3. Studies of Carcinogenicity in Experimental Animals

3.1 Chronic exposure studies

The results of one- and two-year rodent bioassays are summarized in Table 32.

3.1.1 *Mouse*

Groups of 100 male and 100 female B6C3F_1 mice, six to seven weeks of age, were exposed for 18.5 h per day for two years to linearly polarized 60-Hz magnetic fields that were nearly pure, transient-free and had less than 3% total harmonic distortion. [The group-size used in this study was twice that commonly used in chronic rodent bioassays; this enlargement was purposely chosen to increase the statistical power of the experimental design, and thereby increase its ability to identify potentially weak carcinogenic effects (Portier, 1986).] Groups of animals were continuously exposed to field strengths of 2 μT, 200 μT or 1000 μT, or were intermittently exposed (1 h on/1 h off for 18.5 h per day) to a field strength of 1000 μT. Parallel sham control groups were housed within an identical exposure apparatus, but were exposed only to ambient magnetic fields. The exposure system has been described by Gauger *et al.* (1999). The design of these studies allowed for continuous monitoring of magnetic field strength and waveform throughout the two-year exposure period. At termination, all animals received a full necropsy and complete histopathological evaluations were performed on all gross lesions collected from all study animals, in addition to examinations of 43 tissues per animal in all study groups. Body weights were comparable in all groups, but a statistically significant reduction in survival time was observed in male mice subjected to continuous exposure to field strengths of 1000 μT. (This effect was not seen in female mice.) When compared to controls, no increases in the incidence of neoplasms at any site were observed in males or females from any treated group. In fact, a statistically significant reduction in the incidence of malignant lymphoma was observed in female mice exposed intermittently to 1000 μT (32/100 in controls versus 20/100; $p = 0.035$) and significant decreases in the combined incidence of lung tumours were observed in both male (30/100 in controls versus 19/100; $p = 0.04$) and female mice (11/95 in controls versus 2/99; $p = 0.008$) exposed to field strengths of 200 μT. In addition, statistically significant reductions in the total incidence of malignant neoplasms (all sites) were seen in female mice continuously exposed to field strengths of 200 μT (55/100 in controls versus 39/100;

Table 32. Summary of statistically significant findings ($p < 0.05$) from one- and two-year rodent bioassays on carcinogenicity of magnetic fields

Reference	Animal model (species/strain/sex)	Exposure (frequency: field strength)	Statistically significant differences from sham controls (tumour type, trend, field strength)	Comment
McCormick *et al.* (1999); National Toxicology Program (1999a)	Mouse/B6C3F$_1$/ male	60 Hz: 2 μT, 200 μT, 1000 μT or 1000 μT-intermittent	Lung tumours, ↓, 200 μT	
	Mouse/B6C3F$_1$/ female		Malignant lymphoma, ↓, 1000 μT-intermittent Lung tumours, ↓, 200 μT Malignant neoplasms, all sites, ↓, 200 μT Malignant neoplasms, all sites, ↓, 1000 μT	
Mandeville *et al.* (1997)	Rat/Fischer 344/ female (F_0, F_1)	60 Hz: 2 μT, 20 μT, 200 μT, 2000 μT	None	
Yasui *et al.* (1997)	Rat/Fischer 344/ male	50 Hz: 500 μT, 5000 μT	Fibroma of subcutis, ↑, 5000 μT Invasive neoplasms (all), ↓, 500 μT	Histopathology limited to gross lesions identified at necropsy
	Rat/Fischer 344/ female		None	Histopathology limited to gross lesions identified at necropsy
Boorman *et al.* (1999a); National Toxicology Program (1999a)	Rat/Fischer 344/ male	60 Hz: 2 μT, 200 μT, 1000 μT or 1000 μT-intermittent	Leukaemia, ↓, 1000 μT-intermittent Preputial gland carcinoma, ↑, 200 μT Skin trichoepithelioma, ↑, 1000 μT Thyroid C-cell tumours, ↑, 2 μT Thyroid C-cell tumours, ↑, 200 μT Thyroid C-cell carcinoma, ↑, 2 μT	
	Rat/Fischer 344/ female		Adrenal cortex-adenoma, ↓, 1000 μT-intermittent	

↑, increase; ↓, decrease

$p = 0.015$) and 1000 μT (55/100 in controls versus 40/100; $p = 0.024$) (McCormick *et al*., 1999; National Toxicology Program, 1999a).

In a site-specific chronic bioassay, a group of 380 female C57BL/6 mice was exposed to a circularly polarized 60-Hz magnetic field at a field strength of 1420 μT for up to 852 days. The incidence of haematopoietic neoplasms in these mice was compared with that observed in a negative (untreated) control group of 380 female C57BL/6 mice and in a sham-treated control group of 190 female C57BL/6 mice. Chronic exposure to magnetic fields had no statistically significant effects on animal survival or on the incidence or latency of haematopoietic neoplasms in this study. At study termination, the final incidence of lymphomas in mice exposed to magnetic fields was 36.8% (140/380) compared with an incidence of 34.7% (66/190) in sham controls. The incidence of histiocytic sarcomas was 23.7% (90/380) in mice exposed to magnetic fields versus 22.1% (42/190) in sham controls, yielding a total incidence of haematopoietic neoplasia of 56.3% (107/190) in sham controls versus a total incidence of 59.2% (225/380) in the group exposed to circularly polarized magnetic fields. No statistically significant differences were observed (Babbitt *et al.*, 2000). In addition to the investigation of the effects of magnetic fields on haematopoietic tumours, a post-hoc histopathological analysis of brain tissues from animals in this study was performed to investigate the possibility that magnetic fields are a causative agent for primary brain tumours. Consistent with the results for haematopoietic neoplasms, histopathological examination of brains from this study provided no support for the hypothesis that exposure to magnetic fields is a significant risk factor for induction of brain tumour since no brain tumours were identified in any of the three groups described above (Kharazi *et al*., 1999). [The Working Group noted that the primary strengths of this study are that it evaluated the potential carcinogenicity of a previously unstudied type of exposure (circularly polarized rather than linearly polarized magnetic fields), and used very large experimental groups thus increasing its statistical power and its ability to identify effects of modest magnitude.]

Using a unique study design in which three consecutive generations of CFW mice were exposed to extremely high flux densities (25 mT) of 60-Hz magnetic fields, an increased incidence of malignant lymphoma in the second (no statistical analysis given) and third generations (2/41 versus 37/92; $p < 0.001$) of exposed animals was reported (Fam & Mikhail, 1996). [The Working Group noted several deficiencies in the design and conduct of this experiment. These include small and variable group sizes, a very small number of observed malignant lesions in the F_2 generation and weaknesses in exposure assessment. Of particular concern is the inadequate control of environmental factors, including the heat, noise and vibration generated by the exposure system, and the noise and vibration made by the ventilation equipment. Because non-specific stressors have been demonstrated to increase the growth of transplantable lymphomas and other tumours in mice and to decrease survival in several other animal models, inadequate control of environmental conditions may confound the study results. These possible confounders render this study difficult to interpret.]

