis end points with biochemical or other parameters reflective of biological processes known to be associated with carcinogenesis in the different systems. — Environ Health Perspect 105(Suppl 1):81–103 (1997)

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Introduction

Over the past decade, considerable research has addressed the question of whether exposure to electric and/or magnetic fields (EMF) is a risk factor for cancer. Interest in this question has been triggered primarily by residential and occupational epidemiology studies that have suggested an association between EMF exposure and a variety of cancers [e.g., see reviews by Bates (1) and Sagan (2); also see more recent studies by Florides et al. (3), Sahl et al. (4), and Loomis et al. (5)].

Though these epidemiologic data are suggestive, the carcinogenic potential of EMF is still uncertain. As has been the case with numerous environmental chemicals and physical agents, definitive conclusions as to the potential carcinogenicity of EMF are likely to require supporting evidence from whole animal carcinogenesis bioassays along with a greater understanding of relevant biological mechanisms.

The carcinogenic potential of EMF is currently being evaluated worldwide in diverse animal model systems, including 2-year chronic carcinogenesis bioassays and a variety of other, usually shorter term, in vivo assays aimed primarily at examining postinitiation events.

In this review, we discuss the biology of eight major model systems and attempt to clarify the relationship of each to the complex process that we term carcinogenesis. Recent reviews of carcinogenesis mechanisms can be found in Weinstein (6), Weinberg (7), Harris (8), and Barrett (9). Carcinogenesis, though incompletely understood, is usually described in terms of an initiation—promotion—progression paradigm. In this construct, cells that incur one or more genotoxic insults at a critical locus (initiation) may, relative to normal neighboring cells, selectively proliferate into an expanded clone under the nongenotoxic (epigenetic) influence(s) of endogenous growth factors or additional environmental exposures (promotion). Subsequent exposure to genotoxic agents or stochastic genetic errors within the affected tissue would lead to malignancy (progression) (8,10).

The model systems reviewed reflect a range of assays targeted at different stages of this generalized paradigm. Our aim is to provide a biological context for the interpretation of EMF test results from the perspective of the biology of the model systems, their relationship to the general paradigm of carcinogenesis, and their relevance to human cancer. As they are available, we will assess results from tests of EMF using the model systems reviewed; see also Hardell et al. (11) for a recent review of EMF animal test results.

Throughout this paper, physical parameters are given in Standard International (SI) units. Magnetic flux density is expressed in units of tesla (T); electric field strength in volts/meter (V/m). Alternating current (ac) fields cited are sinusoidal unless otherwise noted.

Models of Carcinogenesis

The model systems reviewed are listed in Table 1. In addition to long-term chronic carcinogenesis bioassay protocols in rats and mice, there are a number of protocols targeted at the promotion or progression...
Table 1. Multistage animal models reviewed.

| Model                        | Methodological references and key reviews | EMF Tests\* |
|------------------------------|-------------------------------------------|-------------|
| Rat mammary/DMBA (or NMU)    | Huggins and Yang (12)                      | Completed   |
|                              | Welsch (13)                               |             |
|                              | Russo et al. (14,15)                      |             |
|                              | Nandi et al. (16)                         |             |
|                              | Beniasvili et al. (139)                   |             |
|                              | Mevissen et al. (40,41,42,46)             |             |
|                              | Löscher et al. (34,43)                    |             |
|                              | Löscher and Mevissen (45)                 |             |
|                              | Baum et al. (44)                          |             |
|                              | LeBars et al. (207)                       |             |
|                              | Leung et al. (208)                        |             |
|                              | In progress                               |             |
|                              | Sassar et al. (50)                        |             |
| Rat liver foci/DEN           | Farber (202)                              | Completed   |
|                              | Pereira (54)                              |             |
|                              | Pitot (57)                                |             |
|                              | Dragone and Pitot (52)                    |             |
|                              | Ranug et al. (57-59)                      |             |
| Mouse skin/DMBA              | Eastin (63)                               | Completed   |
|                              | DiGiovanni (61,62)                        |             |
|                              | Yusp (80)                                |             |
|                              | Stuchly et al. (78,80)                    |             |
|                              | McLean et al. (79,81,82)                  |             |
|                              | Ranug et al. (78,77)                      |             |
|                              | Byus et al. (83)                          |             |
|                              | Morris et al. (84)                        |             |
|                              | DiGiovanni et al. (85)                    |             |
| Mouse lymphoma/ X-irradiation| Kaplan and Brown (93)                     | Completed   |
|                              | Janowski et al. (107)                     |             |
|                              | Boniver et al. (102)                      |             |
|                              | Muto et al. (103)                         |             |
|                              | Svendenstol and Holmberg (109)            |             |
|                              | Babitto et al. (109)                      |             |
| Leukemia/cell transplant     | Stromberg (111)                           | Completed   |
|                              | Stromberg et al. (113)                    |             |
|                              | Martens and Hagenbeek (118)               |             |
|                              | Dieter et al. (114)                       |             |
|                              | Schepart (127)                            |             |
|                              | Geran et al. (97)                         |             |
|                              | Venditti (86)                             |             |
|                              | Sasser et al. (131,132)                   |             |
|                              | Thomson et al. (133)                      |             |
| Transgenic models            | Adams and Cory (134)                      | Completed   |
| (ENU/Pim-1 and p53)          | Hanahan (125)                             |             |
|                              | Breuer et al. (98)                        |             |
|                              | Donehower and Bradley (150)               |             |
|                              | McCormick et al. (168)                    |             |
|                              | Repacholi et al. (169)                    |             |
| Rat brain/ENU transplantcetal| Klehues et al. (172)                      | Completed   |
|                              | Maekawa and Mitsuomr (174)                |             |
|                              | Peterson et al. (177)                     |             |
|                              | Brugere et al. (190)                      |             |
|                              | In progress                               |             |
|                              | Mandeville et al. (188,189)               |             |
| 2-year chronic bioassays     | Sontag et al. (192)                       | Completed   |
|                              | Bannasch et al. (193)                     |             |
|                              | Yasui et al. (198-199)                    |             |
|                              | Fam and Mikhail (199)                     |             |
|                              | Mikhail and Fam (200,201)                 |             |
|                              | In progress                               |             |
|                              | Mandeville et al. (188,189)               |             |
|                              | McCormick et al. (195)                    |             |

\*All studies involve extremely low frequency, direct current, or pulsed magnetic fields exposures except as indicated. \*Electric field studies.

Rat Mammary Carcinoma Model

More than 30 years ago, Charles Huggins developed what is today the most widely used animal model for human breast cancer (12). The system has been comprehensively reviewed (13–15). Briefly, mammary tumors (adenocarcinomas and fibroadenomas) develop in rats given low doses of initiator, usually DMBA or N-methyl-N-nitrosourea (MNU), followed by chronic treatment with a promoter. Maximal sensitivity to the initiator treatment is coincident with the period of sexual maturation, about 6 to 8 weeks of age, when the target tissue experiences a high rate of growth. Experiments are usually terminated about 3 months after initial exposure to the initiator. Results are determined by morphological and histological examination and are usually reported as tumor incidence, tumor size or weight, tumor latency, and tumors per tumor-bearing animal.

The rat mammary carcinoma model has been considered an adequate system in terms of its relevancy in many respects to human disease (15). For example, factors that promote tumor development in the rat mammary model, such as reproductive status, also increase breast cancer risk in humans. However, other aspects of the rodent system suggest that comparisons between rodents and humans should be made with caution. Aside from obvious differences between rats and humans, such as different numbers of mammary glands and different cycle lengths (4 vs 28 days), there are a number of more subtle biological differences that could affect the validity of risk extrapolations. For example, the pattern of hormonal sensitivity of mammary tumors in rats and humans is quite different [see Nandi et al. (16) for a recent review].

Though both human and rat breast tumors are hormonally dependent, human tumors are sensitive primarily to estrogens (17) and DMBA-induced tumors in rats are sensitive primarily to prolactin (13). These hormones act through quite different receptor-mediated pathways [discussed in Lucier (18) and Welsch (13)]. Therefore, modulation of tumor response in the rodent system by a mechanism involving the prolactin pathway would not necessarily imply a similar result on tumor development in the human system. On the other hand, MNU-induced rodent mammary tumors are less dependent on prolactin and slightly more sensitive to the direct stimulatory effects of estrogen, suggesting they may be more akin to human breast tumors than DMBA-initiated mammary tumors (19).
Interpreting the relevance to humans of results in the rat DMBA model system can also be complicated because some tested substances may not only interact with mammary tumor pathogenesis, but also may interact with other factors unique to the model system. For example, mutations in the p53 tumor suppressor gene are the most frequently observed genetic lesions in human breast cancers. However, this gene does not appear to be altered in most DMBA-induced mammary tumors (20). Also, in the mammary carcinoma model, DMBA itself affects serotonin (21), melatonin (22,23), and NK (natural killer) function (24), all of which could have some involvement in subsequent tumor development. Interaction of promoting agents with the expression of such DMBA-initiated events may therefore not be generalizable to other systems that do not utilize DMBA.

The literature on tumor promotion in this model system is extensive [see reviews by Welsch (13) and Russo et al. (14)] and indicates that the system is highly sensitive to modulation of tumor development and growth by both endogenous and exogenous substances. Table 3 lists 37 substances or physiological states that act as promoters in this rodent model system. As discussed above, a significant fraction of mammary tumors are hormone sensitive. Estrogens, progestrone, prolactin, and other estrogen-related substances enhance tumor growth rate and incidence. Physiological states that increase serum levels of these hormones, particularly prolactin, also appear to enhance tumor development. Examples are reproductive status (i.e., pregnancy or pseudopregnancy enhances tumor growth), interference with normal circadian rhythms (i.e., constant light increases serum levels of prolactin and increases tumor growth), and even different mental states. For example there is a rapid increase in serum prolactin levels during meditation [reviewed in Jenving et al. (25)] and certain types of stress result in an increase in prolactin secretion. Other endogenous substances, such as insulin, or dietary factors, such as high levels of polyunsaturated fatty acids, carbohydrates, and proteins, also have been observed to enhance tumor growth. In addition, miscellaneous drugs such as haloperidol, perphenazine, and reserpine have demonstrated promoter activity in the rat model. It should be noted that, as indicated in Table 3, evidence for the promoting potential of a number of the listed substances is either conflicting or controversial. In this system, the exquisite sensitivity of tumor development to modulation is underscored by the observation that there are also a variety of substances, both exogenous and endogenous, that inhibit tumor growth. One of these substances, the neurohormone melatonin, is of particular interest because it has been suggested that EMF could act as a promoter by decreasing serum melatonin levels (26). Melatonin is well documented to be oncostatic for mammary cancer. With apparently only one exception (27), all groups that have examined effects of exogenous melatonin treatment on tumor growth in DMBA-induced rats have observed an inhibition of tumor growth (28–31).

