4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Adverse effects other than cancer in humans

4.1.1 *Reproductive and developmental effects*

The effects of two kinds of exposure to magnetic fields are addressed in this section: those associated with power-frequency fields (ELF fields of 50 or 60 Hz) and those associated with video display terminals. The fields associated with video display terminals are typically of varying frequencies in the ELF range as well as higher frequencies (300 Hz–100 kHz). Studies of the possible effects of exposure to such fields on reproductive outcome are discussed below, including some that have examined mutations in maternal or paternal germ cells, investigations of exposure during prenatal development and other possible magnetic field-induced changes in fetal or maternal physiology. The information has been obtained primarily from epidemiological studies and a number of extensive reviews that have been published recently (Chernoff *et al*., 1992; Brent *et al*., 1993; Shaw & Croen, 1993; Huuskonen *et al*., 1998a; Shaw, 2001).

(*a*) *Exposure to ELF electric and magnetic fields during pregnancy*

Studies of maternal exposure to power-frequency fields have focused principally on the use of electric blankets and electrically heated beds, other sources of residential exposure and a few occupational studies.

The use of electric blankets (exclusively older models) and electrically heated beds can add appreciably to total exposure to ELF electric and magnetic fields. It has been estimated that use of electric blankets increases overall exposure to electric fields by 36% over that of non-users (Preston-Martin *et al*., 1988; see also section 1). Although these appliances are frequently used by pregnant women, the available studies present little evidence to support an association of exposure to ELF electric and magnetic fields with adverse reproductive outcomes (Wertheimer & Leeper, 1986; Dlugosz *et al.*, 1992; Juutilainen *et al.*, 1993; Bracken *et al.*, 1995; Li *et al.*, 1995; Belanger *et al.*, 1998). The first suggestion of potential adverse effects came from the study by Wertheimer and Leeper (1986), who reported an increase in the number of spontaneous abortions and of infants who showed below-average fetal growth associated with winter conception, and hence with increased use of electrically heated beds and blankets. However, a number of methodological inadequacies call into question the validity of these results (Hatch, 1992). A subsequent study showed that the use of heated water beds or electric blankets

during pregnancy was not associated with intrauterine growth retardation or reduced birth weight (Bracken *et al*., 1995). The same group also examined the occurrence of spontaneous abortion in pregnant women who used electric blankets or heated water beds: the use of electric blankets did result in an elevated risk ratio (1.8; 95% CI, 1.1–3.1) for spontaneous abortions whereas the use of water beds in homes with wire codes associated with elevated ELF electric and magnetic fields did not increase the risk ratios (Belanger *et al*., 1998).

In a case–control study that examined specific congenital malformations, cleft palate (n = 121), cleft lip (n = 197), anencephalus and spina bifida (n = 224), no effects on odds ratios were evident in the offspring of women who had been exposed to ELF electric and magnetic fields through the use of electric blankets or electrically heated beds (Dlugosz *et al*., 1992). The possible association of neural tube defects with the use of heated beds and blankets by pregnant women was assessed in the offspring of a cohort of 23 491 women (Milunsky *et al*., 1992). No evidence of an association was observed between the use of electric blankets and adverse pregnancy outcomes.

Three studies examining maternal exposure to ELF fields and the risk for cancer in the children exposed *in utero* are reviewed in section 2.2.

In the study of Li *et al*. (1995), the use of electric blankets was not associated with increased anomalies of the urinary tract (odds ratio, 1.1; 95% CI, 0.5–2.3). In a subgroup of women (n = 5) with a history of reduced fertility an odds ratio of 4.4 (95% CI, 0.9–23) was observed.

[The Working Group noted the problem in the interpretation of the data on heated beds arising from the potentially large variability in exposure. This is due both to recall bias on duration and frequency of use and power setting of the appliance and to the large variation in the strengths of the fields produced by the different appliances.]

The studies on the effects of residential exposure (other than from heated beds) on pregnancy outcome have focused primarily on spontaneous abortion and birth weight of the offspring. The first such study (Wertheimer & Leeper, 1989) reported a positive correlation between elevated exposure to ELF magnetic fields at ~ 1 μT in electrically heated homes (from electric heating elements in the ceiling) and fetal loss. In another study, in which ELF magnetic fields were measured in the home, an association was also observed between higher fields (average > 0.2 μT; > 0.6 μT at the front door) and spontaneous abortion in early pregnancy (Juutilainen *et al*., 1993). In contrast, a subsequent study showed no association between exposure to measured magnetic fields above 0.2 μT, or to high wire-codes, and adverse pregnancy outcome, including miscarriage, low birth weight or pre-term delivery (Savitz & Ananth, 1994). Another study that found no relationship between exposure to magnetic fields > 0.2 μT and reduced birth weight or growth retardation was reported by Bracken *et al*. (1995).

Few studies of occupational exposure of women to ELF magnetic fields in relation to pregnancy outcome have been made. In a study of women involved in the manufacturing of semiconductors, no increase in the risk for spontaneous abortion was

observed even for workers exposed to the strongest fields (time-weighted average (TWA) ≥ 0.9 μT) (Swan *et al*., 1995).

A prospective cohort study was conducted to assess the effects of exposure to ELF magnetic fields on spontaneous abortion. Exposure was measured by a dosimeter worn on the body. A weak, non-significant association was observed with exposure to fields above 0.2 μT when the TWA was used as the exposure metric. However, a significantly increased risk (approximately threefold) was found when the exposure metric used was maximum exposure above 1.6 μT. The risk was limited to women who indicated that measurements had been taken during a 'typical' day and was further increased if the subjects had a history of difficulties during pregnancy (Li *et al.*, 2001).

(*b*) *Paternal exposure to ELF electric and magnetic fields*

The investigations of the relationship between paternal exposure to ELF electric and magnetic fields and potentially adverse reproductive outcomes have been almost exclusively conducted in occupational settings. The available studies have largely focused on cancer in the offspring; however, a number of other end-points have also been investigated, including male infertility, perinatal death, spontaneous abortion, congenital anomalies, number of offspring, male:female birth ratio and low birth weight.

The studies examining risk for childhood cancer associated with paternal exposure to ELF electric and magnetic fields are reviewed in section 2.2.

The frequency of abnormal pregnancy outcome (described as congenital malformations and fertility difficulties) was reported to be significantly increased among the wives of workers in high-voltage switchyards (Nordström *et al*., 1983). Buiatti *et al*. (1984) found more cases of male infertility in radioelectrical workers than in controls (odds ratio, 5.9; 95% CI, 0.9–40). No association was seen between semen abnormalities and electrical occupations (Lundsberg *et al*., 1995) and no significant increase in abnormal birth outcome was reported for offspring of power-industry workers (Törnqvist, 1998). Baroncelli *et al*. (1986) reported no effect on the number of children per family when fathers worked in a high-voltage substation. For male workers in industries associated with exposure to ELF fields, the proportion of male offspring was slightly reduced, while the number of offspring of female workers was significantly reduced (Irgens *et al.*, 1997). [The Working Group noted that 'number of children' is a particularly weak end-point with respect to developmental toxicology.]

(*c*) *Exposure to mixed ELF and higher-frequency electric and magnetic fields*

Many of the studies on the relation between exposure to ELF fields and reproductive effects in humans have addressed the question of whether pregnant women are at risk when exposed to the ELF fields associated with video display terminals. Most of these studies have investigated spontaneous abortion and congenital abnormalities in the offspring. There is little evidence for an association between exposure to fields from

video display terminals and spontaneous abortion. Of ten studies (McDonald *et al.*, 1986; Ericson & Källén, 1986a,b; Westerholm & Ericson, 1987; Goldhaber *et al.*, 1988; Bryant & Love, 1989; Windham *et al.*, 1990; Nielsen & Brandt, 1990; Schnorr *et al.*, 1991; Lindbohm *et al.*, 1992), only two showed a significantly increased risk. The study by Goldhaber *et al*. (1988) showed an odds ratio of 1.8 (95% CI, 1.2–2.8), although later analyses suggest that differential reporting of exposure was the source of the association (Hertz-Picciotto *et al*., 1992). Lindbohm *et al*. (1992) observed increased risk ratios among women in Finland who used video display terminals with high-intensity fields (peak-to-peak value, > 0.9 μT) for more than 10 h per week (risk ratio, 3.4; 95% CI, 1.4–8.6). In contrast, a large study conducted in the USA showed no dose–response relationship and no increased risk (Schnorr *et al*., 1991). The strongest fields to which subjects were exposed in this study were weaker than those in the Lindbohm study. Most of the studies reported risk ratios from 1.1 to 1.2 but with 95% confidence intervals that include 1.0. Furthermore, in studies that assessed duration of exposure per day, there was generally no evidence for an increase in risk in association with longer exposure times.

Studies of reproductive health outcomes other than spontaneous abortion have also been made. Low birth weight, pre-term delivery, intrauterine growth retardation and perinatal mortality have been considered when evaluating exposure to fields from video display terminals, but these other end-points have rarely shown any indication of an effect of exposure to ELF fields from video display terminals. Over half a dozen studies (for a review, see Shaw, 2001), including two large studies with more than 1500 cases and 21 000 controls by McDonald *et al*. (1986) and Windham *et al*. (1990), have shown no significant reduction in birth weight associated with use of video display terminals, although intrauterine growth retardation was somewhat elevated (odds ratio, 1.6; 95% CI, 0.92–2.9) with greater use of video display terminals. Elevated risks (odds ratio > 1.5) have occasionally been observed for perinatal death (Bjerkedal & Egenaes, 1987) and congenital abnormalities (Ericson & Källén, 1986a,b); however, the risks were not significantly different from those in controls, and other studies did not confirm the results.

4.1.2 *Immunological effects*

The effects of exposure to magnetic fields on various markers of immune function were studied in two groups of workers: one group comprised 10 hospital personnel operating magnetic resonance tomographs and the other group was composed of 10 industrial workers operating induction heaters. A group of 23 workers served as non-exposed controls. Operaters of magnetic resonance tomographs exposed to static magnetic fields at ≥ 0.5 mT for an indeterminate time showed no significant reductions in their concentration of interleukin-2 or the number of monocytes in their blood. The operaters of induction heaters had been exposed to magnetic fields at either 50–600 Hz (up to 2 mT) or to 2.8–21 kHz (0.13–2 mT) for at least two years, and often longer than five years. The numbers of natural killer cells and monocytes were significantly

increased in the exposed group while monocytes had significantly reduced phagocytic activity compared with those from unexposed personnel. For the two subjects with the highest exposure, the natural killer cell counts were > 700 cells/μL blood compared with 276 ± 124 cells/μL for the controls. Blood samples drawn from these two subjects eight months later still showed elevated counts of natural killer cells (671 and 1202 cells/μL, respectively) while controls had 281 ± 115 cells/μL, indicating that the elevated readings had not been due to an unknown confounding factor at the time of the first blood sampling (Tuschl *et al.*, 2000).

A group of 16 young men aged 20–30 years were exposed to 50-Hz, 10-μT magnetic fields from 23.00 to 08.00. In the first experiment, exposure was continuous for one night, in a second experiment exposure was intermittent, i.e. 1 h 'off' and 1 h during which the field was switched between 'on' and 'off' every 15 s. Sixteen other men were the sham-exposed controls. Blood samples were collected at 3-hourly intervals from 11.00 to 20.00 and hourly from 22.00 to 08.00. No significant differences were observed between exposed and sham-exposed men in haemoglobin concentration, haematocrit, or counts of erythrocytes, platelets, total leukocytes, monocytes, lymphocytes, eosinophils or neutrophils. The numbers of CD3, CD4, CD8, natural killer cells and B cells were also comparable between the two groups (Selmaoui *et al.*, 1996a).

4.1.3 *Haematological effects*

A survey of neurovegetative disorders and haematological effects was conducted in a group of three men and 10 women who had worked near electrical transformers, high-tension cabling (13 kV) and a power generator. In one room the 50-Hz field was 1.2–6.6 μT at floor level and 0.3–1.2 μT at 1.5 m above floor level. The magnetic fields in an adjacent room also used by the group were 0.2–0.3 μT and 0.09–0.12 μT, respectively. The subjects had worked on the premises for at least 8 h per day for one to five years. The occurrence of neurovegetative disorders was assessed from self-rating questionnaires completed by the exposed workers and matched control groups. A comparative analysis of the questionnaires showed that the exposed group suffered a significant increase in physical fatigue, psychological asthenia, lipothymia, decreased libido, melancholy, depressive tendency and irritability. [The Working Group noted the possibility of subjective bias in self-reporting questionnaires.] There was also a significant decrease in total lymphocytes and CD2, CD3 and CD4 lymphocytes, as well as an increase in the number of natural killer cells. Leukopenia and neutropenia were seen in two subjects who were chronically exposed to a field strength of 1.2–6.6 μT. The effects disappeared when exposure stopped, and reappeared when exposure was resumed (Bonhomme-Faivre *et al.*, 1998).

4.1.4 *Neuroendocrine effects*

Melatonin, a hormone produced in the mammalian pineal gland, is secreted in a circadian pattern to give high concentrations at night and low concentrations during the day. The circadian release of melatonin is known to influence certain physiological functions and to modulate the release of other hormones. Although relatively little is known about the mechanism by which changes in melatonin in humans may affect health and well-being, plausible hypotheses exist which suggest that alterations in this hormone may influence the risk for cancer.

On the basis of experimental studies that showed reductions in melatonin concentrations in animals exposed to ELF electric or magnetic fields, Stevens (1987) proposed what has been described as the 'melatonin hypothesis' (Stevens, 1987; Stevens & Davis, 1996). Exposure-related suppression of the night-time rise in melatonin concentration may explain the adverse health outcomes, including cancer — specifically breast or prostate cancer — reproductive problems and neurodegenerative diseases (Wilson *et al.*, 1989). A prerequisite to establishing the relevance and validity of the melatonin hypothesis in humans exposed to ELF electric and magnetic fields would be to determine whether melatonin is indeed suppressed during or following exposure.

(*a*) *Exposure under laboratory conditions*

Studies of endocrine function in humans exposed to 50- or 60-Hz magnetic fields under laboratory conditions have been conducted in four laboratories. As shown in Table 34, the results have been principally negative with respect to the demonstration of exposure-related effects. Night-time exposure of human volunteers to magnetic fields under controlled exposure and lighting conditions had no apparent effect on nocturnal blood concentrations of melatonin when compared with sham-exposed subjects. Endocrine parameters, other than melatonin, have not been shown to be affected by exposure to 50- or 60-Hz electric or magnetic fields.

In the first of a series of double-blind studies, 33 young male volunteers, aged 18–35 years, were exposed to intermittent, circularly polarized magnetic fields of 1 μT or 20 μT, or sham-exposed (11 subjects per group) between 23.00 and 07.00 under controlled environmental and exposure conditions (Graham *et al.*, 1996). Overall, exposure had no effect on melatonin concentrations in serum, as measured by radioimmunoassay. However, men with a pre-existing low melatonin production showed significantly reduced melatonin concentrations when exposed to the 20-μT field. In a second experiment, 40 men were identified who had low melatonin concentrations in their serum. Each of these volunteers slept in the exposure facility for two nights. On one night the men were sham-exposed and on the other they were exposed to 60-Hz, 20-μT magnetic fields. In this experiment, exposure had no effect on melatonin concentrations and the original finding was not replicated in these low-melatonin subjects (Graham *et al.*, 1996). In a third study, using a cross-over design in which each subject served as his own control, 40 young men were sham-exposed for one

Table 34. Melatonin levels in human volunteers

Reference	Assay	Exposure	Response	Comment
Wilson *et al.* (1990)	Early morning excretion of urinary metabolite of melatonin	60-Hz EMFs generated by pulsed alternating or direct current supply to electric blankets at night for 7–10 weeks	No overall effect; transient increases in 7/28 users of one type of blanket	Realistic, but concomitant lack of control over lifestyle
Graham *et al.* (1996)	Night-time serum melatonin concentrations	60-Hz, intermittent fields of 1 or 20 μT for 8 h at night	No effect; possible effect on low-melatonin subjects not replicated in larger study	
Graham *et al.* (1997)	Night-time serum melatonin concentrations	60-Hz, continuous fields of 1 or 20 μT for 8 h at night	No effect	
Selmaoui *et al.* (1996b)	Night-time serum melatonin concentrations and excretion of its major urinary metabolite	50-Hz, continuous or intermittent fields of 10 μT for 9 h at night	No effect	
Wood *et al.* (1998)	Night-time serum melatonin concentrations	50-Hz intermittent sinusoidal or square-wave fields of 20 μT for 1.5–4 h at night	Possible delay and reduction of night-time melatonin concentrations in subgroup	Inconsistent, variable data; incomplete volunteer participation
Graham *et al.* (2000a)	Early morning excretion of urinary melatonin and its metabolite	60-Hz circularly polarized magnetic field of 28.3 μT overnight for 4 consecutive nights	No effect on night-time concentrations	Exposed subjects showed less intra-individual consistency on night 4

From NRPB (2001)
EMF, electric and magnetic field

night and exposed continuously through the second night to a 60-Hz, 20-μT magnetic field. Again, no overall effects on melatonin were found (Graham *et al*., 1997).

Another study conducted in the same laboratory examined 30 healthy young male volunteers who were exposed to 60-Hz, 28.3-μT magnetic fields for four consecutive nights (Graham *et al*., 2000a). Melatonin concentrations were determined by measurement of 6-hydroxymelatonin sulfate (a major melatonin metabolite) in the morning urine samples each day. Again, no overall effect of exposure on melatonin concentrations was observed. The consistency of intra-individual urinary measurements over the four test nights was much higher in the control samples than in those of the exposed subjects.