3.1.2 *Rat*

Groups of 100 male and 100 female Fischer 344 rats, six to seven weeks of age, were exposed for 18.5 h per day for two years to linearly polarized 60-Hz magnetic fields that were nearly pure, transient–free and had less than 3% total harmonic distortion. Groups of animals were continuously exposed to field strengths of 2 μT, 200 μT or 1000 μT, or were intermittently exposed (1 h on/1 h off) to a field strength of 1000 μT. Parallel sham control groups were housed within an identical exposure array, but were exposed to low ambient magnetic fields. The exposure system has been described by Gauger *et al.* (1999). The design of these studies allowed for continuous monitoring of magnetic field strength and waveform throughout the two-year exposure period. At termination, all animals received a full necropsy and complete histopathological evaluations were performed on all gross lesions collected from all study animals, in addition to examinations of 45 tissues per animal in all study groups. Body weight and survival were comparable in all groups. Significant differences from tumour incidences in controls observed in this study included a statistically significant decrease (50/100 in controls versus 36/100; $p < 0.045$) in the incidence of leukaemia in male rats exposed intermittently to field strengths of 1000 μT, a statistically significant increase in the incidence of preputial gland carcinomas in male rats exposed to magnetic field strengths of 200 μT (but not to 1000 μT) (0/100 in controls versus 5/100; $p = 0.032$), a statistically significant increase in the incidence of trichoepitheliomas of the skin in male rats exposed continuously to 1000 μT (0/100 in controls versus 5/100; $p = 0.029$), a statistically significant decrease in the incidence of adenomas of the adrenal cortex in female rats exposed intermittently to 1000 μT (6/100 in controls versus 0/100; $p = 0.02$), and statistically significant increases in the incidence of thyroid C-cell tumours (adenomas + carcinomas) in male rats exposed to field strengths of 2 μT (16/99 in controls versus 31/100; $p = 0.005$) and 200 μT (16/99 in controls versus 30/100; $p = 0.009$). There was also a marginal increase in thyroid C-cell tumours in male rats exposed to a continuous field strength of 1000 μT (16/99 in controls versus 25/100; $p = 0.055$), but no increase in animals intermittently exposed to 1000 μT (16/99 in controls versus 22/100; $p = 0.147$). In male rats, there was also a statistically significant increase in thyroid C-cell carcinomas at 2 μT (1/99 in controls versus 7/100; $p = 0.03$) and a non-significant increase in this rare tumour in animals intermittently exposed to 1000 μT (1/99 in controls versus 5/100; $p = 0.1$) (Boorman *et al.*, 1999a; National Toxicology Program, 1999a). [An examination by the Working Group of the historical controls used by the National Toxicology Program for the 10 most recent bioassays conducted using an identical diet (NTP-2000 diet) showed a historical incidence for thyroid C-cell tumours of 17% (102/603) with a range from 2% (1/50) to 28% (14/50), indicating no discernible problem with the controls for this tumour in this study. In the same historical database, the incidence of thyroid C-cell carcinomas is 1.7% (10/603) varying from 0% (0/50) observed for four of the 10 control datasets to 4% observed for two of the datasets (2/50 and 4/100).] The original authors (Boorman

et al., 1999a) concluded [without the benefit of adequate historical controls] that their finding was equivocal and the peer-review committee of the National Toxicology Program (1999a) reached the same conclusion. [The Working Group noted that the lack of a dose–response relationship and the as yet unknown mechanism of thyroid C-cell carcinogenesis prevent a clear interpretation of this finding. For these reasons, because the results cannot be interpreted as clearly negative and because the data are insufficient to be listed as clearly positive, the Working Group concluded that the evaluation should remain equivocal.]

In another study, groups of gestating Fischer 344 rats were exposed to similar, linearly polarized 60-Hz magnetic fields at field strengths of 2 μT, 20 μT, 200 μT and 2000 μT for 20 h per day from day 20 of gestation. At weaning, groups of 50 female offspring were exposed to the same intensities and magnetic fields as the dams had been for 20 h per day for two years. The experimental design included a group of 50 female rats as cage controls and 50 female sham-exposed controls. During the lifetime of the animals, the study was conducted using a blinded design in which investigators were unaware of group identities. Toxicological end-points included a standard battery of evaluations of the living animals, followed after death by the histopathological evaluation of gross lesions and 50 tissues per animal. The authors noted no statistically significant increases in the incidence of any tumour at any site evaluated (Mandeville *et al*., 1997). [The Working Group noted that this study included exposure during the perinatal and juvenile periods, and its design addressed the possibility of enhanced sensitivity of younger animals to the effects of magnetic fields. The blinded design of the bioassay precluded any possible influence of investigator bias on study results.]

In a third chronic bioassay in rats, groups of 48 male and 48 female Fischer 344 rats, five weeks of age, were exposed for an average of 22.6 h per day to 50-Hz magnetic fields at strengths of 500 μT or 5000 μT for two years; control groups of 48 males and 48 females received sham exposure for the same period. At termination of the study, all animals received a complete necropsy. Histopathological evaluations were performed on all gross lesions, and on all sites suspected of tumoral lesions. Survival was comparable in all study groups, and differential white blood cell counts performed after 52, 78 and 104 weeks of exposure failed to identify any effects of exposure to magnetic fields. The authors reported that exposure to magnetic fields had no effect on survival of animals of either sex or on the total incidence or number of neoplasms. The only histopathological findings that were statistically significant were an increase in the incidence of a benign lesion (fibroma) in the subcutis of male rats exposed to field strengths of 5000 μT (2/48 in controls versus 9/48; $p < 0.05$) and a decrease in the total incidence of invasive neoplasms in male rats in the group exposed to 500 μT (6/48 in controls versus 1/48; $p < 0.05$). When compared with incidence in sham controls (8/48), no increases in the incidence of thyroid C-cell tumours (benign and malignant) were observed in male rats exposed to 500 μT (10/48) or 5000 μT (6/48). The incidence of thyroid C-cell carcinomas was less than 2% in all groups.

Although the increased incidence of fibromas in male rats exposed to 5000 μT was statistically significant when compared with concurrent male sham controls, the incidence of lesions was stated to be comparable to that observed in historical controls in the same laboratory [range not given]. On this basis, the authors concluded that the increase in fibroma incidence observed in rats exposed to 5000 μT was not exposure-related. Because no differences in the incidence of metastatic neoplasms were seen in the study, the observed decrease in the incidence of invasive malignancy appears to be without biological significance (Yasui *et al.*, 1997). [The Working Group noted that this study provided only limited information because of the incomplete histopathological evaluation. Because thyroid C-cell carcinomas frequently involve the entire lobe of the thyroid gland in Fischer rats, this level of histopathological evaluation should be adequate for this tumour. However, since thyroid C-cell adenomas are generally small and difficult to separate from hyperplasia, this evaluation cannot be easily compared with the National Toxicology Program (1999a) study.]

3.2 Exposure in association with known carcinogens

3.2.1 *Multistage studies of mammary cancer*

A number of studies have investigated the effect of exposure to magnetic fields on the incidence of mammary cancer in rodents. All of these have been multistage studies in which female rats were treated with a chemical carcinogen, *N*-methyl-*N*-nitrosourea (MNU) or 7,12-dimethylbenz[*a*]anthracene (DMBA), followed by exposure to magnetic fields of different strengths and for different time intervals.

(*a*) *Multistage studies with* N-*methyl*-N-*nitrosourea*

Groups of 50 female outbred rats (obtained from the Oncology Research Center, Republic of Georgia) between 55 and 60 days of age, were intravenously injected with 50 mg/kg bw MNU. Starting two days after this treatment, the animals were exposed to a 50-Hz, 20-μT magnetic field daily for 0.5 h (group 1) or 3 h (group 2), or to a 20-μT static magnetic field, daily for 0.5 h (group 3) or 3 h (group 4). A fifth group received MNU only. Four control groups each of 25 rats received no MNU, but were exposed to the same magnetic fields as described above for groups 1–4. A 10th group of 50 rats received no treatment at all. The rats were followed for a period of two years after injection of the carcinogen. In comparison with a 59% (27/46) incidence of mammary tumours in rats receiving MNU only, a significant increase was observed in the groups exposed daily for 3 h to a 50-Hz magnetic field (93%, 43/46; $p < 0.05$) or to a static magnetic field (87%, 39/45; $p < 0.05$). These increases were not seen in rats exposed to a 50-Hz field or a static field for 0.5 h per day. In comparison with a 0% (0/48) incidence of mammary tumours in rats that received no MNU and no exposure to magnetic fields, a statistically significant increase was observed ($p < 0.05$) in the incidence of mammary tumours in non-MNU-treated rats exposed for 3 h per day to a

50-Hz magnetic field (30%, 7/23) [$p < 0.01$; χ^2 test]. The authors also noted that exposure to either type of magnetic field for 3 h per day shortened the latent period of tumour development [no statistical analysis given] and led to a change in the morphological spectrum of the mammary tumours, with more adenocarcinomas than fibroadenomas (Beniashvili *et al*., 1991).