Effects of magnetic fields (MFs) on melatonin are less certain. These studies have recently been reviewed (32,33). Though there is uncertainty regarding effective exposure conditions and difficulty in reproducing results, reports from several

### Table 2. Promoter assay protocols.

| Species/strain | Mitogen | Initiator(s) | Method of initiation | Transgenic system | Rat brain/ENU |
|----------------|---------|--------------|---------------------|------------------|--------------|
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
| Rat/Sprague-Dawley | Partial hepatectomy | DMBA, NMU | DEN | Tumor-bearing | ENU (Pim-1, p53) |
|

*For references see Table 1 and text.*

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Table 3. Examples of promoters in the rat mammary/DMBA assay.

| Hormones and adrenal steroids |   |
|------------------------------|---|
| Estradiol                    |   |
| Progesterone                 |   |
| Enovid                        |   |
| Prolactin                    |   |
| Growth hormone               |   |
| Pregnancy                    |   |
| 17-Deoxycorticosterone       |   |

Neuroendocrine system status

Drugs that decrease hypothalamic dopaminergic activity (reserpine, perphenazine, sulpiride, fluphenazine, methylodopa, pimozide, α-methyl paratyrosine, haloperidol)

Stress

Thyrotropin-releasing hormone

Thyroid status

Both hyperthyroid-inducing drugs (thyroxine, triiodothyronine) and hypothyroid-inducing agents (goitrogens, thioracil, propylthiouracil) have been reported as promoters, but results are controversial

Fats, proteins, and carbohydrates

Dietary fat (210–212)

Dietary protein

Sucrose

Immune status

Bacillus Calmette-Guerin

Miscellaneous

Caffeine

Ethanol (213)

Iron deficiency (214)

Glucose

Insulin

*Agents listed are those which have been observed to promote the growth of DMBA-induced mammary carcinomas in rats. Agents observed to inhibit tumor growth or which had no effect are not included. Most reports cited employed Sprague-Dawley rats and used 7,12-dimethylbenz[a]anthracene as inducer. Unless another reference is specified, all promoters listed are from Welsch’s comprehensive review (13). *Whereas low doses of these agents stimulate the growth of DMBA-induced mammary carcinomas, high doses can inhibit growth [see Welsch (13)]. *Results are conflicting.

Table 4. Summary of results reported by the Löschner group for 50-Hz ac magnetic fields.

| Flux density | Reference controls with tumors, % | Sham controls with tumors, % | MF exposed with tumors, % | p-value | Reference |
|--------------|-----------------------------------|-----------------------------|--------------------------|---------|-----------|
| 0.3–1.0 µT   | 58                                | 58                          | NS                       | (40)    |           |
| 10 µT        | 62a                               | 67a                         | NSa                       | (34,41)a|           |
| 50 µT        | 55a                               | 69                          | p < 0.05                  | (46)*   |           |
| 100 µT       | 52a                               | 69                          | p < 0.05                  | (43,44)a|           |
| 30,000 µT    | 67                                | 56                          | 78                        | NS      | (40)      |
| 30,000 µT    | 56                                | 50                          | 40                        | NS      | (40)      |

NS, not statistically significant. *Table entries used for the correlation analysis in Löschner and Mevissen (45).

Significant increases were not observed in rats exposed to the MF for 0.5 hr/day.

Most work with this system is from Löschner and his colleagues (34,40–46). In all experiments, female Sprague-Dawley rats 6 to 8 weeks of age were exposed by gavage to DMBA (four weekly doses at 5 mg/rat/dose) beginning on the first day of MF exposure. MF exposure was 24 hr/day, 7 days/week for 13 weeks. Except as indicated, there were 99 animals in each exposure and sham-exposed group. Exposed and sham-control groups were sacrificed at the end of the exposure period and the number and size of morphologically detectable tumors were determined.

In an early experiment, small groups of animals (18 in each group) were exposed either to 15-mT dc or 30-mT 50-Hz fields (40). Two separate experiments were performed using 30-mT 50-Hz fields. Tumor incidence and weight were reported at the end of the exposure period. In the 15-mT direct current experiment, investigators report a greater than 4-fold increase (p < 0.05) in the weight of tumors in exposed animals compared to sham-exposed controls, but no significant increase when compared to reference controls (age-matched rats treated with DMBA but housed in a separate area). There was also no significant difference in tumor incidence in MF-exposed animals when compared to either sham-exposed or reference controls. This experiment was evidently not repeated. There was also a statistically significant increase (p < 0.05) in the number of tumors (adenocarcinoma) per tumor-bearing animal when compared to the reference control, but not when compared to the sham control, in one of the two 30-mT ac exposure experiments. The other 30-mT experiment was negative, as were the combined results of the two experiments. A statistically significant difference in tumor incidence between exposed and sham-exposed groups was not observed in either of the two 30-mT experiments. As the authors point out, however, the group sizes were very small, limiting the power of the assay.

Löschner and Mevissen have recently reported a highly significant (r = 0.99, p < 0.01) linear correlation between flux density and tumor incidence (45). This correlation is a summary analysis of data compiled from separate experiments conducted over a period of several years. Below, we will review results from each experiment used for the correlation and then comment on the summary correlation analysis. To assist this discussion, we have reproduced the flux densities tested and the tumor incidences reported in Table 4.

As shown in Table 4, results of two experiments at the 0.3 to 1.0 µT flux density have been reported (34,40,41). In each experiment, a group of 36 rats was exposed to the gradient ac sine wave 50-Hz field and a group of 36 was exposed to a sham field. Nocturnal serum melanin was significantly reduced by this exposure, but no statistically significant differences in tumor size, incidence, or histopathology were observed in MF-exposed groups as compared to sham-exposed groups. The investigators caution that results are limited by the relatively small number of animals used in the study.

In abstract form, Mevissen et al. (42) report that exposure to a 10-µT flux density produced no significant differences in tumor incidence or tumor size in MF-exposed animals compared to sham-exposed controls. Tumor incidence in the MF-exposed group was 69% and tumor incidence in sham-exposed controls was 62%. The investigators indicate a tendency toward decreased latency in the MF-exposed group but do not present data. In this experiment, small groups (n = 9) of animals were also MF- and sham-exposed in the absence of DMBA. No tumors were found in either of these two groups.

Results at 50 µT have recently been published (46). Investigators indicate that tumor incidence in the MF-exposed group
was 25.5% above the concurrent sham-exposed control at autopsy (55% in sham-exposed controls and 69% in MF-exposed animals). In a separate publication, the same laboratory (47) reported that this flux density (50 μT) also results in a statistically significant (p<0.05) increase in the mammary gland in ornithine decarboxylase (ODC), an enzyme associated with cell proliferation. They indicate that this observation strengthens their hypothesis that weak 50-Hz magnetic fields promote or co-promote carcinogenesis in the rat mammary model system.

Lösch et al. (43), using 50-Hz, 100-μT exposures, reported statistically significant increases (p<0.05) in the incidence of mammary tumors (as determined by palpation) in MF-exposed animals compared to sham-exposed controls. The difference was observed as soon as 8 weeks after DMBA application and was maintained throughout the 3-month experiment. At termination of the experiment, tumor incidence in the MF-treated group was 52%, versus 34% in sham-exposed controls. A statistically significant (p<0.05) increase in tumor size (as estimated by palpation) was also reported at the end of the exposure period, though not at earlier time points. The average of tumor volumes for MF-exposed animals was also greater than the sham-exposed group, but the difference was not statistically significant.

Recently, Baum et al. (44), supplied additional analysis of data obtained in the 100-μT study (43); they include a detailed histopathological analysis and additional control groups. Investigators report that the increases previously reported in the MF-exposed group were primarily due to effects on tumor growth and malignancy rather than on tumor incidence. Specifically, if macroscopically visible mammary tumors were considered, the difference in tumor incidence between MF-exposed and sham-exposed rats reported previously (43) was observed. However, when the analysis was extended to include a microscopic histopathological analysis that included non-neoplastic as well as neoplastic lesions, no statistically significant differences were observed between MF-exposed and sham-exposed controls.

Evaluation of the linear correlation offered by the Lösch group in its most recently published study (45) is complex primarily because, as noted previously, results for each data point were obtained in different experiments conducted at different times with different groups of animals. Indeed, as shown in Table 4, the sham-control tumor incidences reported range from 34 to 62%, a substantially wider range than the MF-exposed tumor incidences used for the correlation (52-69%). It is true, as Lösch has pointed out (48), that variation in control incidence among different animal groups is expected, and that differences between concurrent control and exposed groups are more significant than differences between exposed incidences and historical control averages. Nevertheless, it is of some concern that the 100-μT tumor incidence value (52%), on which the correlation largely depends, is actually less than the average of the seven sham-control tumor incidences reported in Table 4 (53.9%). It is also of some concern that this 100-μT tumor incidence is so much less than all but one of the other MF-exposed tumor incidences reported in Table 4; see also the comments of Irnich (49).

**Summary.** All rat mammary assays are summarized in Table 5. The positive results reported by Lösch et al. (43), Baum et al. (44), and Mevissen et al. (46) at two flux densities (50 and 100 μT) and by Beniashvili and colleagues at 20 μT (39) suggest the possibility of a promoting effect of MF-exposure at these low flux densities. Though there were significant differences in the protocols employed by these two groups (e.g., different inducers and different exposure periods), both reported significant increases in more than one tumor-associated parameter. However, results are not highly statistically significant and, as discussed above, there was considerable variability in tumor incidence rates. In interpreting the potential significance of these results it is also important to keep in mind the sensitivity of the DMBA/mammary system to modulation by many dietary and other endogenous factors (Table 3). Further investigation with this model may help to clarify results. The U.S. National Toxicology Program (NTP) has initiated MF studies (50) that will attempt to replicate the results obtained by the Lösch group. These NTP studies will, in addition, extend the exposure duration to 26 weeks and slightly vary other aspects of the protocol. The NTP studies will also examine effects of MF exposure on nocturnal melatonin levels and ornithine decarboxylase activity, replicating the reports cited above from the Lösch group.