In a study of the effects of exposure to a 50-Hz, 1-μT magnetic field on sleep patterns in eight women and 10 men, Åkerstedt *et al*. (1999) measured concentrations of melatonin, growth hormone, prolactin, testosterone and cortisol in peripheral blood during a night of sham exposure or a night of exposure to the magnetic field. Exposure to the magnetic field showed effects on sleep patterns, but no significant endocrine or neuroendocrine effects due to exposure were observed.

The other major laboratory study on neuroendocrine effects measured not only melatonin but also pituitary, thyroid and adrenocortical hormones in 32 young men aged 20–30 years who were divided into three groups that were sham-exposed or exposed to continuous or intermittent linearly polarized 10-μT magnetic fields. The exposure-treatments and sampling of serum and urine were conducted over two 24-h periods. No significant differences in serum melatonin and urinary 6-hydroxymelatonin sulfate were found between the test and control groups (Selmaoui *et al*., 1996b). There were also no differences observed in the concentrations or circadian variations of thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, triiodothyronine, thyroxine, thyroxine-binding globulin, cortisol, 17-hydroxycorticosteroids or on thyroxine-binding index (Selmaoui *et al*., 1997).

In another study (Wood *et al*., 1998), the effect of exposure to magnetic fields on melatonin patterns was evaluated in 30 male volunteers. Once the nocturnal melatonin curve had been determined for each individual, the subjects were divided into groups that were either sham-exposed or exposed to magnetic fields (50 Hz, 20 μT) before, during or after the time of peak concentration of melatonin. Exposure preceding the rising segment of the curve significantly delayed the peak in one exposed individual and showed a similar trend in others. The authors noted the suggestive nature of their results but considered them to be preliminary. [The Working Group noted that this is the only laboratory study in humans which sugggested an apparent effect of exposure to magnetic fields on neuroendocrine parameters, including melatonin. Because of the preliminary nature of this study, a number of concerns have been raised over experimental design and statistical analysis.]

(*b*) *Exposure in occupational and residential environments*

A heterogeneous group of epidemiological studies has also evaluated endocrine function in humans exposed to ELF magnetic fields in the relatively uncontrolled

environment of occupational and residential settings. In contrast to the negative results of laboratory studies, all of these studies noted some perturbation in the excretion of 6-hydroxymelatonin sulfate in exposed groups. The perturbations were not, however, consistent across studies and the exposure parameters also differed from one study to the next; they included use of electric blankets, exposure to 16 $^{2}/_{3}$-Hz fields (for railway engineers) as well as exposure to 60 Hz in residential settings and to 50 Hz and 60 Hz in occupational settings.

One of the earliest studies to measure melatonin via the urinary metabolite hydroxymelatonin sulfate (6-OHMS) focused on users of electric blankets. Forty-two volunteers used standard or modified continuous-polymer-wire blankets for eight weeks. The continuous-polymer-wire blankets produced fields that were 50% stronger than those of conventional blankets (0.66 versus 0.44 μT) and they switched on and off twice as often. The subjects in this study served as their own controls. Seven of 28 volunteers using the continuous-polymer-wire blankets were found to have a statistically significant increase in mean night-time urinary excretion of 6-OHMS at the cessation of exposure. This is the only study to report an increase in melatonin or its metabolite in association with higher-than-background exposure to ELF electric or magnetic fields. No changes were seen in the users of conventional electric blankets (Wilson *et al.*, 1990).

A study of Swiss railway workers compared 66 engineers (average exposure of most-exposed workers, 20 μT) with 42 other employees (train attendants and station managers; average exposure of least-exposed workers, 1 μT). Each volunteer served as his own control. Morning and evening urinary concentrations of 6-OHMS were determined during leisure periods and on the day following resumption of work. Evening concentrations of 6-OHMS appeared to be lower by a factor of 0.81 (95% CI, 0.73–0.90) during workdays compared with leisure days in engine drivers, but not in controls. The evening concentrations recovered significantly during leisure periods, which suggests that the effects were reversible. In contrast, morning concentrations of 6-OHMS from engineers and controls did not differ much between workdays and leisure days (Pfluger & Minder, 1996). [The Working Group noted that the interpretation of this study is hampered by the difficulties in accounting for the effects of shift work in some of the subjects and a crude assessment of exposure. It should also be noted that the predominant exposure is to 16 $^{2}/_{3}$-Hz fields, not the 50- or 60-Hz fields considered in most of the other studies.]

The effects of exposure to 60-Hz magnetic fields (and ambient light) were studied in 142 male electric utility workers. Melatonin was measured as 6-OHMS in post-workshift urinary samples over a three-day sampling period. The groups compared were 29 power generation workers (mean exposure, 0.32 μT), 56 linemen and substation operators (mean exposure, 0.23 μT) and 57 utility maintenance and administrative staff (mean exposure, 0.15 μT). In addition to field intensity measured as TWA fields, the temporal stability of the fields was also determined. Exposure intensity, as measured by the geometric mean magnetic field, was not associated with 6-OHMS

excretion. However, evaluation of the temporal stability parameter showed that men in the highest quartile had lower 6-OHMS concentrations on the second and third days of the sampling period when compared with the men in the lowest quartile (Burch *et al*., 1998, 1999).

The same research team studied workers in substations in three-phase environments compared with workers in one-phase environments. Over three consecutive workdays, an apparent field-dependent reduction in mean nocturnal and post-work concentrations of 6-OHMS was reported for men who worked more than 2 h per day in a substation with a three-phase environment. No difference was observed among those men who worked 2 h or less or those who worked in one-phase environments (Burch *et al.*, 2000).

A third occupational study was carried out among female garment-industry workers including 39 production workers and 21 office workers, who served as controls. Exposure assessment varied with the type of machine used and was based on magnetic field measurements made around each type of machine. Exposure to 50-Hz magnetic fields was quite high (> 1 μT) for one group, approximately 0.3–1 μT for a second exposure group and about 0.15 μT for the control group. Morning void urine samples were collected on the Friday and Monday for three consecutive weeks. The average 6-OHMS concentration in the urine on Fridays was lower in the factory workers than in the control group, but no monotonic dose–response pattern was observed. The 6-OHMS concentrations measured in urine samples taken on Monday and Friday were not different for test subjects and controls. Multivariate analysis identified exposure to a magnetic field, smoking and age as significant explanatory variables associated with decreased 6-OHMS excretion (Juutilainen *et al.*, 2000).

The most comprehensive study of the effects of residential exposure to magnetic fields on neuroendocrine response was conducted in Seattle, Washington. The 203 participants were women selected from a group that participated as controls in a case–control study of breast cancer and exposure to electric and magnetic fields. Magnetic fields were measured in the participants' bedrooms, and personal field measurements were made during the same 72 h. Total night-time urine samples collected during the three consecutive nights of the measurement period were used to assess the concentration of 6-OHMS. The results showed that decreasing concentrations of 6-OHMS in the night-time urine were associated with increasing magnetic field strength, as measured in the women's bedrooms at night. The magnetic field effect was seen primarily in women who used medication (e.g. beta blockers, calcium-channel blockers and psychotropic drugs), and was strongest during the times of the year with the shortest nights. These findings were particularly marked when the exposure measure was the proportion of night-time magnetic field measurements ≥ 0.2 μT. The reduction in the concentration of 6-OHMS in urine was not correlated with personal field measurements > 0.2 μT, variability in field measurements, use of electric blankets or wire codes (Davis *et al.*, 2001). [The Working Group noted that

extensive field assessment data were collected and analysed in this study and considerable effort had been made to consider and control potential confounding.]

4.1.5 *Behavioural and physiological effects*

A number of volunteer studies have investigated the effects of static or ELF electric and/or magnetic fields on perception, the electrical activity of the brain, memory and reasoning, mood, hypersensitivity and heart rate.

(*a*) *Static fields*

Prior to the development of magnetic resonance imaging (MRI) techniques, few studies of the effects of static magnetic fields on volunteers had been documented (summarized by WHO, 1987), although various anecdotal reports from laboratories using large accelerators existed. However, with the advent of superconducting magnet technology and MRI in the late 1970s, volunteers could be routinely exposed to static fields ≥ 1.5 T. Most of the reported effects of acute exposure are consistent with known mechanisms of action.

(i) *Perception of electric fields*

The electric charge induced on the surface of a person exposed to a static electric field can be perceived by its interaction with body hair, particularly on the head. Clairmont *et al*. (1989) reported that volunteers had a threshold of perception around 20 kV/m, and that fields above about 25 kV/m produced annoying sensations.

(ii) *Perception of magnetic fields*

Schenck *et al*. (1992) reported dose-dependent sensations of vertigo, nausea and a metallic taste in the mouth in volunteers exposed in MRI systems to static magnetic fields of 1.5 or 4 T; however, gradient and higher-frequency magnetic fields also seem to have contributed to the total exposure. The sensations reported occurred only during movement of the head. In addition, magnetic phosphenes (described below) could sometimes be seen during eye movement in a static magnetic field of at least 2 T.

(iii) *Cognition*

In a static field, Lorentz forces will be exerted on the ion flow through nerve membranes, although these may not be of biological significance at field strengths < 2 T (Tenforde, 1992). The possible cognitive effects immediately after volunteers had been exposed for one hour to a static magnetic field of 8 T were investigated by Kangarlu *et al*. (1999). The written and oral tests comprised a standard ‘mini-mental’ status examination of cognitive function and other standard tests of cognition and motor function. The performance in these tests after exposure did not differ from that in the tests conducted before exposure. An earlier study of a large number of volunteers had reported a lack of effect of exposure to static magnetic fields from magnetic resonance imaging

equipment of 0.15 T in a variety of cognitive tests, although anxiety was increased in the exposed group following exposure (Sweetland *et al*., 1987).

(iv) *Cardiac effects*

On theoretical grounds, Kinouchi *et al*. (1996) noted that the Lorentz force exerted on the blood flow generates an electrical potential across the blood vessel. In practice, so-called 'flow' potentials are readily demonstrated in large animal species, such as dogs, baboons and other monkeys exposed to static fields stronger than ~ 0.1 T. Generally, the largest flow potentials occur across the aorta after ventricular contraction and appear superimposed on the T-wave of the electrocardiogram (Tenforde, 1992).

In addition, a 5–10% reduction in blood flow in the aorta is predicted to occur in static fields of 10–15 T, due to magneto–hydrodynamic interactions (Kinouchi *et al.*, 1996). However, Kangarlu *et al*. (1999) noted that following exposure to an 8-T static field, volunteers showed no change in heart rate or diastolic or systolic blood pressure, compared with values measured before exposure; the values recorded during exposure were also reported as unchanged.

(*b*) *ELF electric and magnetic fields*

The nervous system functions by virtue of electrical signals and may be thought particularly vulnerable to ELF electric and magnetic fields. Various studies have been carried out on the effects of ELF electric and magnetic fields on perception, electrical activity of the brain, cognitive processes (i.e. thinking and memory), mood, hypersensitivity, sleep and heart rate.

(i) *Perception of electric fields*

It is well established that ELF electric fields can be perceived due to the field-induced vibration of body hair. The threshold for perception by hair vibration shows wide individual variation: 10% of exposed subjects were found to have detection thresholds of 10–15 kV/m at 50–60 Hz, and 5% of subjects could detect fields as weak as 3–5 kV/m. Although these effects are not considered to be a hazard, hair vibration and tingling became an annoyance to test subjects at field strengths > 20 kV/m (Deno & Zaffanella, 1975). Of greater biological significance may be the occurrence of capacitive spark discharges or microshocks, generated when two objects of different potential come into close proximity and the electric breakdown field strength of the air is exceeded. The threshold for the perception of spark discharges by a small proportion (10%) of a group of volunteers close to a grounded object has been reported to be 0.6–1.5 kV/m at 50 or 60 Hz, while the threshold was 2.5–6 kV/m for the rest of the group (Bernhardt, 1988).

(ii) *Magnetic phosphenes*

Exposure to power-frequency magnetic fields < 1 mT is generally regarded as imperceptible. In contrast, exposure of the head to magnetic flux densities at 20 Hz above about 5 mT up to about 50 Hz, 15 mT, will reliably induce faint, flickering,

visual sensations called magnetic phosphenes (Lövsund *et al*., 1979, 1980a,b). Similar sensations can be induced by electric currents applied directly via electrodes attached to the head (Lövsund *et al*., 1980b). It is generally agreed that phosphenes result from the interaction of the induced electric current with electrically sensitive cells in the retina. The maximum current density in the retina associated with the generation of magnetically induced phosphenes has been estimated to be about 11 mA/m^2 at 20 Hz (Wake *et al*., 1998), based on calculations using a realistic, electrically heterogeneous model of the human head.

(iii) *Electroencephalograms and event-related brain potentials*

The electrical activity of the brain, recorded as an electroencephalogram, conveys information of a general nature that characterizes the mental state of a person. The electroencephalogram is used in the diagnosis of a variety of pathological conditions. Event-related brain potentials, which are also recorded using electrodes placed on the scalp, convey more specific information concerning brain activity evoked by a sensory stimulus (evoked potentials) and, after about 100 min, by subsequent cognitive processes.

Two double-blind studies on the effects of exposure to 45-Hz, 1000 Amps/m [1.26 mT], magnetic fields on electroencephalograms have been reported. Changes were observed in the alpha, delta and beta frequency bands and in auditory evoked potentials (Lyskov *et al*., 1993a,b). A phase reversal and a slower decrease in the amplitude of the major components of visual evoked potentials have been reported during exposure to very intense (60 mT), pulsed magnetic fields, although they had no effect on visual acuity (Silny, 1984, 1985, 1986). In contrast, no marked effect on visual, auditory or somatosensory evoked potentials was reported during exposure to fields of weaker intensity (28 μT) (Graham *et al*., 1999).

The possible effects of electromagnetic fields on event-related potentials have been investigated mostly in conjunction with various cognitive tests. In general, few effects have been found; those that were noted have tended to be subtle and transitory (Crasson *et al*., 1999). For example, small changes in latency and amplitude of a late component (P300) of the event-related potential associated with cognitive function were observed when subjects exposed to combined electric (9-kV/m) and magnetic (20-μT) fields were asked to discriminate between frequent and infrequent stimuli (Cook *et al*., 1992; Graham *et al*., 1994). When subjects were exposed to a magnetic field of 50 Hz, 100 μT, changes were observed in event-related brain potentials during performance of a listening task, in which auditory discrimination is tested (Crasson *et al*., 1999).

(iv) *Cognition*

A number of studies have looked for evidence of changes in cognitive ability during or after exposure to power-frequency electromagnetic fields. Reaction time, vigilance or sustained attention, memory function, and tasks involving time perception

and information processing have all been tested. Some changes have been reported, but the effects were not consistent between studies. For example, studies have reported both increases (Cook *et al*., 1992; Kazantzis *et al*., 1996) and decreases (Graham *et al*., 1994; Preece *et al*., 1998) in the accuracy of task performance. Similarly, several studies have reported decreased reaction time (Graham *et al*., 1994; Whittington *et al*., 1996), or no effect (Podd *et al*., 1995; Preece *et al*., 1998).

(v) *Mood*

The possibility that environmental exposure to power-frequency electromagnetic fields might be associated with a variety of negative mood states has been assessed in several double-blind laboratory studies in which volunteers have completed mood assessment checklists before and after exposure. None of these studies (Stollery, 1985; Cook *et al*., 1992; Graham *et al*., 1994; Crasson *et al*., 1999) reported any effects although Stollery (1985) reported decreased arousal in one of two participating groups.

(vi) *Hypersensitivity*

It has been reported that some people are sensitive to electric and magnetic fields. The symptoms of sensitivity include sleep disturbance, general fatigue, difficulty in concentrating, dizziness, eye strain, facial skin problems such as eczema and sensations of itching, burning or stinging. Several double-blind laboratory provocation studies have been carried out. Generally, the patients and volunteers who participated in these studies were not reliably able to identify the presence of electric or magnetic fields, and neither subjective symptoms nor biochemical measures were significantly related to the exposure conditions (Andersson *et al*., 1996; Lonne-Rahm *et al*., 2000).

(vii) *Sleep electrophysiology*

Several studies have examined the effect of exposure to electric and magnetic fields on sleep, monitored using electroencephalograms and self-assessment. One study reported a reduction of 'slow-wave' sleep, total sleep time and depth of sleep in subjects exposed to a relatively weak power-frequency magnetic field (50 Hz, 1 μT) (Åkerstedt *et al*,. 1999). In contrast, another study reported that intermittent exposure to 60-Hz, 28-μT magnetic fields was associated with a poor and irregular pattern of sleep (Graham *et al*., 1999).

(viii) *Heart rate*

A statistically significant slowing of heart rate, recorded as the interbeat interval, during exposure to 60-Hz electric and magnetic fields (9 kV/m and 20 μT) has been reported in several studies (Cook *et al*., 1992; Graham *et al*., 1994). However, these effects were not observed at higher or lower field strengths. No effect on heart rate or blood pressure was seen during acute exposure to 50-Hz, 100-μT magnetic fields (Whittington *et al*., 1996).

A more recent study reported an altered heart rate variability during exposure to an intermittent 60-Hz magnetic field at night (Sastre *et al*., 1998). However, in a

pooled analysis of several studies conducted at the same institute, Graham *et al.* (2000b) later reported that this effect was observed only in studies where hourly blood sampling had taken place as part of a different experiment. The authors hypothesized that blood sampling may have altered the arousal of the subjects, allowing interaction with the magnetic field to affect heart-rate variability.