Groups of 40 outbred female rats (obtained from the Oncology Research Center, Republic of Georgia), one month of age, were kept on a 12-h/12-h light–dark cycle, and intravenously injected with MNU (50 mg/kg bw) three times per week. Starting two days after the first dose of MNU, the animals were exposed daily for 3 h to either a 20-μT static magnetic field or a 50-Hz, 20-μT magnetic field. A group of 30 rats received MNU only. Mammary adenocarcinomas were found in 7/22 (32%) of the MNU-treated controls and in 12/30 (40%) and 15/33 (45%) of the animals exposed to the static and 50-Hz magnetic fields, respectively. These differences were not statistically significant. The mean latent period for development of mammary tumours was significantly decreased ($p < 0.05$) in the rats exposed to the 50-Hz fields (125 ± 7 days), but not to the static fields (162 ± 11 days), compared with the latent period observed in the MNU-treated controls (166 ± 4 days). In the same series of studies, experiments were carried out with animals that were kept in constant darkness or in constant light. Incidences of mammary tumours decreased to 1/38 (2.6%), 1/48 (2.1%) and 2/45 (4.4%), respectively, for the MNU, MNU plus static field and MNU plus 50-Hz field groups that had been kept in the dark. Conversely, under conditions of constant light, the tumour incidences were increased: 20/35 (57%), 25/41 (61%) and 34/42 (81%), respectively, for the three groups (Beniashvili *et al.*, 1993; Anisimov *et al.*, 1996).

(*b*) *Multistage studies with 7,12-dimethylbenz*[a]*anthracene*

The results of these studies are summarized in Table 33.

Female Sprague-Dawley rats, 52 days of age, were given 5 mg DMBA by gavage. Administration of DMBA (5 mg by gavage) was repeated at weekly intervals up to a total dose of 20 mg/animal. Beginning immediately after the first dose of DMBA, the treatment groups were exposed 24 h per day for 13 weeks to either a static magnetic field of 15 mT (18 rats), a 50-Hz magnetic field of 30 mT (1 group of 15 and 1 group of 18 rats) or a non-uniform 50-Hz magnetic field ranging from 0.3–1 μT (36 rats). Control groups equal in size to those of the treated rats were sham-exposed. Rats were palpated weekly to assess development of mammary tumours. After 13 weeks, all rats were necropsied and the number and weight or size of the tumours were determined. The exposure system was adequately described and consisted of six identical solenoidal coils and six sham coils of the same dimensions. The DMBA-treated age-matched reference control groups were kept in a separate room (ambient field, 0.05–0.15 μT). In sham-exposed animals and reference controls, the tumour incidence varied between 50 and 78% in the different experiments. The average number of mammary tumours per tumour-bearing animal varied between 1.6 and 2.9. In none of the experiments did exposure to magnetic fields significantly alter tumour incidence

Table 33. Multistage studies of mammary cancer in female Sprague-Dawley rats treated with 7,12-dimethylbenz[*a*]anthracene (4 weekly gavage doses of 5 mg/animal, unless otherwise stated) and exposed to magnetic fields for 13 weeks (unless otherwise indicated)

Reference	Exposure conditions	Exposure and control groups (no. of animals)	No. of animals with tumours	Tumour incidence (%)	Total no. of tumours	No. of tumours per tumour-bearing animal	Remarks
Mevissen *et al.* (1993)	15 mT, static	Exposed (18)	10	56	17	1.7 ± 0.31	
		Sham-exposed (18)	14	78	30	2.1 ± 0.28	
		Reference control (8)	6	75	16	2.7 ± 0.35	
	30 mT, 50 Hz	Exposed (18)	14	78	40	2.8 ± 0.63 *	* $p < 0.05$ compared with sham-exposed animals
		Sham-exposed (18)	10	56	22	2.2 ± 0.47	
		Reference control (18)	12	67	20	1.7 ± 0.23	
	30 mT, 50 Hz	Exposed (15)	6	40	11	1.8 ± 0.34	
		Sham-exposed (18)	9	50	14	1.6 ± 0.17	
		Reference control (9)	5	55	12	2.4 ± 0.37	
	0.3–1 μT, 50 Hz	Exposed (36)	21	58	47	2.2 ± 0.3	
		Sham-exposed (36)	21	58	60	2.9 ± 0.45	
Löscher *et al.* (1993)	100 μT, 50 Hz	Exposed (99)	51	52 *	82	~ 1.6	All figures calculated from curves
		Sham-exposed (99)	34	34	62	~ 1.8	* $p < 0.05$ compared with sham-exposed animals
Baum *et al.* (1995)[a]	100 μT, 50 Hz	Exposed (99)	65	66	134	~ 2.1	No. of animals with adenocarcinoma: exposed, 62; sham-exposed, 49 ($p < 0.05$)
		Sham-exposed (99)	57	58	113	~ 2.0	
		Exposed, no DMBA (9)	0	0	–	–	
		Sham-exposed, no DMBA (9)	0	0	–	–	Tumour volume in exposed animals significantly larger than in sham-exposed animals ($p < 0.05$)
Löscher *et al.* (1994)	0.3–1 μT, 50 Hz	Exposed (36)	24	67	77	3.2 ± 0.54	
		Sham-exposed (36)	22	61	95	4.3 ± 0.83	

Table 33 (contd)

Reference	Exposure conditions	Exposure and control groups (no. of animals)	No. of animals with tumours	Tumour incidence (%)	Total no. of tumours	No. of tumours per tumour-bearing animal	Remarks
Mevissen *et al*. (1996a)	10 μT, 50 Hz	Exposed (99) Sham-exposed (99) Exposed, no DMBA (9) Sham-exposed, no DMBA (9)	66 60 0 0	67 61 0 0	151 129 – –	~ 2.5 ~ 2.5 – –	
Mevissen *et al*. (1996b)	50 μT, 50 Hz	Exposed (99) Sham-exposed (99) Exposed, no DMBA (9) Sham-exposed, no DMBA (9)	69 55 0 0	70 * 56 0 0	193 139 – –	~ 2.7 ~ 2.5 – –	* $p < 0.05$ compared with sham-exposed animals
Mevissen *et al*. (1998a)	100 μT, 50 Hz	Exposed (99) Sham-exposed (99)	83 62	84 * 63	297 230	~ 3.8 ~ 3.8	* $p < 0.05$ compared with sham-exposed animals
Ekström *et al*. (1998)	250 μT, 50 Hz 500 μT, 50 Hz	Exposed (60) Exposed (60) Sham-exposed (60)	42 42 43	70 70 72	102 90 111	2.4 2.1 2.6	A single intragastric dose (7 mg/animal) of DMBA was given one week before exposure to the magnetic field for 21 weeks, 15 s on/15s off
Anderson *et al*. (1999); National Toxicology Program (1999b)	100 μT, 50 Hz 500 μT, 50 Hz 100 μT, 60 Hz	Exposed (100) Exposed (100) Exposed (100) Sham-exposed (100)	86 96 96 92	86 (carc.) 96 96 92	528 * (carc.) 561 692 691	5.3 ± 4.4 * 6.5 ± 4.9 6.9 ± 4.8 6.9 ± 4.8	* $p < 0.05$, decrease

Table 33 (contd)

Reference	Exposure conditions	Exposure and control groups (no. of animals)	No. of animals with tumours	Tumour incidence (%)	Total no. of tumours	No. of tumours per tumour bearing animal	Remarks
Anderson *et al.* (1999); National Toxicology Program (1999b)	100 µT, 50 Hz 500 µT, 50 Hz	Exposed (100) Exposed (100) Sham-exposed (100)	48 38 43	48 (carc.) 38 43	90 (carc.) 79 102	0.9 ± 1.3 0.8 ± 1.3 1.0 ± 1.9	DMBA-treatment with 4 × 2 mg/ animal at weekly intervals
Boorman *et al.* (1999b); National Toxicology Program (1999b)	100 µT, 50 Hz 500 µT, 50 Hz 100 µT, 60 Hz	Exposed (100) Exposed (100) Exposed (100) Sham-exposed (100)	90 95 85 96	90 (carc.) 95 85 * 96	494 * (carc.) 547 433 * 649	4.9 ± 4.2 * 5.5 ± 3.9 4.3 ± 3.9 * 6.5 ± 4.8	* $p < 0.05$, decrease. A single intra-gastric dose of 10 mg/rat followed by exposure to magnetic field for 26 weeks
Thun-Battersby *et al.* (1999)	100 µT, 50 Hz	Exposed (99) Sham-exposed (99)	64 50	65 * 51	166 116	~ 2.3 ~ 2.6	* $p = 0.044$ compared with sham-exposed animals DMBA-treatment with a single oral dose (10 mg/rat), at one week after the start of exposure to magnetic field for 27 weeks

carc., carcinoma; DMBA, 7,12-dimethylbenz[*a*]anthracene

[a] This study provided histological confirmation of the results reported in Löscher *et al.* (1993).

although in one of the groups exposed to the 50-Hz magnetic field of 30 mT, the number of tumours per tumour-bearing animal was significantly increased ($p < 0.05$). This increase was not seen in the other experiment at 30 mT or in the combined analysis. Furthermore, exposure to the static magnetic field of 15 mT significantly enhanced the tumour weight. Exposure to the non-uniform magnetic field (50 Hz; 0.3–1 μT) had no significant effects on tumour multiplicity or tumour sites. The authors concluded that these experiments suggest that magnetic fields at high flux densities may act as a promoter or co-promoter of mammary cancer. However, they considered this interpretation to be tentative because of the limitations of this study, particularly the small sample size used to study exposure to a magnetic field. Confirmation would require further experiments with larger groups of animals (Mevissen *et al.*, 1993).