**Rat Liver Foci Model**

The rat liver foci system is the animal model most commonly used for the study of multistage carcinogenesis in extrapapillary tissues. The model has been comprehensively reviewed by Pitot (51), Dragnet and Pitot (52), and Farber (53). The following summary has been compiled primarily from these reviews. Development of the model stemmed from observations in the 1960s and early 1970s of hyperplastic nodules in the liver and their possible role as precursors in the development of malignant neoplasia. Since that time, several model protocols have been described using altered hepatic foci (AHF) in assays examining the stages of hepatocarcinogenesis in the rat.

The most commonly used protocol requires a mitogenic stimulus, usually a partial hepatectomy, in conjunction with a single subcarcinogenic dose of an initiator (often dieethylaminoethanol [DEA]) for maximal sensitivity (54). (The assay can also be done in neonates, which do not require external mitogenic stimulation.) Carcinogenesis is believed to permit fixation by replication of DNA lesions produced by the inducer prior to repair. Other factors may also be involved, such as chromatin accessibility to the carcinogen. The parameters most commonly quantified are the number of AHF, the volume percentage of AHF in liver, and a phenotypic fingerprint of the AHF, usually determined by use of enzyme marker or immunohistochemical assays. Pitot et al. have also described a methodology for quantitatively distinguishing the initiating and promoting potential of agents tested by this protocol (55).

In this model system, treatment with an initiator (usually DEA) at a subcarcinogenic dose will not result in the appearance of AHF unless a promoter is present. Chronic treatment with a promoter following initiation results in clonal growth to AHF, but only a small fraction of initiated cells develop into AHF. If the promoter is removed, the population of AHF decreases, but does not disappear altogether. The persistence of some AHF could be due to the numerous endogenous factors known to act as promoters of hepatocarcinogenesis (see discussion below). Progression, believed to involve additional genetic events, results ultimately in malignant neoplasms, which may manifest as malignant foci within existing AHF. Only a small fraction of AHF actually progresses to malignant neoplasia.

There are considerable morphologic variations in AHF, which are identified using a number of histological, histochemical, and immunohistochemical techniques. Altered gene expression has been detected in AHF using more than 40 different
| Test system | Rodent strain | Initiator/(promoter) | EMF exposure | Results reported by authors | Reference |
|-------------|---------------|----------------------|--------------|-----------------------------|-----------|
| Rat mammary gland | Rats (strain not specified) | NMU | 20 μT, 50 Hz ac or dc, 0.5 or 3 hr/day exposure for 2 years | Positive: 56% (ac) or 47% (dc) increase in tumor incidence for 3 hr/day exposure groups (p<0.05) | Beniashvili et al. (39) |
| | Sprague-Dawley (18/group) | DMBA | 15 mT (dc) | Equivocal: 4-fold increase in tumor weight (p<0.05) when compared to sham controls but no difference when compared to reference controls | Mevissen et al. (40) |
| | Sprague-Dawley (19/group) | DMBA | 30 mT (50 Hz, ac) (homogeneous) | Equivocal: 65% increase in tumors/tumor bearing animal when compared to ref. control (p<0.05). Not observed when compared to sham control or in a second experiment | Mevissen et al. (40) |
| | Sprague-Dawley (36/group) | DMBA | 0.3-1.0 μT (50 Hz, gradient ac sine wave) | Negative, but nocturnal melatonin reduced | Löscher et al. (34) |
| | Sprague-Dawley (99/group) | DMBA | 10 μT (50 Hz) | Negative | Mevissen et al. (42) |
| | Sprague-Dawley (99/group) | DMBA | 50 μT (50 Hz) | Positive: statistically significant increase in tumor incidence in exposed animals as compared to sham-exposed controls (p<0.05) | Mevissen et al. (46) |
| | Sprague-Dawley (99/group) | DMBA | 0.1 mT (50 Hz) (homogeneous), continuous exposure for 13 weeks | Positive: 50% increase in tumor incidence (52% in EMF-exposed versus 34% in sham-exposed controls) (p<0.05) | Löscher et al. (43) |
| | Sprague-Dawley (130/group) | DMBA | 0.1 or 0.5 mT (50 Hz); 0.1 mT (60 Hz) 18.5 hr/day exposure for 13 or 26 weeks | In progress | Sasser et al. (50) |
| Rat liver focus | Sprague-Dawley | DEN | 0.5, 5, and 50 μT or 0.5 mT (50 Hz) | Predominantly negative | Rannug et al. (57) |
| | Sprague-Dawley | DEN | Field strength not specified but in the 0.5 μT to 0.5 mT range (50 Hz, intermittent on/off cycle) | Positive: quantitative data and statistical significance not reported | Rannug et al. (59) |
| | Sprague-Dawley | DEN (PB) | 0.5 μT or 0.5 mT (50 Hz) | Possibly inhibitory: decrease (p<0.05) in number of foci (34%, 0.5 μT), mean focus area (16%, 0.5 mT), and volume of foci (29%, 0.5 mT) | Rannug et al. (58) |
| Mouse skin | NMRI/HAN | DMBA (25.6 ug) | 50 or 500 μT (50 Hz) | Negative | Rannug et al. (76) |
| | SENCAR | DMBA (2.56 ug) | 50 or 500 μT (50 Hz), continuous or intermittent—15 sec on/off | Suggestive: dose trend (p=0.045) for intermittent exposure groups | Rannug et al. (77) |
| | SENCAR | DMBA | 2 mT (60 Hz) | Negative at 22 weeks | Stuchly et al. (78) |
| | SENCAR | DMBA (TPA, 1 μg/week) | 2 mT (60 Hz) | Negative at 22 weeks | McLean et al. (79) |
| | SENCAR | DMBA (TPA, 0.3 μg/week) | 2 mT (60 Hz) | Positive: increase in rate of tumor development in field-exposed mice (p<0.05) | Stuchly et al. (80) |
| | SENCAR | DMBA (TPA, 0.3 μg/week) | 2 mT (60 Hz) | Negative at 23 weeks: replication of Stuchly et al. (80) | McLean et al. (81) |
| | SENCAR | DMBA (TPA, 0.3 μg/week for 23 weeks) | 2 mT (60 Hz), for 52 weeks | Suggestive positive: non-statistically significant increase in overall tumor incidence at 52 weeks. Significant (p<0.03) increase in the fraction of mice in the field-exposed group with malignant tumors (8 of 48) compared to sham controls (1 of 48) | McLean et al. (82) |

(Continued)
Table 5. Continued.

| Test system | Rodent strain | Initiator/promoter | EMF exposure | Results reported by authors | Reference |
|-------------|---------------|---------------------|--------------|----------------------------|-----------|
| SENCAR      | 2 mT (60 Hz)  | Negative at 23 weeks| Morris et al. (84) |
|             | 2 mT (60 Hz)  | Increased tumor incidence at 43 weeks in 0.25 μg TPA treated MF-exposed group | DiGiovanni et al. (85) |
| Mouse lymphoma | CBA | X-irradiation (0.45 Gy/min; 5.24 Gy in 4 fractional doses) | 0.015 mT (20 kHz, pulsed, sawtooth, vertical) | Negative after lifetime exposure (131 weeks) | Svedenst6l and Holmberg (108) |
|             | C57BL/6J (195-450/group) | X-irradiation (0.34–0.37 Gy/min), Total radiation doses: 0, 350, 475, 600 R | 1.4 mT (60 Hz, circularly polarized with 1-mT horizontal and vertical components) | In progress | Babbitt et al. (109) |
| Rat leukemia transplant | F344 | 1 mT (60 Hz, continuous) (20 hr/day) | Negative | Sasser et al. (131, 132) |
|             | F344 | 1 mT (60 Hz, intermittent, off/on at 3-min intervals, 20 hr/day) | Preliminary results negative | Sasser et al. (132) |
|             | DBA/2 | 1.4 μT, 200 μT, 500 μT (60 Hz) (6 hr/day, 5 days/week) | Negative | Thomson et al. (133) |
| Transgenic mouse | pim and TSG-ps3 | 2 μT, 0.2 mT, 1 mT continuous (18.5 hr/day) and 1 mT pulsed (60 Hz, 1 hr on/off cycle) | Negative | McCormick et al. (168) |
|             | Ep-pim-1 | 1 μT, 0.1 mT, 1 mT continuous (60 Hz) and 1 mT pulsed (50 Hz, 15 min on/off) | In progress | Repacholi et al. (169) |
| Rat brain/ENU | Fischer | ENU (trans-placental) | 2 μT, 20 μT, 0.2 mT, and 2 mT (60 Hz) (20 hr daily exposure) | In progress | Mandeville et al. (188, 189) |
|             | Sprague-Dawley | ENU (trans-placental) | 1, 10, 100 μT (50 Hz) | Negative (no effect on mortality) | Brugere et al. (190) |
| Long-term chronic bioassays | F344 rats and B6C3F1 mice | 2 μT, 0.2 mT, 1mT continuous, 16.5 hr/day (60 Hz) and 1 mT intermittent (60 Hz, 1 hr on/off cycle) | In progress | McCormick et al. (195) |
|             | F344 rats | 0.5 mT, 5 mT (50 Hz) | Negative | Yasui et al. (196–198) |
|             | F344/N rats | 2, 20, 200, 2000 μT (60 Hz) | In progress | Mandeville et al. (188, 189) |
|             | Mice | 25 mT (60 Hz) (363–418 days exposure); sham was <0.05 mT | Positive: 13-fold increase in incidence of premalignant or malignant lymphoma | Mikhail and Fam (201) |
| Multigeneration | Exposure A: 24 second-generation mice | 25 mT (60 Hz) (Exposure A: 120 days; Exposure B: 133–257 days; exposure began prior to conception) | Positive: limited data provided | Fam and Mikhail (199) |

We do not discuss results of two electric field exposure studies that have been reported in abstract form (207, 208). Results of both studies are predominantly negative, but are difficult to evaluate without further information. *Ac fields are sinusoidal unless otherwise noted.

marker assays; however, all AHF do not express all altered phenotypes. The most commonly expressed marker is glutathione S-transferase (GST-P), but even this is not expressed by all AHF. Often a second enzyme marker is also employed, usually γ-glutamyltranspeptidase (GGT).

The markers expressed are dependent upon the promoting agent employed. For example, the two most common enzyme markers, GST-P and GGT, are not expressed when peroxisome proliferator promoters such as ciprofibrate or Wy-14643 are employed. Also, AHF induced by X- and γ-radiation are GGT negative.