(*c*) *Epidemiological studies*

Several epidemiological studies have been carried out over the past 20–30 years on the incidence of neurodegenerative diseases, suicide and depression, and cardiovascular disease in relation to occupational or residential exposure to ELF electric and magnetic fields (reviewed in Portier & Wolfe, 1998; International Commission on Non-Ionizing Radiation Protection, 1998).

(i) *Neurodegenerative diseases*

Many studies have focused on amyotrophic lateral sclerosis, a progressive degenerative motor neuron disease, and Alzheimer disease, a progressive irreversible degenerative disease of the brain, in groups of people occupationally exposed to ELF electric and magnetic fields.

Several studies on amyotrophic lateral sclerosis have been published (Deapen & Henderson, 1986; Gunnarsson *et al*., 1992; Davanipour *et al*., 1997; Johansen & Olsen, 1998; Savitz *et al*., 1998a,b). The combined results from the two studies of utility workers (Johansen & Olsen, 1998; Savitz *et al*., 1998a,b) show a clear increase in mortality from amyotrophic lateral sclerosis in association with exposure to ELF magnetic fields. This increase is unlikely to be due to chance but may be confounded by exposure to electric shocks.

Five studies have been conducted on Alzheimer disease in relation to exposure to ELF electric and magnetic fields (Sobel *et al*., 1995, 1996; Feychting *et al*., 1998b; Savitz *et al*., 1998a,b). When all studies are considered together, there appears to be an association between the occurrence of the disease and estimated exposure to ELF electric and magnetic fields. However, since this result is mainly confined to studies with weaker designs, support for the hypothesis of a link between Alzheimer disease and exposure to ELF electric and magnetic fields is weak (International Commission on Non-Ionizing Radiation Protection, 1998).

(ii) *Suicide and depression*

A number of studies have examined possible associations between the incidence of suicide and residential or occupational exposure to ELF electric and magnetic fields (Reichmanis *et al*., 1979; Perry *et al*., 1981; Baris & Armstrong, 1990; Baris *et al*., 1996a,b; Johansen & Olsen, 1998; van Wijngaarden *et al*., 2000). Only the most recent study provides some support for the original findings of Reichmanis *et al.* (1979) and Perry *et al.* (1981) suggesting a relation between suicide and exposure to

magnetic fields from overhead power lines (International Commission on Non-Ionizing Radiation Protection, 1998).

The relationship between the prevalence of depressive symptoms and residential or occupational exposure to ELF electric and magnetic fields has been investigated in several studies (Dowson *et al*., 1988; Poole *et al*., 1993; McMahan *et al*., 1994; Savitz *et al*., 1994; Verkasalo *et al*., 1997). Overall, the findings are inconsistent and difficult to interpret (International Commission on Non-Ionizing Radiation Protection, 1998).

(iii) *Cardiovascular disease*

Reduced heart rate variability after exposure to 60-Hz magnetic fields has been reported (Sastre *et al*., 1998). Although inconsistent with the findings of others (Graham *et al*., 1999), the results suggested that such exposure might be associated with an increased incidence of cardiovascular disease and death. Two studies have examined mortality from cardiovascular disease among electric utility workers (Baris *et al*., 1996b; Savitz *et al*., 1999). The overall mortality from cardiovascular and ischaemic disease was generally lower in the study cohorts than in the general population, although the most recent study (Savitz *et al*., 1999) found that longer duration of employment in jobs with elevated exposure to ELF magnetic fields was associated with an increased risk for death from arrhythmia-related conditions and acute myocardial infarction. Nevertheless, the International Commission on Non-Ionizing Radiation Protection (1998) considered the evidence relating cardiovascular effects to elevated exposure to magnetic fields as weak, and the possible association between exposure and altered autonomic control of the heart is speculative.

4.2 Adverse effects other than cancer in experimental systems

4.2.1 *Reproductive and developmental effects*

(*a*) *Static magnetic fields*

(i) *Homogeneous fields*

The results obtained in studies on reproduction and development and exposure to relatively homogeneous static magnetic fields (fields without strong gradients) consistently fail to indicate any strong, easily detectable adverse effects. No effects have been seen on frog embryos exposed to a static magnetic field of 2.5 kG (0.25 T) (Hansson Mild *et al*., 1981); on prenatal development, based on standard teratological and several postnatal evaluations in gestating mice exposed at 1 T (Konermann & Mönig, 1986); on the development of the testis and epididymis in mice after exposure to 0.5–0.7 T *in utero* (Tablado *et al*., 2000); on reproductive performance in mice exposed to 0.49 T (Grzesik *et al*., 1988), or on spermatogenesis in male mice exposed to 0.3 T (Withers *et al*., 1985). When male and female mice were mated in a 3.5-T magnetic field, the number of gestating mice decreased to 21% compared with 68% after matings under sham-exposure conditions (Zimmermann & Hentschel, 1987). The effect was not seen

if mating occurred after removal from the magnetic field, suggesting that only mating behaviour in the strong magnetic field was affected. No adverse effects on fetal development were observed. In contrast, Strand *et al*. (1983) observed a significant enhancement of fertilization when ova or sperm of rainbow trout (*Salmo gairdneri*) were exposed to a 1-T static magnetic field.

Only two studies have reported significant effects of static magnetic fields on embryonal development.

Batches of fertilized eggs from two species of sea urchin (*Lytechinus pictus* and *Strongylocentrotus purpuratus*) were exposed to fields produced by permanent magnets. Static fields delayed the onset of mitosis in both species by a length of time that was dependent on the time interval between exposure and fertilization. Fields of 30 mT, but not 15 mT, caused an eightfold increase in the incidence of exogastrulation in *L. pictus*, whereas neither of these fields produced exogastrulation in *S. purpuratus* (Levin & Ernst, 1997).

Light microscopy and electron microscopy showed changes in chick embryo cerebella when the embryos were exposed to a 20-mT static field either on day 6 of development or during the first 13 days of development (Espinar *et al*., 1997).

(ii) *Static fields with strong gradients*

Two studies have reported effects of static magnetic fields with high spatial gradients on the embryonic development of frogs. The early embryonic growth of *Rana pipiens* was strongly inhibited in a 1-T field with a gradient of 0.84 T/cm (Neurath, 1968). The rate of malformation was increased in *Xenopus laevis* embryos grown in 1-T magnetic fields with gradients from 10–1000 T/m (Ueno *et al*., 1984). The authors of both studies discussed possible mechanisms related to the effects of magnetic forces on iron-containing molecules and oxygen molecules.

(*b*) *Strong static magnetic fields combined with weaker time-varying fields*

Magnetic resonance imaging produces a combination of strong static magnetic fields, radiofrequency fields and time-varying ELF and very low-frequency gradient fields. Few studies have addressed possible developmental effects of the combined fields typical of magnetic resonance imaging.

No effects were seen on embryonal development of frogs when frog spermatozoa, fertilized eggs or embryos were exposed to a combination of a 7.05-kG (0.705-T) static magnetic field and a 30-MHz radiofrequency field (Prasad *et al*., 1982).

Groups of 15 gestating C57BL/6J mice were subjected to magnetic resonance imaging conditions on day 7 of gestation for 36 min, using a 1.5-T static magnetic field combined with a radiofrequency field of 64 MHz (Tyndall & Sulik, 1991). The incidence of eye malformations — towards which this mouse strain is genetically predisposed — was significantly increased in exposed animals compared with a sham-exposed group. A similar exposure to magnetic resonance imaging conditions also

produced statistically significant effects on crown–rump length and craniofacial perimeter, which are less sensitive teratological parameters in these mice (Tyndall, 1993).

Chick embryos were simultaneously exposed to a static magnetic field of 1.5 T for 6 h and to 64-MHz radiofrequency field pulses and switched magnetic field gradients for 4 h. Hatching time and the migration, proliferation and death of motoneurons in the lateral motor column in the chick were unaffected by exposure under conditions of magnetic resonance imaging. Embryo development proceeded normally. There were no obvious adverse effects of exposure to magnetic resonance on differentiation of the major organs, no increase in the incidence of gross abnormalities and no evidence of lesions and malformations (Yip *et al.*, 1994).

(*c*) *ELF electric fields*

Several studies have addressed possible effects of 60-Hz electric fields on reproduction and development in rats (Charles River CD and Sprague-Dawley), using field strengths of 80 kV/m (Seto *et al*., 1984), 100 kV/m (Sikov *et al*., 1984; Rommereim *et al*., 1987), 112–150 kV/m (Rommereim *et al*., 1989) or 10–130 kV/m (Rommereim *et al*., 1990). The studies involved large group sizes and exposure over several generations. Overall, the studies did not reveal any consistent adverse effects. Malformations were increased and fertility was decreased in one study (Rommereim *et al*., 1987). These effects were not confirmed in a companion replicate experiment or in further studies by the same group.

Exposure to 50-Hz electric fields at 50 kV/m did not have any significant effects on the growth and development of eight-week-old male rats exposed for 8 h per day for four weeks. Negative results were also obtained in rabbits exposed for 16 h per day from the last two weeks of gestation to six weeks after parturition (Portet & Cabanes, 1988).

A three-generation study was conducted on Hanford Miniature swine kept in a 60-Hz, 30-kV/m electric field for 20 h per day, seven days per week. Two teratological evaluations were performed on the offspring of the F_0 generation. The incidence of malformations was decreased in the first teratological evaluation after four months of exposure (significant only if analysed by fetus), but was increased in the second evaluation, after 18 months of exposure. An increased number of malformations was also found in one group of offspring of the F_1 generation at 18 months (exposed *in utero* and from birth), but not in another group of F_1 offspring 10 months later. A complete teratological evaluation was performed only for the latter group of offspring. The inconsistency of these results precludes any conclusion that there is a causal relationship between exposure to an electric field and developmental effects (Sikov *et al.*, 1987).

(*d*) *ELF magnetic fields*

(i) *Mammalian teratological studies*

Mouse

In the experiments of Rivas *et al*. (1985), 25–27 gestating Swiss mice per group were exposed to 50-Hz pulsed magnetic fields at 83 μT or 2.3 mT. The number of live births per litter and the mean birth weight were slightly lower in the exposed animals, but the differences from the controls were not statistically significant.

Gestating CBA/Ca mice were exposed from day 0 to day 18 of gestation to 50-Hz or 20-kHz magnetic fields in two independent experiments. In the first experiment, 55 females were exposed to a field of 50 Hz, 13 μT (sinusoidal) or a field of 20 kHz, 15 μT (peak-to-peak). A group of 45 sham-exposed animals served as controls. The second experiment involved 33 females exposed to a 50-Hz, 130-μT field and 34 controls. In addition to standard teratological evaluation, micronuclei were determined in erythrocytes from maternal bone marrow. The numbers of skeletal variations were increased consistently in all exposed groups. The variations were similar in all exposure groups, and suggestive of decreased ossification. The incidence of fetuses with at least three skeletal variations showed a statistically significant increase in all exposed groups compared with corresponding controls. No other significant differences were found in any other maternal or fetal parameters (Huuskonen *et al.*, 1998b).

Gestating CD-1 mice were either exposed from day 0 to day 17 of gestation to a 50-Hz sinusoidal magnetic field at 20 mT or sham-exposed, and the development of the fetuses was evaluated. A total of 90 exposed and 86 sham-exposed control females were analysed. Exposure to magnetic fields was associated with longer and heavier fetuses at term, even when adjusted for litter size, and the fetuses had fewer external abnormalities. The incidence of fetuses with one or more cervical ribs was significantly increased, but the finding was no longer significant when analysed using methods accounting for possible litter effects. The incidences of external and internal abnormalities and resorptions, and of other parameters measured were unaffected (Kowalczuk *et al*., 1994).

In Hebrew University mouse pre-implantation embryos (94 to 303 per group) exposed to 1-Hz, 20-Hz or 50-Hz magnetic fields, a significant increase in the percentage of embryos with arrested development was seen after 72 h of exposure at 20 Hz or 50 Hz. Inhibition of hatching and further development was seen in more than 50% of blastocysts. No exposure-related differences were noted in the rate of development in those embryos that continued to develop (Zusman *et al.*, 1990).

No consistent effects were seen in preimplantation CBA/S mouse embryos exposed to 50-Hz magnetic fields at 13 μT. The vitality of the embryos was not affected by the exposure, and the timing of the development up to the blastocyst stage was similar to that in controls (Huuskonen *et al.*, 2001b).

Rat

Zecca *et al*. (1985) exposed groups of 10 gestating Sprague-Dawley rats to a 50-Hz, 5.8-mT magnetic field for 3 h per day during the period of organogenesis (days 6–15). No malformations were observed, and the numbers of visceral or skeletal variations were not increased. Resorptions and total post-implantation losses were doubled in the exposed group, but these differences were not statistically significant. [The Working Group noted that the small group sizes meant that the study had very little statistical power to show any effects.]

Huuskonen *et al*. (1993) exposed gestating Wistar rats (70–72 per group) to a 50-Hz, 35.6-μT sinusoidal magnetic field or to a 20-kHz, 15-μT (peak-to-peak) saw-tooth magnetic field on days 0–20 of gestation for 24 h per day. The number of fetuses with minor skeletal anomalies was significantly higher in both exposed groups compared with controls. The number of implants and living fetuses per litter showed a statistically significant increase after exposure to the 50-Hz fields. No effects on the incidence of external or visceral malformations or resorptions were found.

The effects of 50-Hz sinusoidal magnetic fields on embryo implantation, maternal serum estradiol, progesterone, testosterone and melatonin concentrations, and on estrogen and progesterone receptor densities in the uterus were studied during pre-implantation and implantation periods in rats (Huuskonen *et al*., 2001a). Groups of 60 gestating Wistar rats were exposed to the magnetic fields at 10 or 100 A/m (13 or 130 μT) or sham-exposed for 24 h per day from day 0 of gestation, and killed at regular intervals between 70 h and 176 h after ovulation. No effects on the total number of implantations were seen, although there were statistically significant differences in the estrogen-receptor and progesterone-receptor densities at some time points.

The incidence of minor skeletal anomalies in fetuses was significantly increased when Wistar rats (12 dams per group) were exposed continuously to a 50-Hz magnetic field with a flux density of 30 mT from day 1 to day 20 of gestation. Increased skeletal ossification was noted, possibly indicating accelerated prenatal development (extra-thoracic ribs, particularly comma-shaped). Compared with controls a significantly lower number of fetuses with reduced ossification of pelvic bones was also observed, indicating that ossification was accelerated by exposure to a magnetic field (Mevissen *et al*., 1994).

No effects were reported in 175 gestating Sprague-Dawley rats exposed throughout gestation for 20 h per day to a 60-Hz magnetic field at 1000 μT, or in a second group of 174 animals exposed to an average field of 0.6 μT (0.33–1.2 μT) as a result of leakage from the system used to expose the first group. The 170 control animals were exposed to an ambient field of 0.1 μT. A decrease in the number of fetuses per litter was found in the group exposed to 1000 μT in the first study, but this decrease was not repeated in a replicate group in that study. Fetal body weight and incidences of external, visceral and skeletal malformations and variations were similar in all groups, and there were no signs of maternal toxicity (Rommereim *et al*., 1996).

Continuous or intermittent exposure to 60-Hz magnetic fields during the period of major organogenesis had no adverse effects on fetal development or maternal toxicity in Sprague-Dawley rats. In this study, 46–55 gestating females per group were either sham-exposed or exposed for 18.5 h per day to linearly polarized, sinusoidal 60-Hz magnetic fields at flux densities of 2, 200 or 1000 μT, or to intermittent fields (1 h on/1 h off) at 1000 μT from gestation day 6 to day 19. Some statistically significant differences between the exposed and sham-exposed animals were seen among the many parameters measured, but no dose–response relationships or any other consistent patterns suggestive of adverse effects were observed. In contrast, a clear response to a positive control (ethylenethiourea) was reported (Ryan *et al*., 1996).

The possible developmental effects of 180-Hz magnetic fields (third harmonic of 60 Hz) alone or in combination with 60-Hz fields were evaluated in groups of 18–20 Sprague-Dawley rats exposed for 18.5 h per day from gestation day 6 to day 19 to a 60-Hz field or a 180-Hz field at 0.2 mT, or to a 60 + 180-Hz field (10% third harmonic; total field, 0.2 mT). Exposure to a magnetic field had no effects on maternal health, litter size, litter weight or fetal development. The incidence of fetal anomalies was comparable in all groups, with the exception of rib variants, which were increased in the exposed groups, with a statistically significant increase in the group exposed to 60 + 180 Hz. The increase in the number of rib anomalies was within the variation observed in historical controls, and the authors concluded that the effect was not biologically significant (Ryan *et al*., 2000).

In an in-vitro study, Hebrew University Sabra strain rat embryos (10.5 days old) were exposed for 48 h to pulsed magnetic fields at frequencies of 20, 50 or 70 Hz. The number of embryos was 32–40 in the treated groups and 60 in the control group. [The field intensities were not reported.] Exposure to the magnetic fields resulted in retarded development, and an increased incidence of malformed embryos was seen after exposure to 50 and 70 Hz. The main malformations observed were absence of telencephalic, optic and otic vesicles and of forelimb buds (Zusman *et al.*, 1990).

(ii) *Mammalian perinatal exposure and behavioural effects*

One of the most sensitive systems for investigating the impact of putative toxic agents employs the perinatal exposure of animals and the assessment of anomalies in the subsequent adult expression of neural and behavioural responses (Lovely, 1988). However, few studies have been conducted on the potential neurobehavioural teratological effects of in-utero exposure to ELF electric and magnetic fields.