A group of 99 female Sprague-Dawley rats, 52 days of age, was exposed to a homogeneous 50-Hz magnetic field of 100 μT for 24 h per day for a period of 13 weeks; another group of 99 rats was sham-exposed. The exposure chambers (Merritt coils, adequately described) were identical for both groups of animals. DMBA (5 mg) was administered by gavage to both groups on the first day of exposure to the magnetic field and at weekly intervals thereafter up to a total dose of 20 mg/rat. The animals were palpated once weekly to assess the development of mammary tumours. Eight weeks after the first dose of DMBA, the incidence of palpable mammary tumours in rats exposed to the magnetic field was significantly higher than in sham-exposed animals ($p < 0.05$). This difference was observed throughout the period of exposure, at the end of which the tumour incidence in exposed rats was 52% (51/99) versus 34% (34/99) in the sham-exposed controls ($p < 0.05$). The tumour size ($p = 0.013$) and the number of tumours per animal ($p < 0.05$) were also increased in the exposed group (Löscher *et al.*, 1993). To provide histopathological confirmation, data on palpable tumours were examined separately for the study described above. Interrupted step sections were prepared from all mammary glands from all animals, yielding 50–60 sections/rat. The incidence of mammary tumours (all types) was 66% (65/99) in magnetic field-exposed and 58% (57/99) in sham-exposed rats ($p > 0.05$). The percentage of animals with mammary adenocarcinomas was significantly higher in the group exposed to DMBA plus a magnetic field than in the sham-exposed controls treated with DMBA (62/99 versus 49/99, $p < 0.05$). Forty-five other organs or tissues were also examined and no significant changes in tumour incidence were noted (Baum *et al.*, 1995).

Using the same protocol (exposure to DMBA and magnetic fields) as Löscher *et al.* (1993) with the addition of a full histopathological review, one group of 36 female Sprague-Dawley rats was exposed to a magnetic field of 0.3–1 μT at 50 Hz and a second group was sham-exposed. In this study, 67% (24/36) of the animals exposed to the magnetic field versus 61% (22/36) of the sham-exposed controls had mammary tumours ($p > 0.05$) and there were also no differences observed in the number of tumours per animal or in average tumour size (Löscher *et al.*, 1994).

Using the same experimental protocol (DMBA, magnetic fields) as Löscher *et al.* (1994), one group of 99 female Sprague-Dawley rats was exposed continuously to

50-Hz, 10-μT magnetic fields for 13 weeks and a second group of 99 rats was sham-exposed. At autopsy, 61% (60/99) of the sham-exposed and 67% (66/99) of the magnetic field-exposed rats had developed macroscopically visible mammary tumours ($p > 0.05$). The average size of the individual tumours and the average sum of all tumours per tumour-bearing rat were similar in both groups (Mevissen *et al.*, 1996a).

The experimental design described above was used to study the effects of a higher field strength of 50 μT. Within eight weeks after the first DMBA administration, the group of rats exposed to a 50-Hz, 50-μT magnetic field exhibited significantly more ($p = 0.028$) palpable mammary tumours than sham-exposed animals. Autopsy revealed significantly more ($p < 0.05$) macroscopically visible mammary tumours (69/99) in rats exposed to magnetic fields than did controls treated with DMBA alone (55/99). No differences in the numbers of tumours per tumour-bearing animal or tumour size were seen (Mevissen *et al.*, 1996b).

Löscher and Mevissen (1995) published a regression analysis of the four studies described above (Löscher *et al.*, 1993, 1994; Baum *et al.*, 1995; Mevissen *et al.*, 1996a,b) in which exposure level was compared with the percentage increase in incidence over controls of palpable mammary tumours after 13 weeks of treatment. This analysis demonstrated a highly significant ($p < 0.01$) trend.

A previous study (Löscher *et al.*, 1993; Baum *et al.*, 1995) was replicated in the same laboratory under the same experimental conditions (exposure to DMBA and a 50-Hz, 100-μT magnetic field). After nine weeks of treatment, the incidence of palpable mammary tumours in the group exposed to a magnetic field was significantly higher than that in the sham-exposed group ($p < 0.05$). This difference was maintained throughout the remainder of the period of exposure. At 13 weeks, the incidence of macroscopically visible mammary tumours was 63% (62/99) in controls and 84% (83/99) in exposed rats ($p < 0.05$). No differences were observed in the number of tumours per tumour-bearing rat or in the average tumour size. The addition of this data point to the previous regression analysis by Löscher & Mevissen (1995) did not markedly alter the significant trend ($p < 0.05$) (Mevissen *et al.*, 1998a).

In a study performed in another laboratory, female Sprague-Dawley rats, 52 days of age, were randomly allocated to one of three groups of 60 animals each. All rats received a single gavage dose of 7 mg DMBA on day 1 of the experiment. Beginning one week later, groups were exposed to intermittent (15 s on/15 s off) transient-associated 50-Hz magnetic fields at a field strength of 250 μT or 500 μT and another group was sham-exposed. The exposure treatment was continued for 24 h per day for 21 weeks, with intermissions for animal care and observations. Animals were palpated twice weekly to identify mammary tumours, but no histological analysis was carried out. The tumour incidence in the two groups exposed to the magnetic fields was 70% (42/60 in both groups), and the incidence in the DMBA-treated controls was 72% (43/60). The total numbers of tumours were 102 (250 μT exposure group), 90 (500 μT exposure group) and 111 (sham-exposed). These values were not statistically different.

Total tumour weight and total tumour volume were not statistically different between groups (Ekström *et al.*, 1998).

Groups of 100 female Sprague-Dawley rats, 50 days of age, received four weekly gavage doses of 5 mg DMBA per animal. After the first DMBA dose, exposure to ambient fields (sham exposure), 50-Hz magnetic fields (field strength of 100 or 500 μT) or 60-Hz fields (field strength of 100 μT) was initiated. The animals were exposed to magnetic or sham fields for 18.5 h per day, seven days per week for 13 weeks. In a second study, groups of 100 female Sprague-Dawley rats received lower doses of DMBA (2 mg/animal per week for four weeks). After the first dose of DMBA, rats were exposed to ambient fields (sham exposure) or 50-Hz magnetic fields at a field strength of 100 or 500 μT for 18.5 h per day, seven days per week for 13 weeks. Rats were weighed and palpated weekly for the presence of mammary tumours. Palpable mammary tumours were examined histologically. Exposure to magnetic fields had no effect on body weight gains or on the time of appearance of mammary tumours in either study. In the first study, the mammary cancer incidences were 92% (92/100), 86% (86/100), 96% (96/100) and 96% (96/100) for the DMBA-treated control, 50-Hz, 100-μT, 50-Hz, 500-μT and 60-Hz, 100-μT groups, respectively. The average numbers of mammary carcinomas per animal were 6.9, 5.3 ($p < 0.05$, decrease), 6.5 and 6.9 for the same groups, respectively. In the second study, the mammary cancer incidences were 43% (43/100), 48% (48/100) and 38% (38/100) for the DMBA-treated control, 50-Hz, 100-μT and 50-Hz, 500-μT groups, respectively. There was no effect of exposure to magnetic fields on the number of tumours per rat or tumour size (Anderson *et al.*, 1999; National Toxicology Program, 1999b). [The Working Group noted that the high tumour incidence observed in the first study effectively precluded the identification of increases in tumour incidence at the end of the study. The decrease in average number of tumours per animal exposed to magnetic fields of 100 μT coincided with a 7% decrease in survival which could have affected this finding.]