A number of substances, both natural and synthetic, have been observed to promote hepatic carcinogenesis in the rat liver foci model. In Table 6, 46 such agents are listed. These agents include a wide variety of substances with diverse chemical structures and biological activities. Examples are: hormones such as ethinyl estradiol and prolatin; dietary or endogenous factors such as sucrose, tryptophan, and bile acids; free radical generating agents and antioxidants; a number of chlorinated compounds.
such as aldrin, DDT, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and polychlorinated biphenyls (PCBs); drugs and pesticides such as phenobarbital and disulfiram; and a variety of miscellaneous agents, including γ-irradiation. Thus, as for the rat mammary model, the liver foci system is highly sensitive to modulation by a wide variety of synthetic and natural substances, both endogenous and exogenous.

The prototype promoter in the liver foci system is phenobarbital (PB). PB is a strong inducer of liver microsomal enzymes active in detoxifying (and in some cases activating) xenobiotics. Certain other promoters of hepatocarcinogenesis also influence microsomal enzyme activity, such as PCBS, TCDD, hexachlorocyclohexane, and even dietary fat and ethanol. Interestingly, PB also has some similarities to TPA (12-O-tetradecanoylphorbol-13-acetate) (56), a potent tumor promoter in mouse skin.

A number of other hepatic promoting agents, such as polybrominated biphenyls (PBBs) and PCBs, are hepatotoxins and they cause rapid cell loss followed by regenerative hyperplasia. For example, a choline-deficient diet causes a cycle of liver cell loss and regeneration, which has been described as a chronic partial hepatectomy. Associated with this effect is the invasion of polymorphonuclear leukocytes, a consequent increase in lipid peroxidation and reactive oxygen species, and the possible activation of protooncogenes. It should be noted, however, that some promoters in rat liver such as estrogens, prolactin, and succrose appear to stimulate hyperplasia in the absence of toxicity.

### Tests of Magnetic Fields in the Liver Foci System

In 1993, using the rat liver focus assay described by Pereira (54), Rannug and co-workers published the results of experiments designed to determine whether 50-Hz MFs promoted the development of chemically initiated liver tumors (57), or acted as a cocarcinogen (58).

In the promotion experiment (57), 200 g male Sprague-Dawley rats (10/group) were initiated with ip DEN at a dose of 30 mg/kg of body weight (bw) 24 hr after they had undergone a 70% partial hepatectomy. Beginning 1 week after DEN exposure, they were exposed for 12 weeks to either MFs or PB, which was provided in the diet at a concentration of 300 ppm. Two sets of MF exposures were administered in two separate experiments: sham, 0.5 μT and 50 μT in the first; and sham, 5 μT and 500 μT in the second. The fields were on for 19 hr/day except for weekends and holidays, when the fields were off for 21 hr/day. After 12 weeks, tumor number and size were assessed through analysis of AHF using GGT and GST-P enzyme markers.

No body weight loss or evidence of cytotoxicity was associated with MF exposure. In the first experiment, using 0.5- and 50-μT fields, slight increases were observed in GGT-positive foci. Similar increases in GGT-positive foci were not observed in the second experiment. Effects on GST-P positive foci were not observed in either experiment. Rannug et al. (57) conclude that the results are predominantly negative but do not conclusively rule out the possibility of an effect.

Recently, in abstract form, Rannug and colleagues reported results of experiments using the same protocol as above except that exposure was to intermittent fields (50 Hz, sinusoidal, field strength not specified but in the 0.5 μT–0.5 mT range) (59). Investigators reported a higher incidence in the exposed animals compared to sham controls of H-ras and GST-P foci, but not of GGT foci. The abstract does not include quantitative data and does not indicate whether results were statistically significant.

In the cocarcinogen study (58), hepatocarcinomy, initiation with DEN, promotion with PB, and tumor assays were as above except that exposure was to intermittent fields (50 Hz, sinusoidal, field strength not specified but in the 0.5 μT–0.5 mT range) (59). Investigators reported a higher incidence in the exposed animals compared to sham controls of H-ras and GST-P foci, but not of GGT foci. The abstract does not include quantitative data and does not indicate whether results were statistically significant.
or coinitiator in this system. Additional information on the intermittent field exposures is not available (B Holmberg, personal communication).

**Mouse Skin Model**

The development of skin tumors in mice is a convenient and versatile model for multistage carcinogenesis. This system has been comprehensively reviewed (60–63). Human epidemiological studies do not suggest that EMF exposure results in dermal tumors. However, the skin model is advantageous, in part because of the relative ease with which tumors can be tracked on the backs of living animals. In addition, a wealth of information has been collected on the biological properties of carcinogen-exposed mouse skin. As a result, morphologic (e.g., hyperplasia), biochemical (protein kinase C [PKC], ornithine decarboxylase activity [ODC], growth factors), and genetic (e.g., ras oncoprotein expression) indices may serve as markers to track tumor development and lend insight into carcinogenic mechanisms.

The mouse skin system also has some relevance to human skin cancer [reviewed by Yuspa (60) and DiGiovanni (62)]. Carcinogenesis in the skin of both humans and mice is a multistep process; both processes appear to involve protooncogene activation; and the major end point in the mouse system, squamous cell carcinomas, is histologically similar to squamous cell carcinomas in humans. However, the most widely employed model, the two-stage protocol, is highly specialized, making extrapolation to humans difficult. The two-stage protocol uses DMBA as initiator and TPA, the most active phorbol diester in croton oil, as a complete promoter. The protocol usually employs specially engineered mouse strains, particularly the SENCAR (sensitive to carcinogenesis) mouse.

An irreversible genetic change in an epidermal stem cell is believed to be associated with the initiation stage. This may involve a point mutation in the c-Ha-ras oncogene. TPA is believed to facilitate two steps: conversion of an initiated cell to a latent tumor cell and propagation of converted cells. Some have suggested the conversion phase of promotion may also involve genetic changes (e.g., Kaina (64)), though it is not clear how this would accommodate the observed reversibility of promotion [see discussion in DiGiovanni (62)].

Shortly after repeated application of TPA begins, histological and morphological changes are observed. Toxic effects such as edema and inflammation are observed as soon as a few hours after TPA treatment, followed by regenerative epidermal hyperplasia, an effect common to skin tumor promoters. The mice are examined, usually on a weekly basis, for the appearance of tumors. The major tumor type found is squamous cell papilloma. These tumors appear as early as 5 to 6 weeks after TPA application begins. Some of these papillomas appear to regress if TPA treatment is terminated; however, others will spontaneously progress to squamous cell carcinomas at about 20 weeks. The eventual ratio of papillomas to carcinomas appears to be similar for different promoters, provided comparisons are made among groups with similar tumor burdens (65). It is thought that one or more additional genetic events are required for progression to malignancy.

Though not fully elucidated, the sequence of biochemical and molecular events in the two-stage skin model is probably more fully understood than for any other carcinogenesis model system. The major mechanism of action of TPA-type promoters appears to be their ability to bind to and/or activate PKC. PKC is widely distributed in mammalian tissues and is thought to be a key enzyme in signal transduction pathways [reviewed in Weinstein (66) and Basu (67)]. It has been suggested that activation of PKC is essential for skin tumor promotion by TPA-type promoters. Activation of PKC depends on a number of factors, not all of which are well understood. For example, PKC is not a single enzyme, but represents a family of isozymes (68); enzyme activity requires phospholipid and calcium cofactors and PKC activation requires enzyme translocation to membrane sites [reviewed in Blumberg et al. (69) and DiGiovanni (62)].

A cascade of biochemical events associated with PKC activation includes release of arachidonic acid, which is involved in prostaglandin synthesis, which in turn leads to an inflammatory reaction resulting in an oxidative burst in inflammatory cells [reviewed in Fischer et al. (70)] and can result in chromosome damage mediated by arachidonic acid metabolites [reviewed in DiGiovanni (62)].

ODC is also induced by TPA-type promoters as well as by other types of promoters, including benzoyl peroxide (71), chrysarobin, okadaic acid, calyculin A (72), and arachidonic acid metabolites (e.g., prostaglandin E2). ODC is the rate-limiting enzyme in the biosynthesis of polyamines, which are required for cell growth. ODC has been directly linked to promotion by experiments observing enhanced papilloma formation in the two-stage skin model in transgenic mice over-expressing ODC (73).

Other biochemical effects of treatment with TPA and other promoters have also been observed in the mouse skin system. These include effects on DNA synthesis; oxidative DNA damage (74); phospholipid turnover; superoxide dismutase, catalase, xanthine oxidase, histidase, and histidine decarboxylase activities; the glucocorticoid receptor; epidermal keratins and keratin expression; and synthesis and phosphorylation of histones.

After TPA treatment, a loss of PKC activity through proteolysis is also observed. Though it is not clear how this down regulation of PKC interacts with the process of promotion, it has been suggested that it may be important in sustaining hyperplasia [reviewed in DiGiovanni (61,62)].

Evidence also suggests that promotion can be elicited in epidermal cells through signaling mechanisms that do not involve PKC [reviewed by Yuspa (60)]. For example, many promoters in the mouse skin system do not directly activate PKC. Such promoters may interact with mechanisms that modulate cell growth through growth factor-dependent processes. These, in turn, may result in the activation of PKC indirectly, or may involve PKC-independent pathways (75).

Many substances have been found to act as promoters or co-promoters in this model system. Table 7 lists 55 promoters and 14 co-promoters. Though precise mechanisms are not certain, promoters in mouse skin can be loosely classified in several categories: a) TPA-type promoters, such as alypsialtoxin and teleocidin, which share with TPA the ability to bind to PKC; b) non-TPA-type promoters, such as chrysarobin and thapsigargin, which do not appear to activate PKC but induce ODC by a distinct mechanism possibly involving Ca2+ mobilization; c) okadaic acid-type promoters, which bind to a receptor that appears to be distinct from the phorbol ester receptor; d) dietary or lifestyle factors such as retinoic acid or tobacco smoke; e) various peroxide free-radical generating compounds; and f) miscellaneous agents such as acetic acid, some polymeric aromatic hydrocarbons (PAHs), ultraviolet light, and skin abrasion.