Mouse

In a study of postnatal development and behaviour after prenatal exposure, 21 CD1 mice were exposed throughout gestation to a sinusoidal 50-Hz, 20-mT magnetic field. Three possibly field-dependent effects were noted: exposed animals performed the air-righting reflex about two days earlier than controls; exposed males weighed

significantly less than controls at 30 days of age; and exposed animals remained on a Rota-rod for less time as juveniles than sham-exposed control mice (n = 23). No field-dependent effect on the surface-righting reflex or eye opening was reported, in contrast to the findings of Zusman *et al*. (1990) (see above). There was a suggestion that exposed animals took slightly longer to avoid a cliff edge, but this difference was of borderline significance. In the activity wheel, a slightly increased activity of exposed females and a slightly decreased activity of exposed males was noted compared with control mice, but these effects were not considered by the authors to be of any biological significance. The reduction in running time on a Rota-rod, observed in juvenile mice, may represent an impairment of motor coordination during adolescence induced by the magnetic field. No gross impairments of postnatal development or behaviour were seen in the exposed mice (Sienkiewicz *et al*., 1994).

Seven gestating CD1 mice were exposed for the whole gestation period to a vertical, sinusoidal, 50-Hz magnetic field at 5 mT. Eight control animals were sham-exposed. The male offspring were raised without exposure to magnetic fields, and 10 males per group (no more than two from each litter) were tested at 82–84 days of age for deficits in spatial learning and memory in a radial arm maze. No effects on performance were observed (Sienkiewicz *et al*., 1996).

Rat

In early studies, rats were exposed to 60-Hz electric fields during gestation. The offspring were tested using operant-avoidance behavioural methods at 80 days of age. Perinatally exposed rats performed the task more slowly, but were able to avoid shocks during these tests equally as well as control animals (Persinger & Pear, 1972). Two related studies on postnatal development were reported in which rats were exposed *in utero* to 60-Hz electric fields. In rats exposed *in utero* from gestation day 0 until day 8 after parturition, movement, standing and grooming were increased when compared to controls at 14 days of age. There was a significant decrease in the percentage of exposed offspring displaying the righting reflex. A negative geotropism was seen in exposed offspring in a parallel study where exposure began at day 17 of gestation and was terminated 4 days after weaning. All differences were transient and were no longer evident when the animals were tested at 21 days of age (Sikov *et al*., 1984).

After continuous exposure of dams to a pulsed 20-Hz electromagnetic field throughout gestation, the weight of Sprague-Dawley rat offspring at day 1 of age was reduced but it was increased after exposure to a 100-Hz field. The weights of the offspring of dams exposed to a 50-Hz field were decreased only from 21–28 days of age. When combined into one group, exposed animals showed a statistically significant delay, compared to controls, in eye opening. No effect was seen on the surface-righting reflex (Zusman *et al*., 1990). [The field intensities were not reported.]

Increased male accessory sex-organ weights were noted in Sprague-Dawley rats prenatally exposed to a 15-Hz pulsed magnetic field with 0.3-ms pulse duration and a

peak intensity of 0.8 mT. Gestating animals (6 per group) were exposed for two 15-min periods on days 15–20 of gestation, a period critical for the sexual differentiation of the male rat brain. At parturition, no exposure-related effects on number of live fetuses, average weight or anogenital distance were noted. At day 120 postpartum, the male offspring of the exposed dams exhibited diminished territorial scent-marking behaviour and increased accessory sex-organ weights. Concentrations of circulating testosterone, luteinizing hormone and follicle-stimulating hormone were unchanged, as were epididymal sperm counts. The authors concluded that in-utero exposure to magnetic fields had caused incomplete masculinization (McGivern *et al*., 1990).

The developmental increase in the activity of choline acetyltransferase was examined in the brains of fetuses and offspring from Sprague-Dawley rats exposed to a 60-Hz, 500-mG (50-μT), sinusoidal, circularly polarized magnetic field for one month before gestation and during gestation and lactation. Choline acetyltransferase activity in the brain was assessed at four time points during fetal development and at five and 10 days after parturition. Six animals per group were examined at each time point. No differences were observed between the exposed and control rats (Sakamoto *et al*., 1993).

Female Sprague-Dawley rats were exposed to 60-Hz combined electric and magnetic fields for 23 h per day, from day 5 to day 19 post-conception, to study the effects of exposure on somatic growth and cortical development, as well as biochemical and morphological maturation of the neopallium. The animals were exposed to fields of 1 kV/m and 1 mT, 100 kV/m and 0.1 mT, and 100 kV/m and 1 mT. Pups were killed at birth or on postnatal days 5, 12 or 19 for biochemical and morphological studies. No macroscopic or microscopic changes were observed. A small but significant reduction in cortical weight was observed in rats exposed to 1 kV/m and 1 mT, and a small but significant increase of cortical weight after exposure to 100 kV/m and 0.1 mT. The concentrations of DNA, RNA, protein and cerebroside were measured in the neopallium. Slight but significant reductions in RNA and protein concentrations were measured during the first days of exposure to 100 kV/m and 0.1 mT, and a small reduction in RNA concentration in animals exposed to 100 kV/m and 1 mT. The authors concluded that the exposure had either no effect or else caused minimal changes in somatic growth and cerebral development (Yu *et al*., 1993).

The effects of postnatal exposure to combined electric and magnetic fields (in the same combinations as above) on the development of the cerebellum were studied in newborn Sprague-Dawley rats. The pups were exposed for 7–8 h per day from the day of birth and killed after one, two or three weeks. No morphological changes were observed in the exposed group. There was a small but statistically significant decrease in brain weight in the group exposed to 1 kV/m and 1 mT. The concentrations of DNA and RNA in the cerebellum showed some statistically significant differences after exposure to 1 kV/m and 1 mT and 100 kV/m and 0.1 mT, but not at 100 kV/m and 1 mT. In animals exposed to 1 kV/m and 1 mT, DNA and RNA concentrations were

elevated at six and 13 days, but not at 20 days. In animals exposed to 100 kV/m and 0.1 mT, DNA and RNA concentrations were initially (day 8) lower than in the control animals; concentrations in the exposed and control groups were approximately the same at 14 days and were higher in exposed animals than in controls at 22 days. Protein concentrations were lower in the exposed animals than in controls at eight days, but higher at 14 and 22 days (Gona *et al.*, 1993).

Altered behaviour after perinatal exposure to an electric and magnetic field has been reported. Groups of rats were either sham-exposed or exposed for 20 h per day to a combination of a 60-Hz (30-kV/m) electric and 100-μT magnetic field for 22 days *in utero* and during the first eight days *post partum*. As adults, male rats were trained to perform a multiple, random-interval operant task. The responses of the rats that had been exposed *in utero* to the electric and magnetic field gradually became significantly slower than those of the sham-exposed controls. Once the difference in response rate was established, it was found to persist even after experimental extinction of the response followed by reconditioning. The exposed rats did not differ from sham-exposed controls in terms of body mass, physical appearance, grossly observed activity level or incidence of disease (Salzinger *et al.*, 1990).

(iii) *Mammalian multi-generation studies*

Swiss mice were exposed to 50-Hz pulsed (5 ms) magnetic fields at either 2.3 mT or 83 mT from day 0 to day 120 *post partum* for the first generation, and from conception throughout embryological development and up to day 120 *post partum* for the second generation. In the first generation, no changes were observed in body weights or serum glucose, protein, cholesterol or triglyceride concentrations. In the second generation, the body weights and serum glucose concentrations of the exposed mice were significantly lower at 60 and 120 days *post partum* and the triglyceride concentration was decreased at 120 days, compared to sham-exposed control mice (Rivas *et al.*, 1987).

A study aimed at reproductive assessment by continuous breeding investigated reproductive performance in rats over several generations. Groups of Sprague-Dawley rats (40 breeding pairs per group) were sham-exposed or exposed continuously for 18.5 h per day to linearly polarized, sinusoidal 60-Hz magnetic fields at field strengths of 2, 200 or 1000 μT or to an intermittent (1 hour on, 1 hour off) magnetic field of 1000 μT. No exposure-related toxicity was observed in any of the three generations examined. Fetal viability and body weight were similar in all groups, and there were no differences between test and control groups in any measure of reproductive performance (number of litters per breeding pair, percentage of fertile pairs, latency to parturition, litter size or sex ratio). Teratological examinations were not performed (Ryan *et al.*, 1999).

(iv) *Effects of paternal exposure on mammalian reproduction*

Male OF1 mice were exposed from the age of 6 weeks until adulthood to a sinusoidal 50-Hz, 15-μT magnetic field to study possible alterations in testis histology

and its endocrine function. Female mice that were exposed chronically to the same field from the age of 6 weeks were mated at 20 weeks with the exposed males. The offspring were kept under the same experimental conditions. When the offspring reached sexual maturity, the testes of 30 exposed and 30 control males were analysed. A significant increase in testis size and weight was observed. This increase was associated with increased testosterone concentrations in the interstitial tissue, as was shown by histological analysis. Complete spermatogenesis occurred in both control and exposed animals (Picazo *et al*., 1995).

A flow cytometric study was performed to monitor the effects of a 50-Hz sinusoidal magnetic field on mouse spermatogenesis. Groups of five male hybrid (C57BL/Cne × C3H/Cne)F_1 mice, aged 8–10 weeks, were exposed to a field strength of 1.7 mT for 2 or 4 h. Flow cytometry measurements to distinguish various cell types were performed 7, 14, 21, 28, 35 and 42 days after exposure. No effects were observed in animals exposed for 2 h. In groups exposed for 4 h, a statistically significant decrease in the number of elongated spermatids was observed 28 days after the treatment, suggesting a possible cytotoxic and/or cytostatic effect of the exposure on differentiating spermatogonia (De Vita *et al*., 1995).

Six weeks of continuous exposure to circularly polarized 50-Hz magnetic fields at 1, 5 or 50 μT did not change the plasma testosterone concentration in groups of 48 male Wistar-King rats (Kato *et al*., 1994a).

The possible effects of 50-Hz magnetic fields on the fertility of male rats were investigated in Sprague-Dawley rats, aged 20 weeks, exposed to a sinusoidal, 50-Hz magnetic field at 25 μT for 90 days before they were mated with unexposed females. Ten males per group were used (13 in the control group), and each male was mated with two females. The number of conceptions was significantly decreased from 24/26 (92%) in the control group to 10/20 (50%) in the exposed group. The effect persisted in a second mating at 45 days after cessation of exposure (12 conceptions; 60%), but not at 90 days (16 conceptions; 80%). There was also a significant increase in the total number of resorptions, from two in the female controls to six in the females mated with the exposed males. [As only the total number of resorptions was reported, possible litter effects could not be evaluated.] The numbers of implantations and viable fetuses per litter were not significantly affected. The effect of exposure on the fertility of females (10 animals per group) was also evaluated in this study. The 90-day exposure was carried out under the same conditions as used for the males and resulted in a statistically significant decrease in the number of conceptions, from 100% in the controls to 60% in the exposed females. The mean number of implantations per litter decreased from 9.9 to 4.7 and the mean number of viable fetuses per litter from 9.6 to 4.3. These differences were statistically significant. The total number of resorptions was similar in exposed and control females (Al-Akhras *et al.*, 2001).

(v) *Chick and quail embryos exposed to magnetic fields* in vitro

The initial report of Delgado *et al*. (1982) stated that pulsed magnetic fields at frequencies of 10, 100 and 1000 Hz (pulse duration, 5 ms, peak flux density, 0.12, 1.2 or 12 μT) resulted in a large increase in the percentage of abnormalities noted in chick embryos incubated for two days. In later experiments by the same group, the teratogenic effect seemed to depend on the waveform used (Ubeda *et al*., 1983, 1985). In still later experiments, the effect seemed to depend on the orientation of the chick embryo relative to the geomagnetic field (Ubeda *et al*., 1987). The effect of 100-Hz, 0.4-μT or 1-μT pulsed fields on chick embryos was not always reproducible. In the combined data from 13 experiments (40–50 eggs per experiment), 35% of the exposed and 30% of the control embryos were abnormal. However, there was a significant correlation between the variations in the results and extremely small time-dependent changes in the local geomagnetic field. This finding was interpreted by the authors as suggesting that the effect might occur only at some specific values of the geomagnetic field (Leal *et al.*, 1989).

In all the experiments described above, the chick embryos were examined directly after incubation for 48 h in the magnetic field. Ubeda *et al*. (1994) incubated the eggs for an additional nine days after exposure for 48 h, and the embryos were then inspected in a blinded manner. The group sizes of the exposed and sham-exposed embryos ranged from 72 to 92, and an additional 276 embryos were used as background controls. The 100-Hz fields were similar to those used in previous studies, with 1 μT peak amplitude and 5 ms pulse duration, but two different pulse waveforms were used with rise times of 1.2 and 85 μs. The number of developmental anomalies was increased in the exposed groups, indicating that the abnormalities seen in the previous studies are irreversible. The increase was significant ($p = 0.007$) only for the waveform with the shorter rise time.

Two studies failed to reproduce the results of Delgado *et al*. (1982) (Maffeo *et al*., 1984, 1988). A large well-designed international study (‘Henhouse project’) aimed at replicating Delgado’s results (summarized in Berman *et al*., 1990) was carried out in six separate laboratories using identical equipment and standardized experimental procedures. The eggs, however, came from different sources, and the local geomagnetic fields were different. While the results were not uniform, the combined data showed a significant ($p < 0.001$) increase in abnormal embryos in the exposed group. [The Working Group noted that these results may be compromised by the different field strengths used in different laboratories.]

The experiments of Juutilainen *et al*. (1986) showed a higher percentage of abnormalities compared to controls in chick embryos exposed during the first two days of development to a 100-Hz magnetic field with a pulsed waveform similar to that used by Delgado, but also with sinusoidal and rectangular waveforms. In another series of experiments with sinusoidal waveforms, similar effects were found upon exposure to a wide range of frequencies (Juutilainen & Saali, 1986). The effects of 100-Hz sinusoidal fields with a field strength of 1 A/m (1.3 μT) were confirmed in

experiments with a large number of eggs (Juutilainen, 1986). Further experiments showed similar effects from exposure to 50-Hz sinusoidal fields, and the results suggested that the field strength–response curve has a sharp threshold at 1 A/m (1.3 μT) (Juutilainen *et al*., 1987).

Apart from the extensive series of experiments by Juutilainen and colleagues, there have been few other studies on sinusoidal fields. Cox *et al*. (1993) attempted to replicate in part the findings of Juutilainen *et al*. Two hundred White Leghorn chick eggs were exposed to a 50-Hz, 10-μT magnetic field for 52 h and a second group of 200 eggs was incubated in a background field of 0.2 μT. The incubation was continued for 68 h after removal of the eggs from the magnetic field after which the embryos were examined. No difference in malformation rate was observed between the exposed and control embryos. Most of the experimental conditions in the laboratories of Cox and Juutilainen were similar. However, the static (geomagnetic) field was only 17 μT in Cox's laboratory compared with 44–50 μT in Juutilainen's laboratory.

An extensive series of experiments was conducted to study the effects of pulsed and sinusoidal magnetic fields on chick embryo development, involving over 2500 White Leghorn chick embryos. The experiments were performed over five years in five separate studies. In four of these, a pulsed 100-Hz field with a peak amplitude of 1 μT was used (similar to the field used in the Henhouse study). In the last study, the embryos were exposed for 48 h to a 60-Hz, 4-μT sinusoidal magnetic field. The number of abnormalities was always higher in exposed embryos, but in one of the pulsed-field studies, the difference was small and not statistically significant. Overall, the number of abnormalities was approximately doubled in embryos exposed to the pulsed 100-Hz magnetic fields and approximately tripled by exposure to the sinusoidal 60-Hz magnetic fields. Both effects were highly significant. According to the authors, the lack of response in one of their studies could have been due to a change in the genetic composition of the breeding stock before the start of that study. The authors proposed that genetic differences in susceptibility to magnetic fields may explain the inconsistent results between laboratories (Farrell *et al*., 1997).

The effects of 50-Hz and 100-Hz magnetic fields on the development of quail embryos were investigated in eggs produced by 10 females. Data were reported separately for each female. In each experiment, two eggs from each female were used: one exposed and one control. Sham experiments conducted with 240 eggs showed that there was no difference between the exposure and control locations in the incubator. The eggs were exposed to 50-Hz or 100-Hz magnetic fields with rectangular waveform and intensities of 0.2, 1.2, 3.3 and 3.2 μT. The embryos were exposed for 48 h and then inspected in a blind manner. The number of abnormalities was higher in the exposed embryos than in the controls. However, the increase did not reach statistical significance for the embryos exposed to 50 Hz. Comparison of the data for the individual females suggests that there might be genetic differences in sensitivity (Terol & Panchon, 1995).

(vi) *Other non-mammalian embryos*

Ramirez *et al*. (1983) reported reduced egg laying in fruit flies (*Drosophila melanogaster*) and reduced survival during development after exposure to pulsed 100-Hz, 1.8-mT and sinusoidal 50-Hz, 1-mT magnetic fields. No effects were seen in a similar study using 60-Hz, 1-mT fields (Walters & Carstensen, 1987).