Groups of 100 female Sprague-Dawley rats, 50 days of age, received a single dose of 10 mg DMBA by gavage, followed by exposure to ambient fields (strength < 0.1 μT; sham exposure), 50-Hz magnetic fields (strength, 100 or 500 μT) or 60-Hz magnetic fields (strength, 100 μT). The animals were exposed for 18.5 h per day, seven days per week, for 26 weeks. Rats were palpated weekly for mammary tumours. After 26 weeks of exposure to magnetic fields or sham exposure, the animals were killed and the mammary tumours counted and measured; mammary tumours were confirmed histologically. Exposure to magnetic fields had no effect on body weight gain or the time of appearance of mammary tumours. The incidence of mammary cancer was 96% (96/100), 90% (90/100; $p = 0.07$)), 95% (95/100; $p = 0.52$) and 85% (85/100; $p = 0.009$) for the DMBA-treated control, 50-Hz, 100-μT, 50-Hz, 500-μT, and 60-Hz, 100-μT groups, respectively. The total numbers of carcinomas were 649, 494 ($p < 0.05$), 547 and 433 ($p < 0.05$) for the same groups, respectively. The number of fibroadenomas varied from 276 to 319 per group, with the lowest number in the 60-Hz, 100-μT exposure group. Measurement of the tumours revealed no difference in tumour size between

groups (Boorman *et al.*, 1999b; National Toxicology Program, 1999b). [The Working Group noted that the high tumour incidence observed in sham control animals effectively precluded the identification of increases in tumour incidence at the end of the study. However, unlike the first National Toxicology Program experiment (Anderson *et al.*, 1999; National Toxicology Program, 1999b), the decreases in average number of tumours per animal were probably not associated with differences in survival.]

Using the protocol of Mevissen *et al.* (1998b) the treatment period was extended to 27 weeks. Groups of 99 female Sprague-Dawley rats, 45–49 days of age, were exposed either to sham fields or to 50-Hz, 100-μT magnetic fields for 24 h per day, seven days per week. A single dose of 10 mg DMBA/rat by gavage was administered one week after study start to both sham-exposed rats and those exposed to magnetic fields (rather than four weekly doses of 5 mg). The animals were palpated once weekly from week 6 onwards to assess the development of mammary tumours. The incidence of palpable mammary tumours in the group exposed to DMBA plus magnetic fields was increased by week 13 ($p = 0.029$) and continued to be elevated throughout the study in comparison with the incidence in the DMBA-treated, sham-exposed group. At study termination, the incidence of histologically verified mammary tumours was 50.5% (50/99) in controls and 64.7% (64/99) in exposed rats, the difference being statistically significant ($p = 0.044$). The incidence of adenocarcinomas was not significantly different between the two groups (42.4%, 42/99 in controls versus 52.5%, 52/99 in exposed rats). When tumour incidence was evaluated separately for each of the six mammary complexes, the most pronounced effect of exposure to the magnetic field was seen in the L/R1 glands, where the overall tumour incidences were 18.2% (18/99) and 30.3% (30/99) for control and exposed rats, respectively ($p < 0.05$). No differences in the size of mammary tumours or number of tumours per animal were noted (Thun-Battersby *et al.*, 1999). [The Working Group questioned the feasibility of attributing the site of origin of a mammary tumour to a specific mammary gland, but agreed that increases in L/R1-3 as a complex appear to be exposure-related.]

3.2.2 *Multistage studies of skin cancer*

(*a*) *Mouse (conventional)*

Groups of 32 female SENCAR mice, six to seven weeks of age, were sham-exposed or exposed to 60-Hz, 2000-μT continuous magnetic fields (Merritt exposure system described in Stuchly *et al.* (1991) [geomagnetic field not given]) for 6 h per day, five days per week for 21 weeks with or without weekly co-promotion with 1 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) [four groups in total]. All animals received a single topical initiation with DMBA on the dorsal skin at a dose of 10 nmol (2.56 μg) dissolved in 200 μL acetone one week prior to exposure to the magnetic field. Any macroscopically visible tumours and other tissue abnormalities such as enlarged spleens and lymph nodes were examined histopathologically. [Minimum size of papillomas was not reported.] The development of papillomas in the magnetic field-exposed mice treated

with TPA was no earlier than in sham-exposed animals treated with TPA ($p = 0.898$). At termination of the experiment, the total number of animals with papillomas was 28/31 in the sham-exposed group treated with TPA versus 29/32 animals in the matching group of animals exposed to the magnetic field. The average number of papillomas per tumour-bearing animal was 10 in both sham-exposed and magnetic field-exposed animals treated with TPA. All lesions were benign but two mice from the group exposed to the magnetic field and treated with TPA were found to have one papilloma each with very mild invasion of the squamous epithelium and another mouse was diagnosed as having a lymphoma. The investigators concluded that exposure to a magnetic field did not act as a promoter since none of the DMBA-initiated mice developed papillomas in the absence of treatment with TPA regardless whether or not they were exposed to a magnetic field. A positive control group (21 mice that received 2 μg TPA twice a week) showed no increase in tumour incidence compared with the control group (mice that received 1 μg TPA once a week) [*p*-value not given]; the author suggested a saturation of the system allowing no opportunity to detect a co-promotional effect (McLean *et al*., 1991).

In a second study, two groups of 48 female SENCAR mice, six weeks of age, were sham-exposed or exposed to a 60-Hz, 2000-μT magnetic field (Merritt exposure system described in Stuchly *et al*. (1991)) for 23 weeks; all animals received a weekly application of 0.3 μg TPA. All animals received a single topical initiation with DMBA (10 nmol dissolved in 200 μL acetone) on the dorsal skin one week prior to exposure to the magnetic field. An increased rate of papilloma development in animals exposed to magnetic fields compared with the sham-exposed controls was reported to be significant at weeks 16 ($p = 0.01$, Fisher exact test), 17 ($p = 0.02$) and 18 ($p = 0.02$), but was not significant at the end of the study ($p = 0.16$). The number of tumours per animal was not statistically different from the controls at the end of the study ($p = 0.21$, Wilcoxon test), but a significant difference was also reported at weeks 16 ($p = 0.01$), 17 ($p = 0.03$) and 18 ($p = 0.03$) (Stuchly *et al.*, 1992). [The authors did not report any significant tests on papillomas per tumour-bearing animal although it is clear that the average number of tumours per tumour-bearing animal was lower than that seen in the first study of MacLean *et al.* (1991)]. The same study was prolonged to 52 weeks; TPA treatment was discontinued after week 24. The authors reported no overall increase in total tumours or papillomas alone in animals exposed to magnetic fields, but more animals exposed to magnetic fields developed squamous-cell carcinoma (8/48) than did sham-exposed animals (1/48) [$p < 0.03$, Fisher exact test two-sided]. [The number of tumours per animal was not reported.] The conclusion of the authors was that exposure to magnetic fields may accelerate progression to malignancy (McLean *et al*., 1995).

Two further studies replicating the 23-week study described by Stuchly *et al*. (1992) and using the same experimental design were performed. Two groups of 47 or 48 female SENCAR mice were used in each replicate. At week 23, tumour incidence in the first replicate was identical in animals exposed to a 2000-μT magnetic field and sham-exposed controls. In the second replicate, tumour incidence in mice exposed to

a 2000-μT magnetic field was significantly reduced compared to the incidence in sham-exposed controls ($p = 0.04$, Fisher's exact test). The total number of tumours seen by Stuchly *et al*. (1992) was higher in the group exposed to the magnetic field (86) than in the sham-exposed group (48) ($p = 0.15$), whereas the opposite effect was seen in the two replicates (33 in the group exposed to the magnetic field versus 50 in the sham-exposed group [$p = 0.26$] and 27 in the group exposed to the magnetic field versus 86 in the sham-exposed group [$p = 0.01$]). The study does not support a role for exposure to a magnetic field as a strong co-promoter in the mouse skin-tumour model (McLean *et al*., 1997).