Co-promoters enhance TPA promotion. Examples include agents that affect polyamine or cyclic nucleotide concentrations, such as α-methylornithine, a reversible
**Table 7. Examples of promoters and co-promoters in mouse skin.**

| Promoters/co-promoters | Reference | Promoters/co-promoters | Reference |
|------------------------|-----------|------------------------|-----------|
| TPA-type promoters     |           |                        |           |
| TPA and various derivatives (also known as PMA (phorbol myristate acetate) | (62) | Promoters |  |
| Aplig dispositivo | (62) |  |
| Croton oil | (226) |  |
| Debramprophaistatin C | (227) |  |
| Debramprophaistatin C | (227) |  |
| Dihydroxyestradiol B | (226) |  |
| Indolactam-V | (227) |  |
| N-geranyl(+) -indolactam-V | (227) |  |
| Oscillatoxin C | (227) |  |
| Telecendire A (lyngbyatoxin) and B | (62,228) |  |
| Non-TPA-type promoters |          |                        |           |
| Anthrin (dithranol; 1,8-dihydroxy-9-anthrone) | (62) |  |
| Chrysoarbin (1,8-dihydroxy-3-methyl-9-anthrone) | (62,229) |  |
| Dinipithinysin-1 | (227) |  |
| Mirex | (220) |  |
| Methyl parathion | (62) |  |
| Thapsigargin (sesquiterpene lactone) | (62) |  |
| Okadaic acid-type promoters | |  |
| Okadaic acid (a polyether of a C_8 fatty acid) | (62) |  |
| Calyculin A | (62) |  |
| 35-Methylkodiacid acid (dimethylsinoxid) | (231) |  |
| Dietary/lifestyle factors | |  |
| β-Carotene | (232,233) |  |
| Citrus oils | (226) |  |
| Dietary fat (corn oil) | (234,235) |  |
| Fatty acid methyl esters | (62) |  |
| Onion oil | (236) |  |
| Retinol | (62) |  |
| Tobacco | (62) |  |
| Tobacco (unburned extracts) | (226) |  |
| Vitamin E (dl-α-tocopherol) | (237,238) |  |
| Peroxides | |  |
| Berogly peroxide | (62) |  |
| Butylhydroxytoluene peroxide | (62) |  |
| Butylhydroperoxidation | (239) |  |
| Cumene hydroperoxide | (239) |  |

*In all cases, DMBA was used as initiator. In co-promotion experiments, TPA was the promoter. Various mouse strains were employed, including SENCAR, CD-1, NMRI, and ICR. Co-initiators and agents demonstrating inhibitory activity are not included. This table was compiled from the reviews of Slaga et al. (226) and DiGiovanni (62) and primary references as indicated. *Also a co-promoter. *Results are conflicting. *Weak activity. *Differential effects depending upon dose. *Primarily a Stage II promoter and is weakly active as a complete promoter.

inhibitor of ornithine decarboxylase, which exhibits slight activity; flurbiprofen and indomethacin, which are cyclooxygenase inhibitors; γ-interferon; and some prosstaglandins.

**Tests of Magnetic Fields in the Mouse Skin Model. Promotion assays.** Two laboratories have reported results of promotion assays in mouse skin (76–79). In Sweden, Rannug and colleagues have conducted two experiments to determine whether 50–Hz sinusoidal magnetic fields promote DMBA-induced skin tumors (76,77). In the first, NMRI/HAN female mice (30/group) were exposed to magnetic fields (50 or 500 μT, 19 hr/day) a week after initiation with 25.6 μg DMBA (topical). No field-related differences in tumors were observed among 103 weeks of promotion, nor were there evidence of field-induced hyperplasia at 9, 26, or 52 weeks. Two observations not discussed by the authors may be of interest: a) Figures 1 and 2 in their paper suggest a delay in the appearance of tumor-bearing animals in both MF-exposed groups; however, neither result was statistically significant; and b) no squamous cell carcinomas were observed in the control group treated with DMBA alone, but squamous cell carcinomas were observed in both exposed groups as well as in the DMBA + TPA control.

In their second promotion experiment, Rannug et al. (77) used the more sensitive SENCAR mouse to evaluate whether the intermittency of MF exposures may be a determinant of tumor development. Female mice were exposed to MFs a week after initiation with 2.56 μg DMBA. MF exposures occurred 19 hr/day for 104 weeks and were either continuous or intermittent (15 sec on, 15 sec off), at magnitudes of 50 or 500 μT. The results obtained using continuous exposure conditions appear to confirm their previous negative results. However, since no tumors occurred in the continuously exposed groups, observations from their previous experiment on latency and squamous cell carcinomas could not be compared. Investigators suggest that the intermittent exposures could be weakly promoting, but that the interpretation is uncertain. Since the fields were turned off by mechanical relay, uncharacterized transient were present and it is unknown whether the weak effect, if real, is due to intermittent exposure or to transients. Although there is an increase in the number of tumor-bearing animals in both intermittent exposure treatment groups, neither increase is statistically significant when compared to controls. When the intermittent groups are combined, the results are still not statistically significant.

Two statistical tests were significant at the p < 0.05 level: first, the comparison of the two intermittently exposed groups to the two continuously exposed groups; and second, a linear regression analysis comparing the cumulative number of skin tumors among skin tumor-bearing animals at different dose levels of intermittent MF exposures.

The investigators consider the linear regression test to be the stronger of the two results (B Holmberg, personal communication). Of additional interest is the fact that there appears to be a decrease in the latency period for the 500-μT intermittent exposure group compared to the DMBA controls. As in their previous experiment (76), the only squamous cell carcinomas observed were in the TPA controls and in an MF-exposed group (500 μT).

Stuchly et al. (78) and McLean et al. (79) exposed female SENCAR mice (32/group) to 2 mT (60 Hz), a flux density four times greater than Rannug et al. (76), but for a shorter duration—6 hr/day, 5 days/week for 21 weeks, beginning 1 week after initiation with DMBA (2.56 μg, topical). The investigators reported (79) that
no tumors were observed at 22 weeks when the experiment was terminated and conclude that, though data are limited, 60-Hz MFs do not act as tumor promoters. However, since the DMBA-treated controls also developed no tumors over the course of the experiment, weak promoter activity could have been obscured.

Also, since mouse strains were different and the McLean et al. (79) experiment was terminated after only 22 weeks, comparisons between this result and that obtained by Rannug et al. (76) are difficult. It does appear clear, however, that if there is any promoting effect of 50 or 60 Hz MFs in this model system, it is weak.

Co-promotion assays. McLean et al. (79), using the same DMBA treatment protocol and MF exposure conditions as above, also treated animals with TPA (1 μg/week) beginning 1 week after DMBA treatment. The mean number of tumors per animal at the termination of the experiment (22 weeks) in MF-exposed and control groups were similar. However, the authors noted a slightly earlier appearance of papillomas in the MF-exposed group, but this difference was not statistically significant. However, the spleens from TPA- and field-exposed mice were enlarged relative to TPA and sham-field mice and NK cell activity associated with TPA + 2 mT was somewhat diminished, suggesting that the MF had caused subtle effects (79). In addition, the positive control group that received 2 μg TPA twice per week had no more tumors than those groups receiving 1 μg once per week, suggesting the system may have saturated at the lower TPA dose, leaving no opportunity to detect a co-promotional effect.

The same investigators undertook a second series of co-promotion experiments using a lower amount of TPA (0.3 μg of TPA per week as compared to 1 μg per week) (80). They reported that, compared to the TPA-only group, the rate of tumor development (papillomas) was significantly greater in the TPA + 2 mT group. Although tumor incidence and mean number of tumors per animal were significantly increased in the field-exposed animals during the latter part of the promotion period (16 to 18 weeks), this difference was not statistically significant by week 23 of promotion. The investigators interpreted these findings cautiously, but concluded that the results were suggestive of a possible co-promotional effect of the MFs. Subsequent replications of this experiment produced no differences in tumor incidence at 23 weeks between treatment and control groups associated with the MFs (81).

A recent report (82) documented results of the Stuchly et al. experiment (80) from weeks 24 to 52 during which field exposure was maintained but TPA was discontinued. McLean et al. (82) describe a higher, though not statistically significant, percent of tumor-bearing animals in the MF-exposed group at 52 weeks compared to sham-exposed controls. They report that a higher fraction of mice in the field-exposed group had malignant tumors (8 of 48 mice) as compared to the sham-exposed group (1 of 48), a statistically significant difference (p < 0.03). Though the authors were cautious in their interpretation of these data, they concluded that MF exposure may have accelerated progression to malignancy.

In abstract form, Byus et al. (83) have recently reported results of a similar experiment employing either of two dose levels of TPA 2 times/week (0.25 or 0.5 μg). Female SEN CAR mice in groups of 60 each were used, and MF exposure was 60 Hz, 2 mT, 7 hr/day, 7 days/week. DMBA (10 nmol) was administered 11 days prior to MF exposure. After 43 weeks of MF exposure a higher incidence of papillomas was observed in the MF-exposed group treated with the lower dose of TPA (52% in the MF-exposed group compared to 30% in sham-exposed controls), but after 33 weeks there were no field-related differences in the higher TPA dose group. The authors conclude that the 2 mT magnetic field may act as a co-promoter.

A co-promotion study was also conducted in SEN CAR mice treated with a single subcarcinogenic dose of DMBA and exposed for 23 weeks with three different doses of TPA (0.52, 1.05, 2.1 μg) administered twice per week (84,85). Mice were exposed to 2 mT (60 Hz) MFs or in an ambient field. In addition to papilloma induction, early biomarkers of tumor promotion, including epidermal hyperplasia, PKC, and ornithine decarboxylase, were monitored. For all three TPA doses, there were no field-related increases in tumors, expressed either as percent of mice with papillomas or as papillomas per mouse. Biochemical analyses were also negative.

Summary. Results of promotion experiments in the mouse skin assay system are substantially negative. Recent reports suggest the possibility of late or delayed effects associated with MF exposure in co-promotion assays. Evidence appears negative with respect to co-promotion at earlier time points.

Animal Models of Human Leukemia

Though results are not definitive, several epidemiological studies have pointed to the possibility that chronic human exposure to EMF may result in increased risk of childhood and adult leukemia (86–89).

A variety of rodent in vivo models of human leukemia are available [e.g., see Pattengale and Taylor (90) for review]. Most models were developed to study the effectiveness of potential chemotherapeutic drugs and to study postinitiation events in the progression of leukemia. In some of these models, leukemia is initiated by exposure to known genotoxic agents, such as the ENUs (91), DMBA (92), or X-rays (93). In other models, leukemia is initiated by transplantation of leukemic cells into nonleukemic recipient animals (94,95).

We will discuss five leukemia/lymphoma model systems currently in use to test for possible carcinogenic effects of magnetic fields: the mouse thymic lymphoma model of Kaplan and Brown (93); two leukemia transplant models [a large granulocytic (LGL) cell line passaged in F344 rats (95) and a P388 murine leukemia cell line (96,97)]; and two transgenic mouse models (98,99).