Graham *et al*. (2000c) studied the effects of magnetic fields on 'developmental stability', which describes the ability of an organism to maintain a consistent phenotype under given genetic and environmental conditions. *Drosophila melanogaster* were exposed for their entire lives (egg to adult) to 60-Hz magnetic fields at 1.5 or 80 μT. The exposed flies in both groups showed a significant reduction in body weight, compared to controls. The flies exposed to the 80-μT field showed reduced developmental stability measured both by fluctuating asymmetry (asymmetrical wing veins) and frequency of phenodeviants (fused abdominal segments). [The Working Group noted that developmental stability is a new concept, that could potentially be a very useful tool for detecting relatively weak environmental effects.]

Exposure to sinusoidal magnetic fields of 60 Hz, 0.1 mT has been reported to delay the development of Medaka fish embryos (*Oryzias latipes*) (Cameron *et al*., 1985), sea urchin embryos (*Strongylocentrotus purpuratus*) at 60 Hz, 0.1 mT (Zimmerman *et al*., 1990) and zebrafish embryos (*Dario rerio*) at 50 Hz, 1 mT (Skauli *et al*., 2000). No malformations were found in these studies.

(vii) *Interactions with known teratogens*

Cultures of embryonic cells of *Drosophila melanogaster* were used to assess the potential developmental toxicity of exposure to a 60-Hz, 100-μT field for 16–18 h. Exposure to the magnetic field alone was not teratogenic and exposure did not enhance the effects of retinoic acid, hydroxyurea or cadmium, which were all clearly teratogenic in this model. Additional experiments, in which embryos were exposed at 10 or 100 μT for their entire development up to the adult stage, did not produce a significant increase in developmental abnormalities (Nguyen *et al*., 1995).

Exposure to 50-Hz, 10-mT magnetic fields modified the embryotoxic effect of ionizing radiation on chick embryos, but no effects of exposure to magnetic fields alone were observed. In this study, several experiments were performed with X-ray doses of 4 or 5 Gy given on days 3 or 4 of development. The magnetic field was applied either during the first 2–40 h of embryonic development (before X-ray treatment), or during the 12 h immediately after the X-ray treatment. The embryos were examined at day 9 of development. Embryotoxicity was expressed as the sum of embryonic deaths and malformations. Exposure to the magnetic field before the X-ray treatment seemed to protect the embryos from X-ray-induced toxicity, while an enhancement of the embryotoxicity was seen when exposure to the magnetic field followed the X-ray irradiation. Both the protective effect and the enhancing effect were seen consistently in several experiments and were statistically significant (Pafková & Jerábek, 1994). A similar protective effect against subsequent exposure to the chemical teratogens insulin and

tetracyclin was described for 50-Hz, 10-mT magnetic fields. The authors sought to explain the interactions of magnetic fields with X-rays and chemical teratogens on the basis of magnetic-field-induced oxidative stress (Pafková *et al*., 1996).

4.2.2 *Immunological effects*

(*a*) *In-vivo studies*

(i) *Static fields*

The humoral and cell-mediated immune responses were studied in mice (LAF1/J) following exposure to 1.5-T static magnetic fields for six days. The immune response of spleen lymphocytes to sheep erythrocytes was tested by assaying the number of Jerne plaques formed by spleen lymphocytes, and by measuring the concentration of IgM in the serum. The mitogen-stimulated proliferation index of the spleen lymphocytes was also tested using concanavalin A, phytohaemagglutinin and lipopolysaccharide. In no case did lymphocytes from exposed mice respond differently from those from control animals (Tenforde & Shifrine, 1984).

A series of studies investigated the influence of 60-mT magnets implanted over several brain regions on the immune response in male and female Wistar rats. After implantation, the animals were challenged with sheep erythrocytes or bovine serum albumin and tested 14, 24 and 34 days later. Control rats were implanted with iron beads (and sham-operated, when appropriate, to conform to the treatment of exposed animals). The rats were tested for the plaque-forming cell response, local hypersensitivity skin reactions and experimental allergic encephalomyelitis. The authors reported that placing magnets over each of three regions of the brain could have effects on the immune system not seen in controls (Jankovic *et al*., 1991). Furthermore, while surgical induction of lesions in the brain in the nucleus locus ceruleus or pinealectomy caused a reduced immune response, implantation of the magnets reversed these effects (Jankovic *et al*., 1993a,b). Old rats (aged 22 months) that underwent pinealectomy and magnet implantation also showed recovery of immune responses as did the younger animals in the earlier study (Jankovic *et al*., 1994).

(ii) *ELF electric and magnetic fields*

In a study of the effects of electric fields alone, male and female Swiss-Webster mice were exposed for 30 or 60 days (21 h per day) to 60-Hz electric fields of 100 kV/m. No significant differences in serum immunoglobins (IgG and IgM), complement levels or distribution of T or B lymphocytes were found in comparison with sham-exposed control mice. A statistically significant decrease in leukocyte and lymphocyte counts was found after exposure for 60 days but these counts were elevated compared to controls in a subsequent experiment (Morris & Ragan, 1979).

Male C57BL/6 mice were exposed to 60-Hz, 100-μT magnetic fields for 1, 5, 10, 21, 49 and 105 days. For each exposure period, three replicates were evaluated using a battery of 20 immune assays. When these data were analysed using linear statistical

methods, no significant difference in any immune parameter was found (Marino *et al.*, 2000). [Although the authors noted the increased variance in their data for the two longest exposure times, the Working Group considered this analysis too speculative to include in its evaluation.]

The 7,12-dimethylbenz[*a*]anthracene (DMBA) model for breast cancer was used to study immunological effects in rats exposed to horizontal, 50-Hz, 50-μT magnetic fields for 91 days (24 h per day, seven days per week). The geomagnetic field produced a static component of 16 μT parallel to, and 36 μT perpendicular to, the 50-Hz field. After 13 weeks, a marked suppression in T-cell proliferation capacity under concanavalin A challenge was observed. No change in nocturnal concentrations of serum melatonin was seen in exposed animals after 9 or 12 weeks of exposure (Mevissen *et al.*, 1996b).

Female Sprague-Dawley rats were exposed to magnetic fields of 50 Hz, 0.1 mT (24 h per day, seven days per week) for 2, 4, 8 and 13 weeks. Proliferative capacity and production of interleukin-2 were investigated in primary splenic cultures of T and B lymphocytes. Significantly fewer viable splenic lymphocytes were observed at all times in the exposed animals compared with sham-exposed controls. The proliferation rate of B cells, either unstimulated or stimulated with pokeweed mitogen, was comparable in exposed rats and sham-treated animals. In contrast, the proliferation rate of T cells stimulated with concanavalin A from exposed animals showed a statistically significant increase at two weeks, a slight reduction at four weeks, no statistically significant change at eight weeks and a statistically significant reduction at 13 weeks, compared with controls. Non-stimulated T-cell proliferation was unchanged at these treatment times. The addition of melatonin at 1, 10 and 100 nM did not change the T-cell proliferation rate in concanavalin-A-treated cultures from animals exposed to magnetic fields for two and four weeks nor did the addition of melatonin to these cultures change the T-cell proliferation from that observed in cultures treated with concanavalin A alone. The same was true for sham controls except for one experiment with 100 nM melatonin where the response to concanavalin A and melatonin was significantly higher than the reponse to concanavalin A alone. No changes in production of interleukin-2 were observed after any treatment of B lymphocytes. The authors noted that the triphasic alteration in T-cell function during the 13-week treatment period resembled the responses that are seen during prolonged administration of a chronic mild stress, i.e. activation, tolerance and suppression. They concluded that long-term exposure to magnetic fields may lead to impaired immune surveillance in female rats (Mevissen *et al.*, 1998b).

The activation of interleukins by activated T and B lymphocytes was studied in female Sprague-Dawley rats exposed to 50-Hz, 0.1-mT magnetic fields, under conditions described previously by Mevissen *et al*. (1996b). In the first experiment, DMBA-treated rats were exposed for 14 weeks under conditions that suppressed the T cell-stimulated proliferative response, following exposure to magnetic fields for 13 weeks. This experiment failed to show any difference in production of interleukin-1 by mitogen-

activated B cells between exposed and sham-exposed animals. In the second experiment, non-DMBA-treated rats were exposed for 1, 7 and 14 days to 50-Hz, 0.1-mT magnetic fields. No significant changes were observed in the production of interleukin-1 or interleukin-2 by stimulated B or T cells (Haussler *et al.*, 1999).

Natural and adaptive immunity were studied in rats born and raised for six weeks in a 60-Hz magnetic field for 20 h per day. Twenty days after mating, gestating Fisher F344/N rats were exposed to intensities of 2, 20, 200 μT and 2 mT, or kept under control conditions (< 0.02 μT). At weaning, the offspring were separated from their dams and kept under the same field conditions. The following immunological parameters were examined: total T and B cells; CD5+, CD4+ and CD8+ sub-population pattern and natural killer cell activity in splenic lymphocytes; hydrogen peroxide, nitric oxide and tumour necrosis factor production by peritoneal macrophages. In comparison with the control group, there was a significant reduction in the number of CD5+, CD4+ and CD8+ populations, with the greatest reduction occurring at 2 mT. A smaller but nevertheless significant reduction was observed in CD5+ cells at 200 μT. Linear regression analysis showed a dose–response effect with increasing magnetic field intensity. Furthermore, B lymphocyte populations (Ig+ cells) showed a significant reduction ($p < 0.05$) at 20 and 200 μT, but these results did not show a dose-related response. Natural killer cell activity decreased by 50% ($p < 0.05$) at 2 mT. In peritoneal macrophages, no significant changes were observed in the activity of tumour necrosis factor or secretion of nitric oxide, but background and phorbol ester-stimulated production of hydrogen peroxide increased ($p < 0.05$ and $p < 0.001$, respectively) (Tremblay *et al.*, 1996). [The Working Group noted that the significance of these effects was greatly reduced when a comparison was made with sham-exposed animals; only two end-points remained statistically significant.]

Male and female B6C3F_1 mice and female Fischer 344 rats were exposed continuously (18.5 h per day) to 60-Hz, 0.002-, 0.2- and 1-mT magnetic fields; one additional group was exposed to an intermittent (1 h on, 1 h off) 1-mT magnetic field. After exposure periods of 21, 28 or 90 days the parameters examined included spleen and thymus weights and cellularity, antibody-forming cells, delayed-type hypersensitivity, splenic lymphocyte subsets, susceptibility to infection with *Listeria monocytogenes* and natural killer cell activity. No statistically significant differences were found between exposed and sham-exposed mice, except in the activity of natural killer cells and occasional differences in delayed-type hypersensitivity for which there was no clear dose-related pattern. After 28 days of exposure, the activity of natural killer cells in female mice showed a statistically significant increase only at 1 mT, whereas non-significant increases occurred in a dose-dependent manner. Isolated changes in the activity of natural killer cells were seen in three groups of mice exposed for 42 days. A dose-related reduction in activity of natural killer cells was observed in female mice exposed for 90 days, but this was not consistently reproducible (House *et al.*, 1996).

The influence of 60-Hz magnetic fields on the clinical progression of leukaemia was investigated in male Fischer 344 rats. Large granular lymphocytic leukaemia cells from spleens of leukaemic rats were injected intraperitoneally into young rats, which were subsequently exposed to magnetic fields at either 2 μT or 1 mT, for 20 h per day, seven days per week for five, six, seven, eight, nine or 11 weeks. Changes in growth of the spleen and infiltration of large granular lymphocytic leukaemia cells into the spleen and liver were monitored. No significant and consistent differences were observed between groups exposed to a magnetic field and the control group, whereas progression was enhanced in a positive control group exposed to 5 Gy γ-rays (Morris *et al*., 1999).

A model for transplantable acute myeloid leukaemia in rats was applied to examine the influence of 50-Hz, 0.1-mT magnetic fields. Leukaemic cells were injected intravenously into the lateral tail vein of Norway rats, which were subsequently exposed to the magnetic field for 18 h per day (14.00 to 08.00), seven days a week, for up to 27 days. The geomagnetic field was 47 μT, with projection onto the horizontal axis of 8 μT. The investigators measured survival time, body weight, haematological parameters and infiltration of blood, bone marrow, spleen and liver by leukaemic cells. The results showed no significant changes in rats treated with leukaemic cells and exposed to a magnetic field compared with those injected with leukaemic cells, but not exposed to a magnetic field, for any of the parameters involved in leukaemia progression (Devevey *et al*., 2000).

The effects of 7-Hz and 40-Hz square wave magnetic fields on the immunological response were studied in female Lewis rats. Immediately before exposure, the test rats were injected with emulsified spinal column prepared from female Lewis rats. Groups of rats were exposed to magnetic fields of 0.05 μT or 0.5 μT (peak intensity) of either 7 or 40 Hz for 6 minutes every hour for 8 h per day for 15 days. The field patterns were designed to be similar to those that might occur during geomagnetic storms. After exposure, the brains of the rats were examined for infiltration of lymphocytes (mononuclear cells) and mast cells. In rats exposed to 7-Hz, 0.05-μT fields, there were fewer infiltration foci than in any of the other groups. Rats exposed to 40-Hz, 0.5-μT fields had more foci in the right thalamus while those exposed to 7 Hz at the same intensity showed more foci in the left thalamus. The total number mast cells within the thalamus was also increased by the treatments (Cook *et al.*, 2000).

Eight adult male baboons (*Papio cynocephalus*) were exposed for six weeks to 60-Hz vertical electric fields of 6 kV/m and horizontal magnetic fields of 50 μT. Blood samples taken before exposure, at the end of the period of exposure and six weeks after exposure ended were examined by standard immunological methods to determine total leukocyte count and total T cell (CD3+), T helper cell (CD4+), cytotoxic T cell (CD8+), B cell and natural killer cell numbers. A second experiment was conducted in which six of the eight animals used previously were exposed to higher field intensities: 30-kV/m electric fields and 0.1-mT magnetic fields. In the first experiment, exposure-related reductions ($p < 0.05$) in CD3+ and CD4+ counts, interleukin-2 receptor expression and T-cell proliferation in response to pokeweed mitogen were observed. In the second

experiment, there was a similar but reduced response in these parameters compared with the pre-exposure control values. A comparison of the results in the two experiments showed group × period interactions (indication of significance) for total leukocyte count and CD4+ and CD8+ ratios, but the higher exposures did not show greater effects (Murthy *et al.*, 1995).

(*b*) *In-vitro studies*

(i) *Static fields*

Static magnetic fields generated by a 0.5-T magnetic resonance imaging unit were used to study activation markers and interleukin release in mononuclear cells from human peripheral blood. The cells were exposed to the fields for 2 h at 24 °C, then cultured for 24 h at 37 °C with or without PHA stimulation. The cells were assayed for expression of CD25, CD69 and CD71 by immunofluorescence microscopy, and the concentrations of interferon-γ, tumour necrosis factor α and interleukin-4 were measured in the medium using an enzyme-linked immunosorbent assay. Exposure to the magnetic field caused a reduction in CD69 expression, which was enhanced under PHA stimulation compared with controls. An increased release of interferon-γ and interleukin-4 occurred in unstimulated cells, but a reduced release was seen under PHA stimulation compared to controls. The release of tumour necrosis factor α, interleukin-6 and interleukin-10 was unchanged (Salerno *et al.*, 1999).

Apoptosis, intracellular calcium concentrations and lymphocyte and macrophage functions were measured in C57BL/6 mouse macrophages, splenic lymphocytes and thymic cells exposed for 24 h to static magnetic fields ranging from 25 to 150 mT. Cytofluorometric analysis showed a decreased phagocytic uptake of fluorescent latex microspheres, with a concomitant increase in the concentration of intracellular calcium ions in the macrophages. Exposure to the magnetic fields also decreased the concanavalin A-induced mitogenic response in lymphocytes; this was also associated with increases in calcium ion influx. In addition, exposure gave rise to increased apoptosis in thymic cells, as determined by flow cytometry (Flipo *et al.*, 1998).

(ii) *ELF electric and magnetic fields*

Natural killer cell activity in human peripheral blood was examined following in-vitro exposure to 50-Hz magnetic fields. Phytohaemagglutinin or interleukin-2-stimulated lymphocytes or unstimulated control cells were exposed to 50-Hz magnetic fields before or during cytotoxicity tests, and then mixed with different target cancer cell lines (Daudi, Raji, U937, H14, IGROV, SW626, K562, HL60). Exposure to magnetic fields of 0.1, 0.035, 1.8 and 10 mT, with exposure times between 4 h and 7 days, took place in two independent laboratories. The results from both laboratories showed that exposure to 50-Hz magnetic fields with strengths of up to 10 mT did not affect the cytotoxic activity of human natural killer cells (Ramoni *et al.*, 1995).

Peritoneal mast cells from Sprague-Dawley rats were tested for function and histamine release in response to 48/80 (a standard mast cell-stimulating compound).

The cells were exposed to a 60-Hz, 5-mT magnetic field for periods of 30 min to 2 h, either before or during the test. No significant degranulation occurred during exposure to the magnetic field and the cells showed no reduction in sensitivity to the degranulating agent, 48/80 (Price & Strattan, 1998).

The effect of magnetic fields on intracellular free calcium was studied in thymocytes from Sprague-Dawley rats. The cells were exposed to 50-Hz, 0.1-mT horizontal or vertical magnetic fields or to 50-Hz, 0.14-mT circularly polarized fields for 30 min; the effects of consecutive 20-min periods of exposure to vertical and horizontal magnetic fields were also examined. In addition, control or lectin-activated thymocytes, splenocytes and peripheral blood lymphocytes were exposed to a 50-Hz, 5-mT vertical magnetic field for 30 min. No changes in intracellular free calcium concentration were observed in any of these experiments (Nishimura *et al.*, 1999). Intracellular calcium was also monitored in the Jurkat lymphocyte T-cell line. The cells were pre-incubated for 8 min to establish a baseline, and subsequently exposed for 8 min to a 50-Hz, 0.15-mT magnetic field, or sham-exposed in a blinded fashion. No effects on the concentration of intracellular free calcium were found (Wey *et al.*, 2000).