In another study of skin tumour promotion, groups of 30 female NMRI/HAN mice, seven weeks old, initiated with 25.6 μg DMBA in 200 μL acetone on the shaved dorsal skin or uninitiated (acetone only), were exposed to a 50-Hz magnetic field of strength of 50 or 500 μT or to sham-exposure conditions for 19 h per day on weekdays and for 21 h per day during weekends for a period of 104 weeks [six groups in total]. The exposure system has been described by Rannug *et al.* (1993a). In addition, TPA (given topically at a dose of 3.08 μg in 200 μL acetone twice per week), was used as a positive control in groups treated with either acetone alone or DMBA in acetone. The detection limit for the size of papilloma was 2 mm. After 104 weeks, the animals were assessed by complete necropsy and histopathological evaluation of skin tumour types. The survival of uninitiated mice exposed to the 500-μT magnetic field was significantly reduced in comparison to uninitiated controls ($p = 0.029$; 10% versus 23.3%). Exposure to the magnetic field had no significant effect on survival in any other group. Skin samples taken from members of each group at different times and analysed for hyperplasia showed a hyperplastic response in animals treated with DMBA plus TPA and those treated with TPA alone; no increased hyperplastic response was observed in any of the groups exposed to the magnetic field. No skin tumours were found in uninitiated mice in either the sham-exposed group or the group exposed to the magnetic field but one uninitiated TPA-treated animal had two skin tumours. No statistically significant differences in the number of animals with skin tumours or in the total number of skin tumours were reported in the initiated animals. The authors concluded that exposure to magnetic fields did not promote skin tumour incidence, nor act as a complete skin carcinogen. Full necropsy at death showed no significant change in incidence of other tumours associated with exposure to magnetic fields (Rannug *et al*., 1993b).

In a second study in female SENCAR mice, the tumour-promoting effects of continuous and intermittent exposure to 50-Hz magnetic fields for up to 105 weeks were examined. Starting one week after initiation with 2.56 μg DMBA in 200 μL acetone, groups of 40 mice were exposed to continuous magnetic fields at strengths of 50 or 500 μT or to intermittent fields (15 s on/15 s off) at the same field strengths. Untreated (acetone), sham-treated (DMBA alone) and TPA-treated (initiated and uninitiated) groups were also included. No skin tumours were reported in animals treated with DMBA and then exposed to continuous magnetic fields at either 50 μT or 500 μT. Four skin tumours were found in 4/40 animals in the group intermittently exposed to a field

strength of 50 μT and 13 skin tumours were found in 5/40 animals animals in the group intermittently exposed to 500 μT compared with two skin tumours in 2/40 DMBA-treated sham-exposed animals. These increases were not significantly different when the two intermittently exposed groups were combined and compared with the sham-exposed group [*p*-value not given], but a significant difference was obtained when combined comparisons of continuous versus intermittent exposure were performed [$p = 0.014$, analysis according to Peto (1974)]. [The Working Group noted that it is not clear whether the pooling of data across groups is appropriate.] Similar findings were reported for the total number of skin tumours [no significant difference except when the pooled continuous exposure group was compared to the pooled intermittent exposure group; $p < 0.01$.] A linear regression analysis comparing the cumulative number of skin tumours in skin tumour-bearing animals exposed intermittently to different field strengths with the DMBA-treated controls gave a one-sided *p*-value of 0.045 which the authors noted as suggesting a dose dependency. No hyperplastic response was reported in animals exposed to magnetic fields. Histopathological investigation showed that carcinomas (squamous, spindle or basal) occurred in both the intermittently exposed groups and in the positive control group (DMBA plus TPA), but not in the DMBA-treated control group (where both lesions observed were papillomas). The investigators concluded that intermittent exposure could be weakly promoting, but interpretation is uncertain because of the possibility of induced electric fields due to mechanical switching and associated occurrence of transients (Rannug *et al.*, 1994).

Groups of 56 female SENCAR mice, four to six weeks of age, were initiated with 10 nmol DMBA in 200 μL acetone before being exposed to ambient magnetic fields (mean field strength, 0.11 μT) or continuous magnetic fields of 2000 μT, 60 Hz for 23 weeks. Exposure to magnetic fields was combined with topical application of TPA once a week at doses of 0 (acetone only), 0.85 nmol (1.04 μg), 1.7 nmol (2 μg) or 3.4 nmol (4.2 μg) [a total of eight groups]. An additional group of 40 uninitiated mice was given a weekly dose of 3.4 nmol (4.2 μg) TPA and exposed to an ambient-strength magnetic field. The incidence of skin lesions [minimum size of tumour detected was not reported] was monitored and tumours were histologically evaluated at the end of the study. Tumour incidence [reported only as plots] did not differ between animals exposed to magnetic fields and ambient controls. [The Working Group noted that data on tumour multiplicity, total tumours, time to first tumour and survival of animals were not reported although some were shown in the figures, and *p*-values were given for comparisons of tumour multiplicity and incidence. The Working Group also noted several inconsistencies between the *p*-values and data reported; for example, a *p*-value of 0.32 was given in the Table for the group in which 3.4 (4.2 μg) nmol TPA was used as a promoter compared with the ambient control but a Figure illustrating the same results reported that 55/56 animals in the ambient group had tumours and that 56/56 animals in the group exposed to magnetic fields developed tumours. It was also noted that many of the *p*-values were repeated in both tumour incidence and tumour multiplicity tables.] The conclusion of the authors was that, within the sensitivity limits of

this animal model and the exposure parameters employed, no promotional or co-promotional effects of exposure to a 2-mT magnetic field were observed in the two-stage skin cancer model (Sasser *et al*., 1998).

(*b*) *Mouse (genetically modified)*

Groups of 21–22 female mice of a transgenic hybrid strain (K2) that overexpresses the human ornithine decarboxylase gene and groups of 21–22 female non-transgenic littermates, seven to nine months of age, were exposed to ultraviolet light (200 J/m^2, 35 min/day, three times per week) for 10.5 months in order to induce skin cancer. The investigation of possible promotional or co-promotional effects of ELF magnetic fields was performed in groups of sham-exposed mice and mice exposed for 24 h to intermittent magnetic fields (field strengths of 1.3, 13 and 130 μT, each applied in succession for 20 min, followed by a 2-h pause) or continuous magnetic fields (50 Hz, 100 μT). The field generator used for this study was well described. Skin tumour incidence and tumour multiplicity were monitored, and the tumours were histologically evaluated at the end of the study. An increase in the rate of onset of macroscopically detectable tumours, with a minimum size of 2 mm, was reported in both transgenic and non-transgenic animals exposed to magnetic fields. This effect was statistically significant in the combined analysis, i.e. when all animals exposed to a magnetic field were compared with all controls ($p < 0.015$), and also when transgenic animals exposed to intermittent and continuous magnetic fields were compared to the ultraviolet light-treated controls ($p < 0.025$), but this effect was not significant in non-transgenic mice ($p < 0.15$). No significant differences were seen in the individual comparisons between the separate groups exposed to a magnetic field and the controls. Measurements of human ornithine decarboxylase activity did not show any significant changes as a function of exposure to magnetic fields ($p < 0.10$). The authors concluded that exposure to magnetic fields accelerated tumour growth (Kumlin *et al*., 1998a). [The Working Group considered that the evaluation of these findings is difficult for two reasons. Firstly, a new skin cancer model using transgenic mice was used, but the investigators did not include a positive control, which would have been helpful in the interpretation of the findings, and, secondly, the results on tumour incidence as a function of time were presented only as values summed over the groups continuously and intermittently exposed to magnetic fields.]