Lymphoma in Mice Preexposed to X-rays. Over 40 years ago, Kaplan and Brown pioneered the development of a thymic lymphoma model in mice (93). In the C57Bl strain, which has a low (6–8%) spontaneous incidence of lymphoma, fractionated X-irradiation results in a dose-dependent increase in thymic lymphoma incidence. In Kaplan and Brown’s original experiments, they defined an effective X-irradiation dose (283 roentgens) and an optimal interval for delivery of fractionated doses (4–8 days).

This model has commonly been employed to study the process of lymphomagenesis; see reviews by Haran-Ghera and Peled (100), Janowski et al. (101), and Boniver et al. (102). Though lymphomas induced by this procedure are predominantly of thymic origin, the thymus does not appear to be the site of initiation. The most compelling evidence is the observation that thymic lymphomas arise in thymus grafts after transfer to thymectomized irradiated hosts.

A complex process appears to be involved in lymphomagenesis, including interactions between target cells, the bone marrow, and the thymic environment. Irradiation apparently results in preneoplastic cells that then require a period of residence in the thymus before progressing
to lymphoma (103). The initiating events may occur in thymic precursor cells in the spleen or bone marrow that later migrate to the thymus and then become neoplastic when normal T-cell maturation is arrested.

Kaplan suggested a possible mechanism, reviewed by Haran-Ghara and Peled (100) and Janowski et al. (101), involving multiple effects of X-irradiation: activation of a potentially leukemogenic retrovirus; damage to the thymus, followed by regeneration leading to the appearance of virus-susceptible cells; and injury to bone marrow, preventing repopulation of healthy thymocytes.

Janowski et al. (101) have reviewed the role played by viruses in this process. A retrovirus, the radiation leukemia virus (RadLV) can be isolated from cell-free extracts of murine thymic lymphomas induced by X-rays. RadLV is an endogenous C-type oncovirus (104). It is thymotropic and gains potency on serial passage. It has been suggested, since recombinant proviruses are not detected in the majority of X-ray-induced thymic lymphomas until after further clonal growth in vitro or further tumor development after in vivo transplantation, that proviruses may be involved in tumor development subsequent to initiation.

Activated N- or K-ras oncogenes have also been detected in most X-ray-induced thymic lymphomas and it has been suggested that somatic mutations at the ras locus could be causally involved. Also perhaps associated with neoplastic progression is the observation that trisomy of chromosome 15 is frequently associated with X-ray-induced thymic lymphomas in the later stages of development.

In general, it appears that X-irradiation initiates hematopoietic cells, which are then promoted to a neoplastic state. As originally suggested by Kaplan (105), events induced by irradiation are also probably involved in the promotion process. Thus, X-irradiation could interfere with physiological differentiation signals in the thymus, rendering the cells more likely to continue on a path toward neoplasia.

The model may be useful for studying postinitiation processes since manipulation of the protocol apparently can uncouple initiation and promotion stages. Thus, interferon (106) and exogenous cytokines [reviewed by Boniver et al. (102)] can inhibit postinitiation processes and increased immune impairment (100) or treatment with urethane (107), can enhance it. Also, it has been suggested that some leukemogenic agents could exert their transforming effects without necessarily also conferring on the initiated cells the ability to proliferate independently (100). Thus, split-dose X-irradiation (using the Kaplan and Brown protocol) acts as a complete carcinogen, inducing both initiation and postinitiation events. However, infection with an induced endogenous provirus, or treatment with a leukemogenic agent, may be necessary for neoplastic development after treatment with weak leukemogenic agents. For discussion, see Haran-Ghara and Peled (100).

Because EMF is unlikely to act as an initiator, this model may provide a means to ask whether it can interact with the thymic microenvironment to modulate postinitiation events in initiated preleukemic cells. In this regard it may be of interest to use a protocol involving either lower doses of X-irradiation or weaker leukemogenic agents to focus the protocol more clearly on postinitiation events.

Tests of magnetic fields in the mouse lymphoma/X-irradiation model. Svedenstal and Holmberg (108) exposed female CBA mice to sawtooth 15-μT peak to peak pulsed vertical 20-kHz MFs for their lifetime (up to 131 weeks). MF exposure began immediately after the first of four X-ray exposures and continued for the lifetime of the animals. The total X-ray dose rate was 0.45 Gy/min and total irradiation was 5.24 Gy, divided into four exposures administered at 4-day intervals. Blood cell counts and the incidence of thymic and nonthymic lymphomas were recorded.

The data show a significant increase in the number of leukocytes in exposed animals, whether or not animals were treated with X-rays. Svedenstal and Holmberg indicate these high values were due to extreme values in two animals. No statistically significant effects on thymic or nonthymic lymphoma incidence were observed in the groups exposed to MFs.

While this study is of interest, it is difficult to interpret for two reasons. First, as the investigators indicate, experimental and control groups were started at different times and used different batches of animals. The tumor incidences reported for each experimental group, including controls, were totaled over three to four subgroups that entered the study at times differing by as much as a year. Comparisons between such nonconcurrent experimental and control groups should be made with caution. Second, as the investigators also point out, the X-ray-induced incidence of lymphoma was higher than expected (66%), resulting in limited statistical power of the assay.

Babbitt et al. (109) are conducting a large study (195–450 mice/group) using the Kaplan model, in which each of four groups of mice receiving fractionated ionizing radiation (Cobalt 60) at 0, 350, 475, and 600 R, will be chronically exposed to either 60-Hz circularly polarized MFs with 1 mT horizontal and vertical components, or to a sham field. MF exposure began on the first day of irradiation and will continue 18 hr/day for the 2-year duration of the study. Evaluation will be by morphologic and histopathologic analyses.

Leukemia Transplant Models. Large granular lymphocytes passed in vivo in F344 rats. Large granular lymphocytes (LGL) comprise 10 to 15% of peripheral blood mononuclear cells in rats. A class of leukemias involving the proliferation of LGL occurs in both humans and rodents. LGL in humans are believed to be NK cells or in vivo activated cytotoxic T lymphocytes (110). Stromberg (111) and Rosol and Stromberg (112) have pointed out the morphologic, biochemical, and functional similarities between human and rat LGL and have also indicated similarities in the clinical profiles of the LGL leukemias in humans and the Fischer rat.

Stromberg et al. (113) proposed a passed leukemia cell model in which leukemic cell lines are maintained in vivo by serial transplantation in male Fischer F344 rats. Dieter et al. (114) have employed this model as a short-term assay for potential antileukemic agents. In their procedure, passed cells are harvested and injected subcutaneously into 8-week-old syngeneic recipients. The chemical to be tested is usually administered at the time of transplantation. After 70 days, the animals are sacrificed and hematological, histological, biochemical, and clinical data are used to characterize the progression of the disease. The major parameters reported are spleen weight, white blood cell count, and hematological indices.

The morphological, clinical, and biochemical characteristics of the leukemia response in transplant recipients is similar to the spontaneous disease in Fischer rats (115,116). Furthermore, the protooncogene associated with the spontaneous disease (c-fms) is found in transplant recipients (117). The onset of the disease (118) is characterized by LGL infiltration of the spleen resulting in splenomegaly and, often, infiltration of the bone marrow. This is usually accompanied by severe
hemolytic anemia, enlargement of lymph nodes, and a number of other well-defined clinical features (111,119).

Because this model employs previously initiated neoplastic cells, biological factors that may be involved in progression are of primary interest. Proliferation of hematopoietic cells appears to be controlled by a variety of growth factors. Increasing evidence favors an autocrine growth model wherein abnormal production of a growth factor by a cell with receptors for that factor results in neoplastic proliferation (120,121). In fact, it has been shown that the product of the oncogene c-fms, which is associated with LGL leukemia in Fischer rats, is related to the receptor for the mononuclear phagocyte growth factor (CSF-1) (122).

It is also of interest that dietary restriction (DR) significantly slows the proliferation of LGL leukemia cells in the F344 rat transplant model (123), as it does with many other types of neoplasms (124).

The model was used to test five substances previously found to decrease or increase the incidence of leukemia in 2-year chronic carcinogenesis bioassays in F344 rats (114). All five substances had activities in the short-term transplant model similar to those seen chronically. While these results are of interest, they may not be generalizable to the LGL system as usually employed. Dieter et al. (114) used a slow growing LGL cell line and observed only small increases in leukemia end points in transplant controls as compared to nontransplant controls. For example, the difference Dieter et al. (115) observed in white blood cell counts in nontransplant controls and transplant controls was less than 2-fold as compared to the more than 10-fold increases later reported by Dieter (125) and by others (95,126). Perhaps not surprisingly, the result reported by Dieter et al. (114) for one of the five substances, 2,4,6-trichlorophenol (TCP), was not replicated in a recent report from a different laboratory (126).

P388 Leukemia Cell Transplant Model. Since 1968, the P388 leukemia cell transplant model has been a standard pre-screen used in the preclinical drug testing program of the National Cancer Institute (NCI) (96,127). P388 is a rapidly growing leukemia cell line that is highly sensitive to a wide spectrum of cytotoxic drugs. This is an advantage in that the assay is relatively simple to perform, is of short duration (20–30 days), and few cytotoxic drugs are undetected by the system. However, the extreme sensitivity of the system is also considered to make it less clinically relevant than other slower growing tumors [e.g., see Budel et al. (128)], or tumors that have drug resistance markers characteristic of many clinical forms of leukemia [see Yang et al. (129)].

In a typical chemotherapeutic drug prescreening assay, about 10^6 P388 cells, as diluted ascitic fluid, are injected intraperitoneally in BDF_1 or CDF_1 mice (six animals/test group). Drug treatment begins on the day after implantation. The lifespan of treated animals is usually less than 3 weeks (97). Typically, the only parameter monitored is survival time. An increase of at least 25% in median survival time over controls is considered a positive result. An agent positive in this prescreen is then tested further in other screening assays prior to entering clinical trials (130).

Over the years, the drug-testing program of the NCI has screened more than 300,000 chemicals using this system. As of 1984, some 10,000 compounds were routinely tested each year (130).

Tests of magnetic fields in leukemia transplant models. Large granular lymphocyte model. Results of tests of 60-Hz magnetic fields using the LGL model have recently been reported in abstract form (131,132).

In these studies, groups of 18 animals were exposed to 1.0 mT fields (20 hr/day) beginning at the time of injection, with LGL leukemia cells, with exposure continuing for the duration of the experiment. The first set of experiments (131) used continuous fields and the second (132), in addition to replicating the continuous exposure condition, also tested intermittent fields (off/on at 3-min intervals). The 1996 study also examined effects at a 10-fold smaller inoculum in addition to replicating the original experiment using 2.2×10^7 cells. Controls were a null energized field (about 2 μT) (only in the 1994 study), ambient controls (0.1 μT or less), and γ-irradiated positive controls. Blood samples were taken weekly for 10 weeks from serially sacrificed animals or from animals following death. Hematological end points included Hb (hemoglobin) concentration, RBC (red blood cell), differential WBC (white blood cell), platelet cell counts, peripheral blood tumor cell counts, and other parameters. No significant exposure-related differences were observed in hematological parameters or mortality in either study. Further analysis of peripheral nucleated blood cells and survival data for the 1996 study are in progress.