Mononuclear cells from human peripheral blood were exposed to either static or pulsed, 50-Hz, 3-mT magnetic fields and assayed for proliferative responses and production of the cytokines interleukin-2, interferon-γ and tumour necrosis factor α. Pulsed 50-Hz fields with a 120-ns rise time, a 100-μs fall time and a duty cycle of 2/5 were applied for 15 min every 2 h for 6 h, for a total exposure time of 45 min. Proliferative response was measured with and without PHA stimulation, and cytokine concentrations were determined with biological immunoenzymatic assays. There was no effect of the static or pulsed fields on cell proliferation, and the cytokine concentrations, and transcriptional or translational processes in the exposed cells did not differ from those in the controls (Pessina & Aldinucci, 1997).

In a later study, mononuclear cells from human peripheral blood were exposed for 12 h to pulsed magnetic fields of 50-Hz, 3-mT square waves with a rise time of 120 ns, a fall time of 2 ms and a duty cycle of 1/2. In unstimulated cells, a reduction in tumour necrosis factor α was seen immediately after exposure, but no changes were observed in either interleukin-1β or interleukin-2. In contrast, cells stimulated with PHA immediately before exposure to the fields showed progressive increases ($p < 0.05$) in the concentrations of interleukin-1β and tumour necrosis factor α at 24 and 48 h after treatment. The concentration of interleukin-2 was also increased, but only at the end of exposure; proliferation indices were also significantly increased 48 h after treatment (Pessina & Aldinucci, 1998).

Murine cytotoxic T-lymphocytes were used to target B-lymphocytic tumour cells in a standard assay to test chromium release from the B-lymphocytic tumour cells into the medium as an indicator of cell disruption by the murine cytotoxic T-lymphocytes. The latter were exposed through agar bridges to 60-Hz, sinusoidal electric fields for 48 h at intensities of 0.1, 1 and 10 mV/cm in the medium. Following exposure, a 4-h cytotoxicity assay was performed. The results showed a non-significant reduction (7%) in

cytotoxicity after exposure to 0.1 mV/cm, and significant reductions of 19% ($p < 0.0005$) and 25% ($p < 0.005$) after exposure to 1 and 10 mV/cm, respectively (Lyle *et al.*, 1998).

Human leukocytes exposed at 20 °C to 0.2-, 1- or 3-ms pulses from a spark discharge with electric fields of 2.6 kV/cm and higher — as single pulses and with up to 10 pulses at 5-s intervals — showed that breakdown of the membrane barrier was field intensity-dependent (Hansson Mild *et al*., 1982).

Mononuclear cells obtained from the peripheral blood of 25 healthy donors were exposed for 12 h to a 3-Hz pulsed magnetic field of 4.5 mT, with a 1/2 duty cycle and rise and fall times less than 100 μs (the field was somewhat smoothed due to coil inductance). Each pulse had a maximum value of 13 T/s, which produced a maximum induced electric field in the medium of 23 mV/m. Cell proliferation, as measured by the uptake of tritiated thymidine, was inhibited in mononuclear cells from 24/25 donors, by up to 60%. There were no differences in blastogenic response between exposed and control cultures (Mooney *et al.*, 1986).

4.2.3 *Haematological effects*

(*a*) *Static fields*

Female NMRI mice were exposed to a static magnetic field of 3.5 T. The haematological parameters were generally unaffected. Necropsy and histopathological investigations revealed no pathological alterations (Zimmermann & Hentschel, 1987).

The potential adverse effects of subchronic exposure to a strong static magnetic field were evaluated in adult Fischer rats and their offspring. A battery of clinical tests in adult male and female rats and their offspring detected no adverse biological effects that could be attributed to a 10-week period of exposure to a 9.4-T static magnetic field (High *et al.*, 2000).

(*b*) *ELF electric and magnetic fields*

The acute effects of power-frequency magnetic fields on haematopoiesis were studied in 10-week-old CBA/H mice known to be susceptible to the induction of acute myeloid leukaemia after exposure to ionizing radiation. Mice were exposed to 50-Hz, 20-mT magnetic fields for seven days. Samples of blood and bone marrow were taken from three mice in each group immediately after exposure (day 0) and at about the same time on days 2, 4, 7, 10 and 18. Up to 19 days after exposure, no significant effects on peripheral blood characteristics were observed. Assays of bone-marrow stem cells and myelomonocytic progenitor cells also failed to show significant differences between exposed and control mice (Lorimore *et al.*, 1990).

Spleen colony formation was examined in male CBA mice exposed to 50-Hz, 0.022-mT magnetic fields for 1 h, at the same time of day, for five successive days (5 h per five days). The number of colony-forming units was higher in the exposed animals than in the untreated controls but was not higher than that counted in sham-

exposed animals. Significant changes were seen in the thymus weight and thymus index of exposed animals when compared with both control and sham-exposed animals. In a second study, mice were given a sublethal dose of X-rays (6 Gy) followed 2 h later with the same magnetic field treatment as above, i.e. 5 h every five days. The number of colonies per spleen showed a consistent, significant increase with exposure to the magnetic field and the number of colony forming units per femur was decreased. In a third study, bone marrow was taken from mice that had been exposed to 50-Hz, 0.022-mT magnetic fields for 5 h per five days, and injected into mice that had been exposed to a lethal dose of X-rays (9 Gy). The number of colony forming units per femur in the recipient mice was significantly reduced at days 1 and 4 after injection (Korneva *et al.*, 1999).

Two hundred and forty adult male Sprague-Dawley rats were exposed for 8 h per day to 50-Hz, 25-kV/m or 100-kV/m vertical electric fields for 35, 55 and 155 days and the animals were killed after 140, 164 and 315 days, respectively. Irrespective of the duration of exposure, the mode of grounding and the field strength, no statistical differences in body weight, morphology or histology of the liver, heart, mesenteric lymph nodes or blood parameters were found in the exposed animals compared to controls (Margonato *et al.*, 1993).

Adult male Sprague-Dawley rats were exposed to a 50-Hz magnetic field with a flux density of 5 μT for 22 h per day for 32 weeks. Haematological variables were measured in blood samples taken before exposure to the field and at 12-week intervals during exposure. No differences were detected between the exposed and control groups in haematology and haematochemistry, or in the concentrations of the neurotransmitters dopamine and serotonin in the brain (Margonato *et al.*, 1995).

Adult male Sprague-Dawley rats were exposed for 8 h per day on five days per week for eight months to 50-Hz electric and magnetic fields of two different field strength combinations: 5 μT and 1 kV/m and 100 μT and 5 kV/m. The animals were kept under constant controlled illumination for 24 h per day. Blood samples were collected for determination of haematological variables before exposure and at 12-week intervals during exposure. No pathological changes were observed at either field strength combination in animal growth rate, in morphology and histology of the tissue specimens collected from the liver, heart, mesenteric lymph nodes, testes and bone marrow, or in serum chemistry (Zecca *et al.*, 1998).

Haematological and serum chemistry variables were examined in groups of female Sprague-Dawley rats exposed to uniform, vertical 60-Hz electric fields at 100 kV/m for 15, 30, 60 or 120 days. Another group of rats was sham-exposed. Blood samples were collected from all animals within 3 h after exposure and analysed for leukocyte and erythrocyte counts, haemoglobin concentration, reticulocyte and thrombocyte counts, bone marrow cellularity and prothrombin times, serum iron and serum alkaline phosphatase concentrations and serum triglyceride values. Significant differences between exposed and sham-exposed rats were seldom seen. Statistical evaluation of these data did not detect any consistent effect of the electric field (Ragan *et al.*, 1983).

Groups of Fischer 344/N rats and B6C3F_1 mice were exposed to 60-Hz magnetic fields or sham-exposed for eight weeks. Magnetic field strengths were 0.002, 0.2 and 1 mT. The whole-body exposure was continuous for 18.5 h per day, seven days per week. An additional group of rats and mice was exposed intermittently (1 h on/1 h off) to 1-mT magnetic fields for the same period of time. The animals were kept on a 12 h/12 h light/dark cycle. There were no gross, histological, haematological or biochemical lesions that could be attributed to magnetic field exposure (Boorman *et al.*, 1997).

4.2.4 *Neuroendocrine effects*

The hypothesis that reduced pineal function may promote the development of breast cancer in humans was initially suggested by Cohen *et al.* (1978). Several mechanisms whereby changes in pineal or serum melatonin concentrations may affect the risk for breast cancer have been proposed. These include the observation, at least in some animal species, that decreased melatonin concentrations cause elevations in circulating levels of estrogen and progesterone which increase cell proliferation in the stem cell population of the breast and may thus increase the risk for cancer in this tissue (Cohen *et al.*, 1978). Other authors have suggested that melatonin may directly suppress the growth of human mammary tumour cells (Blask & Hill, 1986) and of cells of other cancer types, particularly melanoma, prostate cancer, ovarian cancer, bladder cancer and leukaemia (Stevens, 1993) and that melatonin may act as a scavenger of free radicals, preventing oxidative damage to DNA (Reiter *et al.*, 1995), at least at pharmacological levels (Cridland *et al.*, 1996). It has also been suggested that melatonin may modulate immune responsiveness (Maestroni *et al.*, 1986).

Studies of the effects of electric and magnetic fields on melatonin concentrations have mostly been carried out in rats but have also used mice, Djungarian hamsters (*Phodopus sungorus*), sheep and primates, including humans. The experimental details of the animal studies are given in Table 35. Human data are discussed in section 4.1.4 and summarized in Table 34.

(*a*) *Electric fields*

Several studies by one group of authors (Wilson *et al.*, 1981, 1986*;* Reiter *et al.* 1988), reported that exposure to electric fields significantly suppressed pineal melatonin and the activity in the pineal gland of the enzyme *N*-acetyltransferase, which is important in the synthesis of melatonin. These effects appeared within three weeks of exposure, but disappeared within three days after cessation of exposure. A similar suppression of pineal melatonin was reported by these authors to occur after the prenatal and neonatal exposure of rats to power-frequency electric fields; no simple dose–response relationship was apparent. Grota *et al*. (1994) reported that exposure of rats to power-frequency electric fields had no effect on pineal melatonin concentrations

Table 35. Studies in animals of melatonin concentrations in response to exposure to ELF electric and magnetic fields

Reference	Assay	Exposure	Response	Comment
ELF electric fields				
Rats				
Wilson *et al.* (1981, 1983)	Night-time pineal melatonin concentrations and NAT enzyme activity in adult rats	60 Hz, 1.7–1.9 kV/m (not 65 kV/m) due to equipment failure; 20 h per day for 30 days	Reduced pineal melatonin and SNAT activity	Data combined in one experiment because of variability
Wilson *et al.* (1986)	Night-time pineal melatonin concentrations and SNAT enzyme activity in adult rats	60 Hz, 65 kV/m (39 kV/m 'effective') for up to 4 weeks	Pineal melatonin and SNAT activity reduced after 3 weeks of exposure; recovered 3 days after exposure ceased	
Reiter *et al.* (1988)	Night-time pineal melatonin concentrations in immature rats	60 Hz, 10, 65 or 130 kV/m during gestation and 23 days postnatally	Night-time peak reduced and delayed in exposed animals	No simple dose–response relationship
Grota *et al.* (1994)	Night-time pineal melatonin concentrations and NAT enzyme activity and serum melatonin in adult rats	60 Hz, 65 kV/m for 20 h per day for 30 days	No effect on night-time melatonin and NAT; serum melatonin decreased	
ELF magnetic fields				
Mice				
Picazo *et al.* (1998)	Serum melatonin concentrations in fourth generation of male mice	50 Hz, 15 μT for four generations	Reduced night-time concentrations	Experimental procedures not fully described
de Bruyn *et al.* (2001)	Night-time plasma melatonin concentrations in mice	50 Hz, 0.5–77 μT (2.75 μT average) 24 h/day from conception to adulthood	No effect	

Table 35 (contd)

Reference	Assay	Exposure	Response	Comment
Rats				
Martinez-Soriano *et al*. (1992)	Serum melatonin concentrations in adult rats	50 Hz, 5 mT for 30 min during the morning for 1, 3, 7, 15 and 21 days	Serum melatonin reduced on day 15 [no values for days 1, 7 or 21]	Technical difficulties; brief description of method
Kato *et al*. (1993)	Pineal and serum melatonin concentrations in adult rats	50 Hz circularly polarized, 1, 5, 50 or 250 μT for 6 weeks	Night-time and some daytime reductions in serum and pineal melatonin	Questionable comparisons with historical controls
Kato *et al*. (1994b)	Serum melatonin concentrations in adult rats	50 Hz, circularly polarized, 1 μT for 6 weeks	Night-time melatonin concentrations reduced, returning to normal within one week	Comparison with sham-exposure
Kato *et al*. (1994c)	Pineal and serum melatonin concentrations in adult, pigmented rats	50 Hz, circularly polarized, 1 μT for 6 weeks	Night-time pineal and serum melatonin concentrations reduced	Comparison with sham-exposed and historical controls
Kato *et al*. (1994d)	Serum melatonin concentrations in adult rats	50 Hz, horizontally or vertically polarized, 1 μT for 6 weeks	No effect	Comparison with sham-exposed and historical controls
Kato *et al*. (1994a)	'Antigonadotrophic' effect of melatonin on serum testosterone in adult rats	50 Hz, circularly polarized, 1, 5 or 50 μT for 6 weeks	No effect	Comparison with sham-exposure
Selmaoui & Touitou (1995)	Night-time serum melatonin concentrations and pineal NAT activity in adult rats	50 Hz, 1, 10 or 100 μT for 12 h once or for 18 h per day for 30 days	Reduced melatonin and NAT activity after 100 μT (acute) and 10 and 100 μT (chronic)	
Bakos *et al*. (1995, 1997, 1999)	Night-time excretion of melatonin urinary metabolite in adult rats	50 Hz, 1, 5, 100 or 500 μT for 24 h	No significant effects compared with baseline pre-exposure controls	

Table 35 (contd)

Reference	Assay	Exposure	Response	Comment
Mevissen *et al.* (1996b)	Night-time pineal melatonin concentrations in non-DMBA-treated adult rats	50 Hz, 10 µT for 13 weeks	No effect	A small part of a larger, well-planned mammary tumour study
Löscher *et al.* (1998)	Night-time serum melatonin concentrations in adult rats	50 Hz, 100 µT for 1 day or 1, 2, 4, 8 or 13 weeks	No consistent efects on melatonin	The few positive effects could not be replicated
John *et al.* (1998)	Night-time excretion of melatonin urinary metabolite in adult rats	60 Hz, 1 mT for 20 h/day for 10 days or 6 weeks; 1 mT intermittent for 1 h or for 20 h/day for 2 days	No effect	
Djungarian hamsters				
Wilson *et al.* (1999)	Night-time pineal melatonin concentrations	60 Hz, 100 µT for 15 min, 2 h before dark	Suppression of night-time peak	
Yellon (1994)	Night-time pineal and serum melatonin concentrations	60 Hz, 100 µT for 15 min, 2 h before dark	Reduced and delayed night-time peak; less effect and absent in 2nd and 3rd replicates, respectively	Considerable variation between replicate studies
Yellon (1996a)	Night-time pineal and serum melatonin concentrations	60 Hz, 100 µT for 15 min, 2 h before dark	Reduced and delayed night-time peak; less effect in second replicate	Considerable variation between replicate studies
Yellon (1996b)	Night-time pineal and serum melatonin concentrations; adult male reproductive status	60 Hz, 100 µT for 15 min, 2 h before dark for 3 weeks	No effect on pineal or serum melatonin; no effect on melatonin-induced sexual atrophy	Second part of above study

Table 35 (contd)

Reference	Assay	Exposure	Response	Comment
Truong *et al*. (1996)	Night-time pineal and serum melatonin concentrations; male puberty, assessed by testes weight	60 Hz, 100 μT for 15 min, 2 h before dark from 16 to 25 days of age	Reduced and delayed night-time peak; this effect absent in second replicate; no effect on development of male puberty	Considerable variability in melatonin concentrations between replicate studies
Truong & Yellon (1997)	Night-time pineal and serum melatonin concentrations	60 Hz, 10 or 100 μT (continuous) or 100 μT (intermittent) for 15 or 60 min before or after onset of dark period	No effect	
Yellon & Truong (1998)	Night-time rise in pineal and serum melatonin concentrations; testes weight	60 Hz, 100 μT in complete darkness; 15 min/day for up to 21 days	No effect, even in the absence of photoperiodic cue	
Niehaus *et al*. (1997)	Night-time pineal and serum melatonin concentrations; testis cell numbers	50 Hz, 450 μT (peak) sinusoidal or 360 μT (peak) rectangular fields, 56 days	Increased cell number and night-time serum melatonin concentrations after exposure to rectangular field	Animals on 'long-day' schedule; difficult to interpret
Wilson *et al.* (1999)	Night-time pineal melatonin concentrations and testis and seminal vesicle weights in short-day (regressed) animals	60 Hz, 100 or 500 μT; continuous and/or intermittent, starting 30 min or 2 h before onset of darkness; for up to 3 h on up to 42 days	Reduced pineal melatonin after acute (15 min) exposure; reduced gonad weight but not melatonin after 42-day exposure	Authors suggest a stress-like effect

Table 35 (contd)

Reference	Assay	Exposure	Response	Comment
ELF electric and magnetic fields				
Suffolk sheep				
Lee *et al*. (1993, 1995)	Night-time serum melatonin concentrations and female puberty, detected by rise in serum progesterone	60-Hz, 6-kV/m and 4-μT fields generated by overhead power lines; 10 months	No effect of electric and magnetic fields; strong seasonal effects	Two replicate studies; open-air conditions
Non-human primates				
Rogers *et al*. (1995a)	Night-time serum melatonin concentration in baboons	60-Hz, 6-kV/m and 50-μT fields (6 weeks), 30-kV/m and 100-μT fields (3 weeks)	No effect	
Rogers *et al*. (1995b)	Night-time serum melatonin concentration in baboons	60-Hz, irregular and intermittent sequence of 6-kV/m and 50-μT fields or 30-kV/m and 100-μT fields accompanied by 'transients'	Reduced serum melatonin levels	Preliminary study on two animals

Data from National Radiological Protection Board (1992, 2001)
DMBA, 7,12-dimethylbenz[*a*]anthracene; NAT, *N*-acetyltransferase; SNAT, serotonin-*N*-acetyltransferase

or activity of *N*-acetyltransferase, although concentrations of serum melatonin were significantly depressed.