3.2.3 *Multistage studies of liver cancer*

(*a*) *Mouse*

Groups of 50 female CBA/S mice, 3–5 weeks of age, were exposed to ionizing radiation (using a 4- or 6-MV linear accelerator). The total body dose was 4 Gy delivered as three equal fractions of 1.33 Gy at one-week intervals. Simultaneously, the animals were exposed either to a 50-Hz vertical magnetic field of field strength 1.26, 12.6 and 126 μT (each applied in succession for 20 min) or were sham-exposed. A third group

served as cage controls. The mice were exposed to the magnetic field or sham-exposed for 24 h per day for 1.5 years. An increase in the incidence of basophilic liver foci in mice exposed to magnetic fields was statistically significant when compared to sham-exposed mice ($p = 0.002$; Poly-3 test). The incidence of liver carcinomas showed a slight, but not statistically significant ($p = 0.147$) increase in the mice exposed to a magnetic field (14/48, 28%) compared to the sham-exposed group (7/50, 14%) (Heikkinen *et al*., 2001).

(*b*) *Rat*

Two studies in rats have been conducted to evaluate the possible tumour promoting and/or co-promoting effects of a 50-Hz magnetic field on chemically initiated liver tumours. The rat liver foci assay was used for the experiments (Rannug *et al*., 1993b,c). Preneoplastic lesions were initiated in male Sprague-Dawley rats weighing approximately 200 g [age not specified] by intraperitoneal administration of 30 mg/kg bw *N*-nitrosodiethylamine (NDEA) 24 h after a 70% partial hepatectomy. These studies reported the number, the volume percentage and the mean area of altered hepatic foci in the liver, as determined by use of two enzyme markers, the placental glutathione-S-transferase (GST-P) and γ-glutamyltranspeptidase (γGT). Markers were measured by immunohistochemical methods using sections from the right lateral lobe of the liver.

In the first study, rats were exposed to either a 50-Hz magnetic field (4 groups of 10 rats) or to phenobarbital (2 groups of 10 rats) given in the diet at a concentration of 300 ppm for 12 weeks, beginning one week after administration of NDEA. Two separate experiments were conducted. In the first experiment, the flux densities to which the animals were exposed were 0.5 and 50 μT and for the second experiment 5 and 500 μT were used. The fields were switched on for 19–21 h per day, seven days per week. The exposure system is described in Rannug *et al*. (1993a). The experiments were performed in a blind fashion and sham controls (2 groups of 10 rats) were used as matching controls. The body weight gains and liver weights did not differ between animals exposed to magnetic fields and controls whereas the liver weight was significantly increased in rats used as positive controls (phenobarbital). The number of γGT-positive foci per cm^2, the mean focus area ($cm^2 \times 10^{-4}$) and the number of foci per cm^3 in groups exposed to magnetic fields did not differ significantly from the number seen in the controls. A significant difference was seen when the positive control (NDEA + phenobarbital) was compared to the control rats (NDEA) ($p < 0.01$). A significant increase in the percentage volume occupied by γGT-positive foci was observed at a field strength of 50 μT compared with the control rats ($p < 0.05$). In the second experiment, no such increase in γGT-positive foci was seen in association with exposure to magnetic fields. However, the percentage volume occupied by foci was lower in the first experiment (experiment 1: NDEA, 0.002 ± 0.001; experiment 2: NDEA, 0.006 ± 0.003), and the magnitude was similar to that seen in the first experiment in animals treated with NDEA and exposed to a magnetic field of 0.5 μT (0.005 ± 0.002) and in animals treated with NDEA and exposed to a magnetic field of

50 μT (0.005 ± 0.001). No effects on GST-P-positive foci were observed for any parameters measured in either experiment when compared with the NDEA control (Rannug *et al*., 1993c).

A second study, undertaken to investigate the possible interaction between exposure to a 50-Hz magnetic field, partial hepatectomy, initiation with NDEA and promotion with phenobarbital, was performed as described above, but the exposure to a magnetic field began immediately after partial hepatectomy. Four groups (9–10 rats each) were included in the study and treated as follows: one group was treated with NDEA alone, one group with NDEA followed by phenobarbital and two groups were given NDEA and phenobarbital and were exposed to 50-Hz magnetic fields, at field strengths of 0.5 and 500 μT. No effects on the liver:body weight ratio or body weight gain in the treated animals were observed after the 12-week exposure period when compared with controls. In the group exposed to magnetic fields of 0.5 μT, the number of γGT-positive foci per cm^3 (931 ± 131) was significantly decreased compared with the matching control group (1413 ± 181) ($p < 0.05$), and a reduction in GST-P-positive foci was associated with the higher field strength (500 μT) for the mean focus area and the percentage volume occupied by foci ($p < 0.05$) (Rannug *et al*., 1993a).

3.2.4 *Multistage studies of leukaemia or lymphoma*

(*a*) *Mouse (conventional)*

Groups of female CBA/S mice, 25 ± 5 days of age, were exposed either to X-rays (four fractions of 1.31 Gy at a dose rate of 0.45 Gy/min at four-day intervals; 64 mice in total), to pulsed magnetic field (vertical 20-kHz field with a field strength of 15 μT; 53 mice in total) for the lifetime of the animal or to both X-rays and pulsed magnetic field (exposure to magnetic field started immediately after each X-ray exposure; 63 mice in total) or received no treatment (stray-field, field strength < 0.7 μT; 47 mice in total). The mice were killed when moribund and autopsied; haemoglobin concentration, leukocyte counts and differential leukocyte counts were determined and 10 tissues were examined histologically. The diagnosis of lymphoma by microscopy was confined to thymic and non-thymic types. The difference in mean survival time between mice exposed to X-rays alone versus those exposed to both X-rays and magnetic field was not significant. However, mean survival time in mice exposed to magnetic field alone was significantly reduced in comparison to untreated controls ($p = 0.002$). Lymphoma incidence was increased in animals exposed to X-rays; however exposure to magnetic fields had no effect: the incidence of lymphomas was 42/64 (65.6%) in mice exposed to X-rays alone, 45/63 (71.4%) in mice exposed to both X-rays and magnetic field, 3/53 (5.7%) in mice exposed to magnetic field alone and 3/47 (6.4%) in untreated controls. The body weights of mice exposed to X-rays alone or both X-rays and magnetic field were not significantly different from those of controls, but the body weights of mice exposed to magnetic field alone were significantly greater than those of controls. Under the environmental conditions used, pulsed magnetic fields did not affect the frequency of spontaneous

lymphoma, or the frequency of lymphomas induced by exposure to X-rays (Svedenstål & Holmberg, 1993). [The Working Group noted that animals were introduced to the experimental conditions at different time points, in some cases over a period of nearly one year.]

Groups of newborn Swiss Webster mice were subcutaneously injected with 0 or 35 μg DMBA in 1% gelatin, and, two weeks later, exposed to a 50-Hz magnetic field at field strength of 1 mT for 3 h per day, six days a week for 16 weeks, or were sham-exposed. All surviving mice were killed and autopsied at 32 weeks of age. No thymic lymphoma/leukaemia was found in the 84 sham-exposed mice that received 1% gelatin alone. There was no significant difference in survival times. The incidences of pre-malignant changes in thymus, early thymic lymphoma and advanced lymphoma were: sham-exposed males treated with DMBA, 18/80 (22.5%); males exposed to a magnetic field and treated with DMBA, 24/89 (27.0%); sham-exposed females treated with DMBA, 28/75 (37.3%); and females exposed to a magnetic field and treated with DMBA, 26/76 (34.2%); and demonstrated no statistically significant differences within a sex. The incidence of 'dense' metastatic infiltrations in the livers of the mice treated with DMBA and exposure to a magnetic field was significantly greater ($p < 0.01$) than that in the sham-exposed group treated with DMBA. The number of granulocytic leukaemia-bearing mice in the former group was 5/165 and that in the latter group was 4/155. The authors concluded that the study had found no evidence for a 'striking promotion effect' of this magnetic field on the incidence of lymphoma or leukaemia induced by DMBA (Shen *et al*., 1997).