P388 model. Thomson et al. (133) used the P388 leukemia transplant model in female DBA/2 mice to examine 60-Hz 1.4, 200, or 500 μT MF effects. Groups of 10 mice each were implanted with 1×10^5 P388 cells at about 8 weeks of age and then exposed until death (about 2 weeks) to the MFs (6 hr/day, 5 days/week) beginning 2 to 3 hr after the implant. Sham-exposed controls, both nonimplanted and implanted, were run in parallel to the MF-exposed groups. End points determined were survival, spleen weight, and body weight. No statistically significant effects were reported. Though no effect on the incidence or progression of P388 leukemia was apparent, this model, because of the rapid development of leukemia, may not be sensitive enough for the detection of weak effects.

Transgenic Models. Transgenic models utilize specially constructed mouse strains that are constitutive for the expression of specific oncogenes. A number of models are available, which express most classes of oncogenes; see reviews by Adams and Cory (134), Hanahan (135), and Connelly et al. (136). These classes include genes encoded for growth factors, growth factor receptors, proteins believed to be involved in signal transduction pathways, cytoplasmic protein kinases, genes that affect transcription or cell division, and tumor suppressor genes [reviewed by Adams and Cory (134)].

In this brief discussion we will focus on two murine transgenic models currently in use to test for potential effects of magnetic fields on lymphomagenesis: the ENU/Pim-1 and TSG-p53 systems.

ENU/Pim-1 system. The Pim-1 oncogene was originally discovered because it is activated by the Moloney murine leukemia virus in conjunction with induction of T-cell lymphomas (137). This oncogene encodes two cytoplasmic protein kinases (138) and is highly expressed in many human hematopoietic malignancies (139,140). It is primarily expressed in hematopoietic tissues and gonads (141,142).

About 10% of transgenic mice over-expressing the Pim-1 oncogene in lymphoid cells develop T-cell lymphomas (143). If such mice are treated at day 15 after birth with initiating doses of ENU as low as 15 mg/kg bw (ip) and followed for 34 weeks, the latency period is significantly decreased and the frequency of lymphomas is greatly increased (98). Overexpression of c-myc is observed in the ENU-induced lymphomas (but not in spontaneous tumors) in the Pim-1 strains, suggesting that both c-myc and Pim-1 are involved in lymphomagenesis in this system. Studies not involving ENU with double transgenic mice containing
both Pim-1 and myc oncogenes also suggest a strong synergism between these two oncogene classes in both B- and T-cell lymphomagenesis (148).

Studies using either Eμ-Pim-1 strains, which overproduce Pim-1, or Pim-1-deficient strains, have sought to elucidate the mechanism of oncogenesis induced by Pim-1. Although a precise mechanism is still unclear, several studies have provided clues. First, Pim-1 does not appear to increase susceptibility to mutagenesis, at least as measured by the micronucleus assay (145). Second, Pim-1 may play an important role in growth signaling from the erythropoietin receptor (146), or in modulation of the interleukin-3 (IL-3) signal transduction pathway (147). In fact, Pim-1 is induced by IL-3 (148). Third, Pim-1 may inhibit apoptosis (149).

The p53 system. The complex functions of p53 have recently been thoroughly reviewed (150–152). p53 is a tumor suppressor gene. Its inactivation is associated with the progression stage of carcinogenesis (153–155) and with enhanced genetic instability (99). p53 induces transcription of a number of genes and is part of a checkpoint system that prevents damaged DNA from being replicated by arresting the cell cycle in G1 [reviewed by Hartwell and Kastan (151)]. Inactivation of this checkpoint is believed to enhance genomic instability, thus furthering the likelihood of cellular progression to malignancy (99). The association of p53 inactivation with progression is based on studies using the multistage mouse skin model (153), erythroleukemia development in mice (154), and observations in humans (155). As reviewed by Danehower and Bradley (150), p53 may also act prior to the progression stage in some other animal models of carcinogenesis.

The interactions of p53 with cellular processes is complex and incompletely understood. p53 accumulates in the cytoplasm during G1 and migrates to the nucleus when the S phase begins (156). It appears to regulate transcription by binding directly to DNA in concert with certain coactivators, transmitting a transcription activation signal in a transcription initiation complex (157). Enhanced susceptibility to tumorogenesis has been linked to inhibition of this DNA binding capacity.

p53 has also been linked to maintenance of asymmetric division kinetics of renewing cells, the primary mode of cell division in adults (158), apoptosis (159,160), DNA repair (161), gene amplification (162), and mutator and hypermutable phenotypes (163).

Mutations at the p53 locus are common in a wide variety of human cancers (164). It is interesting that both transgenic mice overexpressing a mutated form of p53 (165) and p53-deficient mice (99) have enhanced susceptibility to the development of spontaneous tumors, particularly soft-tissue tumors and lymphomas. Almost 80% of p53-deficient mice develop malignant lymphomas involving a variety of organs, including the thymus, heart, lung, spleen, liver, kidney, and brain by about 5 months of age, compared to 30% of transgenic mice expressing a mutated form of p53 in 18 months (165). The characteristics of tumors observed in p53-deficient mice are similar to those of human childhood tumors, which has led some to suggest that these mice may be useful models for childhood cancers (166). Mice heterozygous for p53 are also susceptible to spontaneous tumors, but less so than the homozygous mice. It has been suggested that the longer latency period and the lower spontaneous tumor frequency in heterozygous mice make them more suitable for use in carcinogenesis test protocols (166). This suggestion was borne out in comparative tests of homozygous and heterozygous strains using dimethylnitrosamine (DMN) as an initiator (166). Both p53 transgenic mice and p53-deficient mice are also more sensitive to carcinogenesis induced by ionizing radiation (167).

Tests of magnetic fields in transgenic systems. Studies have recently been completed using Pim-1 and TSG-p53 transgenic mice to assess the potential effects on lymphomagenesis of continuous exposure (18.5 hr/day to 60-Hz MFs at 0, 2 μT, 0.2 mT, and 1 mT) or intermittent exposure (1 mT, 1-hr on/off cycle) (168). Animals (30–40 of each sex) were used at each exposure condition and parameters evaluated were lymphoma incidence and latency period. No increases in lymphoma incidence over controls were observed under any MF exposure condition.

Repacholi et al. (169) recently presented an in-progress report of a carcinogenicity study using Eμ-Pim-1 transgenic mice in which groups of about 100 mice were exposed to 50-Hz continuous magnetic fields of 1 μT, 0.1 mT, and 1 mT and intermittent fields of 1 mT (50 Hz, 15 min on/off) 20 hr/day for up to 18 months. The final evaluation will include a full pathological analysis as well as immuno-phenotyping of lymphoid cells.

Rat Brain Model

A number of human epidemiological studies have reported an association between exposure to EMF and increased brain cancer risk, most recently a large study of electrical utility workers (170). There are a number of brain cancer animal models available, including those involving carcinogen- or virus-induction, cell or tissue transplantation, and transgenic systems; see reviews by Peterson et al. (171) and Kleihues et al. (172). In this brief summary, we will focus on an ENU-induction model in rats.

The effectiveness of the nitrosoureas, particularly MNU and ENU, in inducing brain cancer after transplacental administration, was demonstrated some 25 years ago (173). The nitrosoureas induce gliomas in the brain or spinal cord, most commonly oligodendrogliomas and Schwannomas (also called neurinomas) in peripheral nerves.

The nitrosoureas are mutagens that alkylate guanine at the O6 position. Their efficiency in inducing brain tumors may be due to the slow repair of these lesions in the brain [reviewed by Maekawa and Mitsumori (174)]. Target genes for ENU mutagenesis have not yet been identified with certainty. However, mutations in tumor suppressor genes have been associated with brain tumors in humans [reviewed in Brüstle et al. (175)]. These could be target genes for ENU mutagenesis. A variety of oncogenes have also been associated with malignant gliomas [reviewed by Peterson et al. (171), Radner et al. (176), and Bigner and Vogelstein (177)] and these are also candidate target genes for ENU.

As is the case for humans, spontaneous tumors in the rat brain are relatively rare, though there is considerable variation in both incidence and tumor type among rat strains (178). The frequency and type of neurogenic tumors produced by the nitrosoureas also are quite variable depending upon the route of administration, rat strain, and age. Newborns and neonates are particularly sensitive to induction. It has been proposed that target cells are either embryonal matrix cells or oligodendrocyte precursor cells [reviewed by Maekawa and Mitsumori (174)].

Indirect evidence suggests that carcinogenesis in rat brain is a multistage process (175). For example, O6-ethylguanine lesions in brain are similarly induced and repaired in two strains of rats with significantly different sensitivities to neurogenic tumor induction by ENU (178), suggesting that O6-ethylguanine is not sufficient for tumorigenesis.
In a typical protocol, ENU is administered to female rats in the third week of pregnancy [e.g., iv, 10 mg/kg (179)] or to newborn rats [e.g., sc, 17 mg/kg (180)]. If initiator treatment was transplacental, promoter treatment begins in newborn pups; if initiator treatment was postnatal, promoter treatment begins several weeks after initiation. Early lesions appear at about 2 months of age following transplacental ENU exposure. Tumor incidence and histopathology are evaluated after about 6 months.

The heavy metal zinc acetate (180) and the skin tumor promoter TPA (181) have been reported to increase the incidence of brain tumors after transplacental administration of ENU, though others (179) have not considered these results definitive. Several substances including the drugs buformin [reviewed by Maekawa and Mitsumori (174)], the cytokine TNF-α (182), and NGF (nerve growth factor) (183) have an inhibitory effect on transplacental ENU-induced neurogenesis. Chronic stress (184), which has been associated with enhancement of some forms of cancer, and phenobarbital (185) were not observed to increase the incidence of transplacental ENU-induced brain tumors in rats.