The difficulties in reproducing some of the earlier findings on the effects of power-frequency electric fields on melatonin have been discussed by Brady and Reiter (1992).

(*b*) *Magnetic fields*

(i) *Studies in mice*

One large-scale study (National Toxicology Program, 1996) reported that exposure to continuous or intermittent 60-Hz magnetic fields (up to 18.5 mT) had no effect on serum or pineal melatonin concentrations in mice. Chronic exposure to 50-Hz magnetic fields of varying intensity (1–130 μT), as part of a tumour promotion study, was reported to have no effect on the night-time excretion of a urinary metabolite of melatonin in mice that had been pre-exposed to ionizing radiation (4 Gy) (Heikkinen *et al.*, 1999).

(ii) *Studies in rats*

An extensive series of studies was conducted in male rats to assess the effects of exposure to circularly or linearly polarized power-frequency magnetic fields of up to 250 μT for up to six weeks on pineal and serum melatonin concentrations. [The Working Group noted that a major difficulty with the interpretation of the results of many studies by this group was that the sham-exposed rats were sometimes treated as 'low-dose' groups because they were exposed to stray magnetic fields (< 2%) generated by the exposure system; thus, statistical comparison was sometimes made with historical controls. Such procedures fail to allow for the interexperimental variability (Kato *et al.*, 1993, 1994b,c,d).]

In the first study, night-time pineal and serum melatonin concentrations were shown to be significantly reduced after exposure for 6 weeks to circularly polarized power-frequency magnetic fields of up to 250 μT, compared with melatonin concentrations in historical controls; in contrast, there was no difference between the concentrations measured in the exposed and concurrent, sham-exposed groups (Kato *et al.*, 1993). The effect had disappeared one week after cessation of exposure (Kato *et al.*, 1994b). A further study with a different, pigmented, rat strain showed a night-time suppression of serum and pineal melatonin in exposed animals compared with both sham-exposed animals and historical controls (Kato *et al.*, 1994c). In contrast to these results, exposure to horizontally or vertically polarized power-frequency magnetic fields for six weeks had no effect on pineal or serum melatonin when compared to sham-exposed animals and historical controls. The reason for this difference between the effects of circularly polarized and horizontally or vertically polarized fields was not clear (Kato *et al.*, 1994d). The last study of this series tested the hypothesis that a reduction in serum melatonin might be correlated with an increase in serum testosterone. However, animals exposed to circularly polarized 50-Hz magnetic fields

were found to have serum testosterone levels similar to those in their sham-exposed counterparts (Kato *et al.*, 1994a).

Four other groups investigated the effects of magnetic fields on serum and pineal melatonin concentrations in rats and came to inconsistent, but generally negative, conclusions. One study reported that acute or chronic exposure of rats to horizontally polarized power-frequency magnetic fields significantly depressed night-time serum melatonin concentrations and activity of *N*-acetyltransferase in the pineal gland (Selmaoui & Touitou, 1995). In another study, exposure to a vertical or horizontal power-frequency magnetic field (50 Hz, 100 μT) had no effect on the circadian excretion of 6-sulphatoxymelatonin, the major urinary metabolite of melatonin (Bakos *et al.*, 1995, 1997). As part of a larger study on the effects of electromagnetic fields on mammary tumours induced by 7,12-dimethylbenz[*a*]anthracene and pineal function in rats, no effect of exposure to a magnetic field of 50 Hz, 10 μT, continuously for 13 weeks, was found on pineal melatonin concentrations in animals treated with 7,12-dimethylbenz[*a*]anthracene (Mevissen *et al.*, 1996b; see also Löscher *et al.*, 1998). Exposure of rats to power-frequency magnetic fields (60 Hz, 1 mT) for up to six weeks under a variety of conditions intended to maximize magnetic field sensitivity had no effect on the circadian excretion of the major urinary metabolite of melatonin (John *et al.*, 1998).

(iii) *Studies in seasonal breeders*

Four laboratories have investigated the effects of exposure to ELF electric and magnetic fields on pineal activity, serum melatonin concentrations and reproductive development in animals that breed seasonally. Three research groups examined these effects in Djungarian hamsters in which the duration of the night-time rise in melatonin secretion during the shortening days of autumn and winter inhibits reproductive activity.

The most complete data come from a series of studies by Yellon and colleagues. Acute exposure to a power-frequency magnetic field (60 Hz) two hours before the onset of darkness reduced and delayed the night-time rise in serum and pineal melatonin concentration, but this effect was diminished in a second replicate study and absent in the third (Yellon, 1994). Similarly variable results on pineal and serum melatonin concentrations were reported by Yellon (1996a,b) and Truong *et al.* (1996). In addition, both studies found that exposure to a magnetic field had no effect on reproductive development, even in reproductively repressed hamsters kept on ‘short-day’ (winter) light/dark schedules, which might be thought to be sensitive to reduced and delayed night-time melatonin elevation. A fourth study, under experimental conditions that were different from those in the previous studies found no effect of exposure to magnetic fields on the night-time melatonin concentrations (Truong & Yellon, 1997). Finally, a brief exposure to power-frequency magnetic fields before the night-time rise in pineal and serum melatonin concentrations had no effect even in complete darkness, i.e. in the absence of a strong photoperiodic cue (Yellon & Truong, 1998).

In studies from a different laboratory, chronic exposure of Djungarian hamsters, which were kept on 'long-day' (summer) light/dark schedules, to 'rectangular' power-frequency magnetic fields (50 Hz; 360 or 450 μT) was reported to increase testis cell numbers and night-time concentrations of melatonin in serum, whereas exposure to sinusoidal power-frequency magnetic fields had little effect. The authors concluded that the in-vivo effects of magnetic fields may have been dependent on their waveform, and that the rapidly changing waveform of the rectangular fields was a more effective biological stimulus (Niehaus *et al.*, 1997). [The Working Group noted that the results are not easy to interpret: increased melatonin concentrations in the Djungarian hamster are usually accompanied by decreased testicular activity.]

More recently, the effect of exposure to power-frequency (60 Hz) magnetic fields on pineal melatonin concentration, serum prolactin concentration and testicular and seminal vesicle weights have also been studied in Djungarian hamsters that had been shifted to a short-day light/dark regime in order to induce sexual regression. Night-time pineal melatonin concentrations were reduced after acute exposure, but this effect diminished with prolonged exposure. In contrast, induced sexual regression, as indicated by the reduction in testicular and seminal vesicle weights, seemed to be enhanced rather than diminished by prolonged exposure to the magnetic field, suggesting a possible stress response (Wilson *et al.*, 1999).

The fourth set of studies of the effects of electric and magnetic fields on seasonal breeders was conducted with Suffolk sheep, which have a long gestational period and become reproductively active in the autumn, as the day-length shortens. In two replicate studies, Suffolk lambs were exposed outdoors to the magnetic fields generated by overhead transmission lines for about ten months. No effect of exposure was observed on serum melatonin concentrations or on the onset of puberty (Lee *et al.*, 1993, 1995).

(iv) *Studies in non-human primates*

Chronic exposure of three male baboons (*Papio cynocephalus*) to a combination of 60-Hz electric and magnetic fields (6 kV/m, 50 μT and 30 kV/m, 100 μT) for 6 weeks had no effect on night-time serum melatonin concentrations (Rogers *et al.*, 1995a). A preliminary study, based on data from two baboons, showed a marked suppression of the night-time rise in melatonin after exposure of the animals for three weeks to an irregular, intermittent sequence of combined electric and magnetic fields in which switching transients were generated (Rogers *et al.*, 1995b).

(v) *Cellular effects*

The effects of magnetic fields on the function of the serotonin receptor, 5-HT1B, were studied in tissue samples of rat and guinea-pig brain and in Chinese hamster ovary cells transfected with the human form of the receptor. The tissue and cell samples were exposed to 50-Hz magnetic fields (0.01–10 mT) for 30 or 60 min before specific assays were performed. The authors observed an effect of the field on the

affinity constant of 5-HT1B receptors, which decreased (in a sigmoidal fashion at field intensities between 0.05 and 2 mT, with a threshold at 0.6 mT) when the response saturated. Functionally, the magnetic fields inhibited the action of a 5-HT1B agonist to produce cyclic adenosine monophosphate (cAMP) and also caused a change in the cellular activity of the receptors, as demonstrated by the inhibition of synaptosomal release of 5-HT1B receptors from rats, guinea-pigs and humans (Massot *et al.*, 2000). [The Working Group noted that it is unclear how these results relate to changes *in vivo*.]

4.2.5 *Behavioural effects*

Studies on the effects of ELF electric and magnetic fields on behaviour have included tests of:

— perception and detection;
— arousal and aversive activity responses; and
— learning and memory.

The studies of specialized response systems that operate in various animal species and are associated with exposure to electric and magnetic fields, such as communication of food location (in honeybees), electroreception systems (as found in certain fish species) and homing and navigation (found in several species of birds), are not discussed here.

(*a*) *Static fields*

Davis *et al*. (1984) observed that neither exposure of mice to a 60-Hz, 1.65-mT nor a 1.5-T static magnetic field resulted in any behavioural alterations in a passive avoidance learning test.

Exposure to a strong static magnetic field (600 mT) for 16 h per day for 14 weeks inhibited avoidance behaviour in rats (Nakagawa & Matsuda, 1988). A taste-aversion study was conducted in rats exposed to very high-intensity static magnetic fields (9.4-T magnet) for 30 min. The exposure was significantly associated with taste aversion (Nolte *et al.*, 1998) and the effect lasted throughout the eight days of post-exposure follow-up.

The effects of exposure of rats to static magnetic fields were also investigated in a maze test to assess alterations in learning ability. Newborn male and female rats exposed to a field of 0.5 T for 14 days postnatally showed no significant change in learning ability compared with sham-exposed controls when tested one month after exposure (Hong *et al.*, 1988).

If given a choice, rats preferred to stay out of a high-voltage static electric field when the field strength was ≥ 55 kV/m, whereas they showed no such aversion at field strengths ≤ 42.5 kV/m. Changes in the air concentrations of either positive or negative ions had no effect on aversive or non-aversive behaviour (Creim *et al.*, 1993).

(*b*) *ELF electric and magnetic fields*

(i) *Behavioural effects related to perception of fields*

Behavioural studies in several animal species provide evidence that the animals perceive the presence of electric and magnetic fields and suggest that electric fields may directly alter behaviour. A number of investigations have reported the threshold of detection of a vertical 60-Hz electric field to be in the range of 4–10 kV/m in rats (Stern *et al*., 1983). Male rats were trained to press a lever in the presence of the field and not to press in its absence. Control procedures showed that the behaviour required the rat to be in the electric field and that the behaviour was not controlled by any of several potentially confounding variables. Female rats, evaluated in a similar experimental signal-detection system showed comparable detection thresholds of 3–10 kV/m (Stern & Laties, 1985).

The thresholds for the perception of electric fields by animals other than rats have also been evaluated: they ranged from an average of 12 kV/m in baboons (with one animal perceiving a field as weak as 5 kV/m) (Orr *et al*., 1995b) to 35 kV/m in miniature swine, as determined by use of preference aversion measurements (Kaune *et al*., 1978). The perception threshold for mice was 25 kV/m, using arousal as the response indicator (Rosenberg *et al*., 1983), and that for chickens and pigeons was approximately the same (Graves *et al*., 1978; Graves, 1981). Human volunteers in certain postures were able to perceive a 9-kV/m electric field (Graham *et al*., 1987). It appears that changes in various environmental factors, such as relative humidity, can alter perception thresholds (Weigel & Lundstrom, 1987). Cutaneous sensory receptors that respond to 60-Hz electric fields have been identified in the cat paw (Weigel *et al*., 1987).

Detection of magnetic fields by animals is presumed to be quite different from that for perceiving electric fields and a wide divergence of results has been reported in various studies designed to evaluate detection of and response to magnetic fields by animals. By use of conditional suppression techniques to measure the response, rats were shown to be able to perceive the presence of a magnetic field as weak as 0.2 mT, with a 7–65-Hz frequency range (Smith *et al.*, 1994).

(ii) *Activity, aversion responses*

The arousal response of animals exposed to a stimulus is a less precise index of perception than the responses discussed above. Arousal and preference or avoidance responses have been determined at several field strengths for ELF electric and magnetic fields. Such responses were observed in mice exposed to 60-Hz electric fields. At 25, 50 and 100 kV/m the responses were transient and not sustained with prolonged or repeated exposure (Hackman & Graves, 1981; Rosenberg *et al*., 1981). When exposed to field strengths of 25 kV/m, rats preferred to spend their inactive period in the field (60 Hz). At 75–100 kV/m, they avoided exposure (Hjeresen *et al.*, 1980). To determine the strength of the aversion in rats, Creim *et al*. (1984) examined taste-aversion associated with exposure to electric fields. The animals showed no taste-aversion behaviour when exposed to electric fields up to 133 kV/m. Static fields

(approximately 75 kV/m) were also ineffective in producing taste aversion in rats (Creim *et al*., 1995).

There was no indication of aversive behaviour in mice exposed for 72 h to 1.65-mT static or 60-Hz magnetic fields (Davis *et al.*, 1984). These results were confirmed in other experiments showing a lack of aversive behaviour in rats exposed for 1 h to a 60-Hz, 3.03-mT magnetic field (Lovely *et al*., 1992). The internal body currents induced by this level of exposure were comparable to those from strong electric fields. Because aversion was not demonstrated with magnetic fields, but was observed with electric fields, these results suggest that the aversive behaviour is not due to internal body currents. One study, using special combinations of parallel static and alternating magnetic fields (at cyclotron resonance frequencies), reported a reduction in exploratory behaviour at much lower intensities of static fields (500 and 50 μT) (Zhadin *et al*., 1999).

At higher field intensities, both static (490 mT) and 50-Hz, 18-mT magnetic fields caused a decrease in irritability of rats after extended (2 h per day, 20 days) exposure (Trzeciak *et al*., 1993). No other significant behavioural effects were observed.

Swine exposed to 30-kV/m electric fields were reported to prefer the field during the day and to avoid exposure during the night (Hjeresen *et al*., 1982). At comparable exposures to 60-Hz, 30-kV/m electric fields, minor behavioural changes were observed in baboons, which appeared to be related to the animals' perception of the fields (Rogers *et al*., 1995c). Even at field strengths up to 65 kV/m, no aversive behaviour was noted in non-human primates, although some increase in social stress was induced in groups of baboons exposed to 60-kV/m electric fields (Easley *et al*., 1991). [The Working Group noted that only a few other behavioural changes have been reported after exposure of animals to electric fields up to 100 kV/m. Furthermore, the alterations that were seen in most studies were not persistent. Indeed, the animals quickly habituated to the presence of the electric field.]

(iii) *Neurobehavioural teratology*

Neurobehavioural teratology studies are reviewed in section 4.2.1.

(iv) *Learning, performance and memory*

As early as 1970, studies were conducted to examine the effects of ELF electric and magnetic fields on learning and performance. Macaques (*Macaca nemestrina*) exposed to weak electric fields of 4–10 Hz showed a disruption of the timing behaviour of an operant schedule response (Gavalas *et al*., 1970; Gavalas-Medici & Day-Magdaleno, 1976). Operant behaviour in baboons was studied after exposure to 30- and 60-kV/m electric fields, but other than an initial work stoppage in exposed animals, no effect on operant behaviour was observed (Rogers *et al*., 1995c). No effects were seen on response rate, number of errors or extinction of a simple task motivated by appetite (Rogers *et al*., 1995d). Social behaviour of baboons was not

affected by exposure to a 30-kV/m electric field for 12 h per day for 6 weeks (Coelho *et al*., 1991).

The spatial learning task is considered to reflect the 'working' or 'short-term' memory. Tests of the performance of rodents in a maze are the usual methods of assessing spatial learning during exposure. In adult male mice exposed for 45 min to a 50-Hz, 0.75-mT magnetic field immediately before testing in a radial arm maze, the rate of learning the task was significantly reduced, compared with unexposed controls, although the overall accuracy of the memory was not affected (Sienkiewicz *et al*., 1998). A similar test performed by rats exposed to 60-Hz, 0.75-mT magnetic fields showed a significant retardation of learning compared with sham-exposed controls. Treatment of the animals with the cholinergic agonist, physostigmine, before the exposure to the magnetic field reversed the field effect (Lai, 1996). Rats were also tested in a water maze to evaluate performance after exposure to a 1-mT magnetic field for 1 h. No differences between exposed and sham-exposed animals were observed in learning (ability to locate the platform); however, the swimming speed of the exposed rats was significantly lower than that of controls (Lai *et al*., 1998).