Groups of female C57BL/6 mice, 28–32 days of age, were either sham-exposed (190 mice) or exposed to ionizing radiation (^{60}Co γ-rays) at 3.0, 4.0 or 5.1 Gy (dose rate, ~ 0.2 Gy/min; 3 groups of 190 mice) or to a 60-Hz magnetic field at a strength of 1.4 mT (380 mice) or to both ionizing radiation and magnetic fields for 18 h per day (3 groups of 380 mice). A group of 380 negative controls was exposed to ambient magnetic field only. All animals were kept until natural death or were killed at 852 days (the mean lifespan of the negative controls). All animals were necropsied and sections of thymus, spleen, lymph nodes, lungs, mainstem bronchi, sternum, kidneys, liver and brain as well as gross lesions in these tissues were examined. Lymphomas were classified as lymphoblastic, lymphocytic, immunoblastic, plasma cell and follicular centre cell. Non-lymphoid tumours included myelogenous leukaemia and histiocytic sarcoma. Because of considerable overlap, the follicular-centre-cell, immunoblastic and plasma-cell lymphomas were combined. There were no significant differences in mortality between the groups. The relative frequencies and general occurrence of haematopoietic neoplasia were similar for both animals exposed to magnetic fields and sham-exposed mice that had received the same ionizing radiation treatment. An exception was the reduced incidence rate of lymphoblastic lymphoma at death in the group exposed to 5.1 Gy and the magnetic field compared to the group exposed to 5.1 Gy only ($p = 0.05$). The total tumour incidences for groups exposed to magnetic fields were not significantly different from those of unexposed animals ($p = 0.55$, χ^2 test). The authors concluded that the

results establish a lack of any overall effect of treatment with a single high level of exposure to magnetic field on the incidence of haematopoietic tumours (Babbitt *et al.*, 2000).

Groups of 50 female CBA/S mice, 3–5 weeks of age, were exposed to ionizing radiation (using a 4- or 6-MV linear accelerator), The total body dose was 4 Gy delivered as three equal fractions of 1.33 Gy at one-week intervals. Simultaneously, the animals were exposed either to a 50-Hz vertical magnetic field of strength 1.26, 12.6 and 126 μT (each applied in succession for 20 min) or were sham-exposed. A third group served as cage controls. The mice were exposed to a magnetic field or sham-exposed for 24 h per day for 1.5 years. In this study, survival until the end of the study in the group exposed to ionizing radiation plus the time-varying magnetic field was 66%, in comparison to a survival until the end of the study of 54% in the group that received ionizing radiation plus sham exposure. The incidence of lymphoma in the group exposed to radiation plus a magnetic field was 22% in comparison to an incidence of lymphoma of 30% in the group treated with radiation plus sham exposure. The authors concluded that the data from this study did not support a role for magnetic fields as a tumour promoter (Heikkinen *et al.*, 2001).

(*b*) *Mouse (genetically modified)*

Groups of 103–111 female (C57BL/LiA × CBA × C57BL/6)fBR-(TG)pim-1 transgenic Eμ-*Pim*-1 (PIM) mice, which carry the *pim*-1 oncogene and are highly sensitive to lymphoma induction by *N*-ethyl-*N*-nitrosourea (ENU), 38–52 days of age, were sham-exposed or exposed to a 50-Hz magnetic field at a field strength of 1, 100, 1000 μT (continuous) or 1000 μT (intermittent 15 min on, 15 min off) for 20 h per day for up to 18 months; a group of 97 female wild-type C57BL/6Ntac mice was sham-exposed. No differences in body weights were observed throughout the experiment. Thirty Eμ-*pim*-1 mice and 30 wild-type mice each received an intraperitoneal injection of 50 mg/kg bw ENU. After nine months of treatment, the incidence of lymphoblastic lymphoma in these controls was 60% in the Eμ-*pim*-1 mice and 5% in the wild-type mice. The incidence of lymphoma (lymphoblastic and non-lymphoblastic) was 2/97 in wild-type mice and 32/111, 31/105, 27/103, 32/105 and 36/104 in the sham-exposed mice and in those exposed to continuous magnetic fields of 1 μT, 100 μT and 1000 μT and those exposed intermittently to 1000 μT, respectively. The incidence of a renal glomerular disease, not associated with lymphoma incidence, varied from 9% to 19% between the groups. All of 12 representative cases of lymphoma expressed the T-cell marker Thy 1, none carried B-cell markers. The authors concluded that, compared to the results of the sham exposure, there was no statistically significant increase in the incidence of lymphoma or its subtypes or in the time to appearance of the tumours in any of the groups exposed to magnetic fields (Harris *et al.*, 1998). [The Working Group noted that the 235 'healthy' survivors from the six groups were not autopsied. This is unlikely to have had an effect on the results obtained using this model since the lymphomas are likely to have been rapidly lethal.]

Groups of 30 male and 30 female Eμ-*pim*-1 mice (see definition above; 'high-incidence' model) [age unspecified] received a single intraperitoneal injection of 25 mg/kg bw ENU followed one day later by exposure to a 60-Hz continuous magnetic field at a field strength of 0 (sham controls), 2, 200 or 1000 μT or to an intermittent field of 1000 μT for 18.5 h per day for 23 weeks. The exposure system has been described by Gauger *et al.* (1999). Groups of 30 male and 30 female TSG-*p53* mice (knock-out; 'low-incidence' model) [age unspecified], not pre-treated with ENU, were either sham-exposed or exposed to a continuous magnetic field of 1000 μT for 18.5 h per day for 23 weeks. All animals underwent a limited gross necropsy. Survival until the end of the study was similar for both strains in all treatment groups, except in Eμ-*pim*-1 males exposed continuously to a magnetic field of 1000 μT (77% versus 60% in sham controls). The incidence of lymphomas in male Eμ-*pim*-1 mice in the sham-exposed group was 59% compared with 47%, 43% and 57% in the groups exposed to fields of 2 or 200 μT, or to the intermittent field of 1000 μT, respectively (not significant). Continuous exposure to the magnetic field at 1000 μT resulted in a decreased lymphoma incidence (23%), which was statistically significant ($p = 0.041$, Fisher's exact test; $p = 0.054$, life-table test). There was no significant difference in the incidence of lymphomas between groups of female Eμ-*pim*-1 mice. The incidence was 49% in sham controls and 45%, 45%, 47% and 53% in the four groups exposed to magnetic fields. In the TSG-*p53* mice, the incidence of lymphoma was 3% in male controls, 0% in exposed males, 3% in female controls and 7% in exposed females (not significant). The authors concluded that their study demonstrated no increased risk of lymphoma in either Eμ-*pim*-1 or TSG-*p53* (knock-out) mice exposed to 60-Hz magnetic fields (McCormick *et al*., 1998).

(*c*) *Other studies*

Although studies with fully transformed cells are outside the immediate scope of this monograph, the Working Group briefly considered a series of in-vivo studies conducted to determine the influence of magnetic fields on the growth and proliferation of transplantable tumour cells. In these studies, mice or rats received injections of leukaemia cells and were subsequently exposed to magnetic fields. The results of these studies were uniformly negative: no effects of exposure to magnetic fields were identified in any study reported (Thomson *et al*., 1988; Sasser *et al*., 1996; Morris *et al*., 1999; Devevey *et al*., 2000).

3.2.5 *Multistage studies of neurogenic cancer*

One study has been conducted to determine the influence of 60-Hz magnetic fields on the induction of neurogenic tumours by transplacental exposure to ENU. On day 18 of gestation, female Fischer 344 rats received a single intravenous dose of ENU (5 mg/kg bw) and were randomized into groups of 32 dams which were either sham-exposed or exposed to magnetic fields at strengths of 2 μT, 20 μT, 200 μT and

2000 μT. The magnetic field exposure system has been described in detail by Mandeville *et al.* (1997). After parturition, dams were exposed to magnetic fields together with their pups until weaning. At weaning, female pups were selected into groups of 50, and exposure to the magnetic fields was continued until study termination at age 65 weeks. A blinded histopathological analysis was performed on the brain (three levels) and spinal cord (three levels) of all animals. Exposure to magnetic fields had no influence on survival in ENU-treated animals. No significant differences between the sham-exposed group and the groups exposed to magnetic fields were seen in the incidence of all neurogenic tumours, glial tumours in the central nervous system or schwannomas in the peripheral nervous system. When compared with a total incidence of neurogenic tumours of 61% (30/49) in sham controls, the incidences of all neurogenic tumours in groups exposed to 60-Hz magnetic fields were 53% (26/49), 56% (28/50), 48% (24/50) and 46% (23/50) in the groups exposed to 2, 20, 200 and 2000 μT, respectively. None of these differences was statistically significant. The authors concluded that their study provided no evidence that 60-Hz magnetic fields have a promoting effect on neurogenic tumours (Mandeville *et al.*, 2000).