Though the rat model system has some similarities to the human disease, there are also significant differences. Thus, the primary tumor types induced in the rat brain, particularly oligodendrogliomas and astrocytomas, also occur in adult humans. However, in humans, there is an increased incidence of brain cancer in childhood that decreases in early adulthood and is not observed in rats unless they have been exposed transplacentally to carcinogens [reviewed by Maekawa and Mitsumori (174)]. (Some have suggested the young-age peak in humans may be due to prenatal carcinogen exposure.) There are also significant sex differences in human brain cancer (186) but not in rats (174). Additionally, the predominant childhood brain tumor type in humans is not induced by the nitrosoureas in rats. Rat esthesioneuroepitheliomas, which are induced by nitrosamines but not the nitrosoureas, are histologically similar to the human tumors and have been suggested as a model for human cancer (187). (However, this tumor does not occur in the brain, but in the sensory epithelium of the nasal cavity.)

**Tests of Magnetic Fields in the Rat Brain System.** Tests are currently in progress using the ENU transplacental model in Fischer rats. Experiments are planned in which animals will be chronically exposed 20 hr/day to 2, 20, 200, and 2000 µT (188,189).

Brugere et al. (190) report, in abstract form, no effect on survival time after chronic treatment of weanlings with homogeneous 50-Hz magnetic fields of 1, 10, or 100 µT. In this experiment, Sprague-Dawley females were treated with ENU (50 mg/kg, iv) on day 19 of pregnancy and weanlings (60 males and 60 females in each exposure group) were exposed to MFs for their lifetimes. Though the authors conclude from these results that there was no effect on ENU-induced brain tumors, this conclusion is unwarranted without supporting histopathological analysis. Investigators do not state whether any animals in the study had central nervous system tumors.

**Two-year Chronic Bioassays in Rats and Mice**

Under the auspices of the NTP, more than 400 chemicals have been evaluated for carcinogenic potential using 2-year chronic bioassays in F344 rats and B6C3F1 mice over the past 15 years (191). NTP studies specify a rigorous methodology (192,193), which, while not uncontroversial [e.g., Ames and Gold (194)], is considered to be the most thorough rodent carcinogenesis bioassay available. The protocol is primarily directed at detection of initiator activity. However, since the animal strains employed develop a variety of spontaneous tumors, the protocol is also suitable for detection of agents capable of promoting neoplastic processes involved in spontaneous neoplasia.

In the NTP protocol, relatively large numbers of animals of both sexes in both species are exposed to at least two doses of test substance in addition to a sham control. The animals are monitored over the course of their lifetimes for survival, body weight, and clinical signs of neoplasia. At death, or at termination of the experiment, all animals are evaluated by complete necropsy and histopathological examinations.

**Tests of Magnetic Fields Using Chronic Bioassay in Rats and Mice.** (See also “Tests of Magnetic Fields in Lymphoma/X-Irradiation.”) Studies to assess potential carcinogenic effects of MFs using the standard NTP 2-year chronic bioassay protocol have been initiated (188,189,195). In the McCormick et al. study (195), some 2000 male and female F344 rats and B6C3F1 mice will be exposed continuously (18.5 hr/day for 2 years) to 60-Hz MFs at 0, 2 µT, 0.2 mT, or 1 mT in exposure groups of 100 animals each. One intermittent exposure to 1 mT (1 hr on, 1 hr off) will also be included. Exposure will be completed in 1996 and final results are expected in 1998.

In the Mandeville et al. study (188,189), 5 groups of 50 female F344/N rats will be exposed 20 hr/day from birth to 2 years of age to linear sinusoidal continuous-wave 60-Hz MFs at sham plus four intensities (2, 20, 200, and 2000 µT). Histopathology will be performed on all tissues following NTP protocols.

Yasui et al. (196–198) reported, primarily in abstract form, that continuous exposure of F344 rats (48 male and 48 female) for 2 years to 0.5 mT or 5 mT 50-Hz sinusoidal alternating MFs did not result in a statistically significant difference in neoplastic end points compared to sham-exposed controls. Analysis involved a comprehensive histopathological examination of organs, including hematomal tests. Unfortunately, there is insufficient detail in published reports for a thorough evaluation.

Mikhail and Fam have reported, mostly in abstract form, that continuous multigeneration exposure of mice to a 60 Hz, 25-mT MF results in highly statistically significant increases in lymphomas in exposed animals compared to unexposed controls (199–201). The authors indicate that positive results were obtained in separate experiments involving 24 second-generation mice exposed for an average of 120 days, 53 third-generation mice exposed for 133 to 257 days, and 41 third-generation mice exposed for 363 to 418 days. Unfortunately, there is insufficient detail in published reports for an adequate evaluation.

**Conclusion**

We have reviewed eight major animal models currently in use to test for potential carcinogenic properties of EMF (Table 1). For each model we have discussed its development, multistage characteristics, relevant biological mechanisms, and test protocol characteristics (summarized in Table 2). We have also compiled a referenced list of substances that have tested positive for promoter activity in three widely used models (Tables 3,6,7).

The models we have reviewed represent an imaginative and diverse collection of methods for detecting agents capable of enhancing the carcinogenic process. With the exception of the 2-year chronic bioassay protocols, the models discussed focus primarily on detecting agents that affect the promotion or progression stages of
carcinogenesis. Some of these systems use engineered strains of rodents (e.g., SENCAR or transgenic strains of mice). Others result in assay end points that have uncertain relevance to malignant disease (e.g., AHF, papillomas), or may be dependent upon a highly specialized protocol (e.g., the two-stage mouse skin protocol involving use of both an initiator and promoter).

It will be important to continue to address the relevance of results in these systems to human cancer risk. A number of factors involving species differences, the relationship of the induced neoplasm to human disease, and protocol-specific effects must be considered.

Tests of MFs that are completed or in progress in these systems have also been reviewed (results are summarized in Table 5). These include all completed animal studies and those currently in progress that use animal cancer models to test for the effects of static and low-frequency EMF.

Nearly all studies cited used MFs at the common power delivery frequencies of 50 or 60 Hz. These frequencies fall in the extremely-low-frequency spectrum, defined as 3 Hz to 3 kHz. A few other studies used either 60-Hz electric fields, static MFs, or MFs at frequencies of 15 to 20 kHz, typical of video display terminals. Typical time-averaged residential exposures are around 0.1 μT and seldom exceed 0.5 μT. However, some individuals, especially in occupational environments, are occasionally exposed to much higher fields—up to 1 to 2 mT. These high exposures usually involve only a portion of the body. Most animal studies use MFs on the order of 1 mT, which are 3 to 4 orders of magnitude higher than average residential exposures, but in the upper range of occupational human exposures.

Though most results are negative, weakly positive or equivocal results involving one or more test parameters have been reported in several systems: the rat mammary gland system; the rat liver focus system; and the mouse skin system. It may be of interest that positive effects reported in both the rat mammary gland and the mouse skin systems suggest that EMF at low flux densities may enhance the latter stages of carcinogenesis. The positive reports of Fam and Mikhail (199) and Mikhail and Fam (200,201) are very difficult to evaluate (see "Two-year Chronic Bioassays in Rats and Mice"). As shown in Table 5, some experiments are still in progress [mouse lymphoma (109), transgenic mouse (169), rat brain (189), and 2-year chronic bioassays (189,195)].

It should be noted that interpretation of negative results in EMF carcinogenesis studies could be complicated by the possibility of window effects, though their existence is controversial; see Bowman et al. (202) for discussion. If EMF effects on carcinogenesis were to involve such phenomena, it would be difficult to conclude that negative results at relatively high exposures implied negativity at lower exposures, such as is routinely assumed for chemical effects on carcinogenesis.

The interpretation of the weakly positive results discussed above is complex. First, results reported positive are at a weak level of statistical significance (p < 0.05). That such results could be the result of uncontrolled variability is a concern, particularly because of the sensitivity of the promotion response to modulation by numerous endogenous and exogenous substances. It is therefore especially important that weakly significant results be independently corroborated before any definitive conclusion is drawn. Second, in some cases, positive results are in one measured parameter and not in other related parameters measured in the same assay. Taken together with the weak (p < 0.05) statistical significance, the absence of an effect on more than one related parameter also tends to decrease confidence in the validity of the positive results.

The results reported by Lösch and his colleagues require special commentary. These authors have provided a series of experimental neoplastic and related biochemical evidence that they believe strongly suggests that weak 50-Hz MFs in the 50 to 100 μT range act as a promoter or co-promoter in the rat/DMBA mammary carcinoma model. It is important to note that their experiments are currently undergoing independent replication.

Though testing is incomplete, based on results so far available it appears that any effect of EMF in these model systems is weak. This should be carefully considered in the protocol design of future studies. Realistically, it may be difficult to fine tune these assays enough to demonstrate unequivocally the presence or absence of very weak effects. It may therefore be advantageous to expand end points in these systems to include biochemical or biophysical parameters relevant to possible carcinogenic mechanisms of EMF. Because EMF does not appear to have genotoxic potential at environmentally relevant exposures [reviewed by McCann et al. (203) and Murphy et al. (204)], any carcinogenic potential would most likely involve nongenotoxic pathways [for discussion see Cohen and Ellwein (205) and Kavet (206)]. Further, since EMF does not appear to have toxic effects that could lead to regenerative hyperplasia, any such effect of EMF on carcinogenesis would most likely be through a nontoxic proliferative stimulus mechanism.

As discussed in the text, there are many biochemical or other parameters reflective of biological processes known to be associated with carcinogenesis in the different model systems. A number of these parameters may be appropriate for use in expanded test protocols. Parameters that are also known to be possibly associated with EMF bioeffects may be of particular interest. Examples are effects on membrane function (such as may affect the phospholipid-dependent enzyme PKC), reactive oxygen species, calcium ion mobilization, or the hormonal environment (particularly melatonin). Whereas such expanded protocols may permit more definitive conclusions as to the potential adverse health effects of EMF in animal systems, the additional parameters added to already complex protocols will also raise statistical issues that will need to be taken into account in protocol design.

**Note Added in Proof:** After this paper was in press, Lösch and colleagues reported that they had successfully replicated the 100 μT experiment (Lösch W, Mevissen M, Häußler M. Exposure of rats to a 50-Hz, 100 μT magnetic field increases the development and growth of mammary tumors in a DMBA-model of breast cancer replicate study. In: The Annual Review of Research on Biological Effects of Electric and Magnetic Fields from the Generation, Delivery & Use of Electricity, San Antonio, TX, November 19, 1996. W/L Associates, Ltd., Frederick, MD. pp 7–8). In addition the in press work of C. Graham et al. cited on MF exposure and plasma melatonin in humans has been published [Graham C, Cook MR, Rife DW, Gerkovich MM, Cohen HD. Nocturnal melatonin levels in human volunteers exposed to intermittent 60 Hz magnetic fields. Bioelectromagnetics 17:263–273 (1996)].
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