Thomas *et al*. (1986) and Liboff *et al*. (1989) reported that timing discrimination in rats was disrupted even by a very weak 60-Hz magnetic field of 26 μT. These results, however, could not be replicated in later studies conducted under the same exposure conditions (Stern et al., 1996).

Rats exposed overnight to a 7-Hz, ~ 50-nT magnetic field coupled with a known geomagnetic activity showed no significant differences in number of errors made or speed of acquisition of the learning task when compared with sham-exposed controls (McKay & Persinger, 1999).

Studies have also been conducted to evaluate learning, performance and memory in animals given operant tasks after exposure to combined electric and magnetic fields.

In a study in rats, Salzinger *et al*. (1990) exposed animals to 60-Hz fields of 30 kV/m and 100 μT. When the performance of complex operant tasks was tested at various times during the light/dark cycle, a slightly slower response was observed in exposed animals at one point in the cycle.

Groups of eight baboons were sham-exposed or exposed to 6-kV/m, 50-μT, and subsequently to 30-kV/m, 100-μT fields at 60 Hz (Orr *et al*., 1995a). In contrast to the effect seen with exposure to the electric field (30 kV/m) alone, there was no decrement of performance in the animals exposed to the combined fields. The authors suggest that the 100-μT magnetic field may have blocked the transient loss of performance. Other behavioural end-points were also unaffected in exposed baboons at these combinations of exposure to electric and magnetic fields (Coelho *et al*., 1995). Macaques (*Macaca nemestrina*) were exposed to combined 60-Hz fields of 3–30 kV/m and 10–90 μT for 18 h per day for three weeks. No changes were observed in the performance of the exposed animals in a food-motivated operant task compared with sham-exposed controls (Wolpaw *et al*., 1989).

There is clear evidence that animals can perceive electric fields at field strengths in the range of 3–10 kV/m and above. The ability to perceive ELF magnetic fields at low intensities is less well established, with both positive and negative indications of perception.

[The Working Group noted that both static and ELF magnetic fields have been shown to influence animals in learning and memory tasks. The data indicate that, for exposure to electric fields, aversive behaviour occurs above approximately 30–50 kV/m, depending on the animal species. For ELF magnetic fields, intensities of up to at least 18 mT do not appear to influence aversion. However, there have also been studies that showed no effects of exposure on either learning or memory acquisition. The results of a number of studies suggest that observation of field-related effects requires that exposure is closely coupled to testing in time, and may be more related to acquisition of the task or even to the state of arousal of the animal than to an effect on the memory itself. Because studies in this area show variable results, demonstrating both decreases in apparent learning ability and memory, and lack of any effect, it is difficult to draw firm conclusions as to the robustness of the effects of exposure to magnetic fields on learning.]

4.3 Effects of ELF electric and magnetic fields on bone healing[1]

In some situations the interaction of electric and magnetic fields with biological systems may be beneficial and lead to new medical applications. Examples include nuclear magnetic resonance imaging (MRI), electromagnetically induced hyperthermia, and bone healing with pulsed electromagnetic fields. This section will briefly discuss the evidence for the beneficial effects of exposure to electric and magnetic fields on bone healing in humans. Similar studies in animals and in-vitro experiments dealing with this topic have been reviewed in detail elsewhere (Portier & Wolfe, 1998) and will not be discussed here.

The therapeutic effects of specific low-energy, time-varying magnetic fields (pulsed electromagnetic fields, PEMF) on bone healing were first documented in 1973. These effects are based on the generation of electrical currents within the bone tissue by magnetic induction. These currents enhance the activity of bone-forming cells, the osteoblasts (Cane *et al.*, 1993). Initially, this form of treatment with athermal energy was used mainly for patients with juvenile or adult non-unions, i.e. bone fractures showing no sign of union nine months after injury, despite the usual forms of surgical treatment, including bone grafting. The biological effectiveness of PEMF therapy in augmenting bone healing has been confirmed by several double-blind and placebo-controlled prospective studies, and supported by laboratory studies (for an early review, see Bassett 1989).

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[1] For the sake of completeness, this section was added by the IARC secretariat after the Working Group Meeting.

A group of 125 patients with non-united fractures of the tibial diaphysis were treated for >10h/day with PEMF (15-Hz pulse burst; 1.5-mT peak flux density; 25-μs decay time; 4-kHz pulse repeat rate; 5-ms burst duration). This treatment resulted in an estimated electric field pulse of 0.1–0.2 V/m in the bone tissue. Healing of the fracture was observed in 87% of the patients who required an average treatment duration of 5.2 months (range, 2–22 months). Failure of the therapy was attributed by the authors to inadequate immobilization of the fractured bone, separation of opposing fractured bone surfaces by more than 1 cm, exposure for less than 10 h/day, or incorrect positioning of the induction coils (Bassett *et al.*, 1981).

A double-blind, randomized, placebo-controlled trial of magnetic field therapy was conducted in 16 patients with tibia fractures that had not healed for at least one year. All patients received full leg plasters and were divided into a treatment group (9 patients; average age, 38 years) and a placebo group (7 patients; average age, 29 years). The patients were instructed to use PEMF stimulators — active or inactive — for 12–16 h/day, with coils designed to fit around the cast of each patient. The active devices produced a 1.5-mT peak intensity, 5-ms burst waveform repeated at 15 Hz. At 24 weeks, the fractures in 5/7 patients in the placebo group and 5/9 patients in the treatment group had healed. The authors concluded that magnetic field therapy is not more effective than the traditional treatment of these fractures (Barker *et al.*, 1984).

A group of 45 patients with tibial shaft fractures were included, over a period of 6 years, in a double-blind multi-centre trial to assess the effect of exposure to PEMF on bone fracture healing. The fractures had not shown union for 16–32 weeks, and some were characterized by severe displacement, angulation or the presence of injury to soft tissue and skin. Plaster immobilization was used in all patients (mean age, 35 years), 20 of whom received active electromagnetic stimulation (15-Hz pulse burst; 200-μs pulse duration; 25-μs decay time; peak flux density not reported). The other 25 patients (mean age, 45 years; significantly different from that of the treatment group) served as controls and used non-functioning stimulators. Treatment continued for 12 weeks, for 12 h/day. In the treatment group, union was observed in five cases, progress to union in five patients, but no progress was reported in 10 patients. In the control group the numbers of patients were one, one and 23, respectively ($p = 0.002$) (Sharrard, 1990).

In two randomized placebo-controlled clinical trials with 32 and 37 patients, respectively, treatment with pulsed magnetic fields (1.8 mT; repeat frequency, 75 Hz; rise time, 1.3 ms; estimated peak intensity of the induced electric field, 50 mV/m) for 8 h/day for up to 90 days induced a significant increase in trabecular bridging after surgical bone transection (Borsalino *et al.*, 1988; Mammi *et al.*, 1993).

A randomized double-blind prospective study was conducted in 195 patients who underwent lumbar interbody fusion, a surgical procedure aimed at connecting adjacent vertebrae. The study comprised 98 patients exposed to 15-Hz pulse-burst signals of 1.5 mT (rise time, 25 μs; repeat rate, 4 kHz; burst duration, 5 ms) and 97 patients in the placebo group. Both groups were asked to wear a brace for 8 h/day. Fusion was

observed in 60 of 65 patients (92%) in the treatment group and in 63 of 97 (65%) patients carrying a placebo device ($p < 0.005$). The success rate in 33 patients who used the active brace for less than 4 h/day was similar to that of the placebo group (Mooney, 1990).

Another double-blind clinical trial was conducted in patients undergoing limb-lengthening surgery, which involves transection of the bone, distraction of the bone ends, and regeneration of bone tissue in the distraction gap. Patients were asked to wear the induction devices — active or placebo — for 4 h/day for up to 12 months. Exposure conditions to the pulsed magnetic fields were similar to those described in the previous study (Mooney, 1990). Bone densities were measured by X-ray analysis at the mid-point of the gap and at the proximal and distal ends. No difference between the treatment and placebo groups was observed either in limb-lengthening rate or in bone density at the distraction gap. However, there was a significant increase of bone density in the proximal segment in the field-exposed group, and a significant reduction of bone loss at the distal side (Eyres *et al.*, 1996).

A recent clinical trial used low-amplitude PEMF on 19 patients with non-union or delayed union of the long bones. The stimulator device produced 0.3-ms pulses repeated at 80 Hz, with maximum magnetic fields of 0.01–0.1 mT, i.e. considerably weaker than in previous studies. Stimulation was applied for 9–12 h/day until mobility at the fracture site had disappeared. Among the 13 patients (age range, 9–90 years; period since injury 8–108 weeks, average 41.3 weeks) who completed the treatment, 11 had successfully healed bones, after treatment periods of 4–27 weeks. The two unsuccessful cases had bone gaps greater than 1 cm after removal of dead bone after infection. The authors concluded that weak magnetic fields may be effective in stimulating bone healing (Satter-Syed *et al.*, 1999).

A recent review discussed studies in which magnetic fields were applied to promote bone-healing, to treat osteoarthritis and inflammatory diseases of the musculoskeletal system, to alleviate pain, to enhance healing of ulcers and to reduce spasticity. The action of magnetic fields on bone healing and pain alleviation was confirmed in most of the trials. In the treatment of other disorders the results have been contradictory. Application times varied between 15 minutes and 24 hours per day for three weeks up to 18 months. There seems to be a relationship between longer daily application time and positive effects, particularly in bone-healing. Of the 12 well-controlled studies dealing with the application of pulsed magnetic fields on bone healing, 11 reported a more rapid and improved healing process, compared to placebo controls. In all these studies, a treatment duration of 8–12 hours per day appeared to be required to produce the beneficial effect. It was noted that optimal dosimetry for this type of therapy has yet to be established (Quittan *et al*., 2000).

4.4 Genetic and related effects

4.4.1 *Genotoxic effects*

(*a*) *Studies in humans*

(i) *Static magnetic fields*

No data were available to the Working Group.

(ii) *ELF electric and magnetic fields*

Several studies were carried out to investigate the clastogenic effects of exposure to power-frequency electric and magnetic fields and transient electric currents.

Chromosome analyses were performed on lymphocytes from 32 workers occupationally exposed for more than 20 years to 50-Hz electric and magnetic fields in 380-kV switchyards. Comparison with a control group of 22 workers of similar age and occupation, who had not been exposed to electric or magnetic fields, showed that neither the numbers of structural chromosomal changes nor the frequencies of sister chromatid exchange were increased (Bauchinger *et al.*, 1981).

Chromosomal aberrations in lymphocytes from three groups of welders were examined. The technology used by one group of welders gave rise to elevated concentrations of nickel in their serum and urine. Although all three groups were exposed to essentially the same electrical discharges, only the welders with the higher concentrations of nickel had increased chromosomal aberrations in their lymphocytes. There were no correlations between the number of aberrations and the concentration of nickel in the serum or the duration of occupational exposure, but correlations were found with the number of cigarettes smoked. It was concluded that certain welding processes produce fumes that seem to have effects on chromosomes, but that fields from welding *per se* do not seem to cause increased aberrations (Elias *et al.*, 1989).

Lymphocytes from the peripheral blood of 20 switchyard workers (9 smokers, 11 non-smokers) were assayed for chromosomal anomalies. The rates of chromatid and chromosome breaks were found to be significantly increased compared to those in lymphocytes from 17 control subjects (7 smokers, 10 non-smokers) (Nordenson *et al.*, 1984).

In a follow-up to the previous study, data were reported on 38 employees of electric power companies, 19 of whom worked on the repair and maintenance of circuit breakers and disconnectors in 400-kV substations. The other 19 served as controls and were exposed only to normal environmental electric and magnetic fields. Coded blood samples were analysed for the presence of chromosomal aberrations, sister chromatid exchanges (SCE), and cells with micronuclei. Compared with the control group, the exposed subjects showed a statistically significant increase in chromosomal aberrations and cells with micronuclei, but not in the frequency of SCE. Because similar results were obtained in studies of lymphocytes exposed *in vitro* to transient electric currents (spark discharges), the increase in chromosomal damage in substation workers may be

associated with exposure to transient electric currents during work (Nordenson *et al.*, 1988).

Chromosomal aberrations, SCEs, replication indices and micronuclei were analysed in lymphocytes from the peripheral blood of 27 non-smoking power linemen who had considerable long-term exposure to 50-Hz electric and magnetic fields. An equal number of non-smoking telephone linemen were matched pair-wise with the exposed workers for age and geographical region, and served as controls. No differences between the groups were observed with respect to SCEs, replication indices or micronuclei. The overall frequency of chromosomal aberrations was higher in the exposed workers than in the controls, but the difference was not significant. However, the mean rate of lymphocytes with chromatid-type breaks was significantly higher in the power linemen than in the reference group. The excess of aberrant cells was observed mainly in lymphocytes from power linemen who had smoked earlier in their life. Although the interpretation is complicated by the confounding effect of having been a smoker, these results suggest that exposure to 50-Hz electric and magnetic fields is associated with a slight increase in chromatid breaks (Valjus *et al.*, 1993).

Thirteen workers in a high-voltage laboratory and 20 controls participated in a cross-sectional, matched-pairs study of cytogenetic damage. During cable testing the workers were exposed to static, alternating (50 Hz), or pulsed electric and magnetic fields. The magnetic field strength was normally 5–10 μT, but was occasionally much higher. Chromosomal aberrations, SCE and aneuploidy were studied in lymphocytes from the peripheral blood of exposed workers and controls. In addition, chromosomal aberrations were investigated in lymphocyte cultures treated with hydroxyurea and caffeine, to inhibit DNA synthesis and repair. Among seven laboratory workers (all smokers) the mean number of chromosome breaks/200 cells was 2.3, as compared with 0.7 for controls matched for job, age and smoking habits. The comparable figures for the cultures treated with hydroxyurea and caffeine were 12.0 and 6.0, respectively. No field-related increase was detected in non-smokers by either method. The other genetic parameters did not differ between the exposed workers and the controls (Skyberg *et al.*, 1993).

A cytogenetic analysis was carried out on cultured (48 h) peripheral lymphocytes of Swedish train drivers exposed to relatively strong magnetic fields up to > 100 μT. A pilot study with lymphocytes from 18 train drivers indicated a significant difference in the frequency of cells with chromosomal aberrations (gaps included or excluded) in comparison with seven concurrent controls (train dispatchers) and a control group of 16 office workers. The frequencies of cells with chromosome-type aberrations (excluding gaps) were about four times higher in train drivers than in office workers ($p < 0.01$) and dispatchers ($p < 0.05$). Seventy-eight percent of the train drivers had at least one cell with chromosome-type aberrations per 100 compared with 29% for the dispatchers and 31% for the office workers. In a follow-up study on a group of 30 train drivers, half of the cytogenetic slides from each subject were examined in one laboratory and the remainder was analysed in another; a statistical analysis showed no difference in results

between laboratories, so the data were pooled. The results showed an increase ($p < 0.05$) in the frequency of cells with chromosome-type aberrations (gaps excluded) in the train drivers compared with that in a control group of 30 policemen. Sixty percent of the train drivers had one or more cells per 100 cells with chromosome-type aberrations compared with 30% among the policemen. These results support the hypothesis that exposure to magnetic fields at mean intensities of 2–15 μT can induce chromosomal damage *in vivo* (Nordenson *et al.*, 2001).

A cross-sectional study was carried out in a Norwegian transformer factory of 24 workers exposed to electric and magnetic fields and mineral oil, and 24 matched controls. The exposure group included employees in the high-voltage laboratory and in the generator-welding department. Electric and magnetic fields and oil mist and vapour were measured. Blood lymphocytes were cultured and analysed for chromosomal aberrations. In addition to conventional cultures, the lymphocytes were also treated with hydroxyurea and caffeine to inhibit subsequent DNA synthesis and repair. The results of the conventional lymphocyte cultures were similar in the exposure group and the controls for all cytogenetic parameters. In the cultures in which DNA synthesis and repair were inhibited, the cytogenetic parameters of the lymphocytes from generator welders did not differ from those of the controls, whereas in lymphocytes from workers in the high-voltage laboratory, the numbers of chromatid breaks, chromosome breaks and aberrant cells were significantly increased compared with control values. More years of exposure and smoking increased the risk of aberrations. No increase in cytogenetic damage in exposed workers compared to controls was detected with the conventional lymphocyte assay. In repair-inhibited cultures, however, there were indications that electric and magnetic fields in combination with exposure to mineral oil may produce chromosomal aberrations (Skyberg *et al.*, 2001).

(*b*) *Studies in animals*

(i) *Static magnetic fields*

Wing spot tests were performed in *Drosophila melanogaster* to examine the possible mutagenic activity of a static magnetic field. A DNA repair-defective mutation was introduced into the conventional test system to enhance the frequency of the mutant spots. Third instar larvae were exposed to a horizontal 5-T static magnetic field for 24 h. After moulting, wings were examined under a microscope to detect hair spots (large single and twin spots) with mutant morphology, indicative of somatic recombination. The exposure caused a statistically significant enhancement of somatic recombination compared with the unexposed control. This enhancement was suppressed to the control level by treatment with vitamin E, a non-specific antioxidant. Enhancement of non-disjunction, terminal deletions and gene mutations was not detected (Koana *et al.*, 1997). [The Working Group noted that there is limited information on the genetic effects of static magnetic fields.]

(ii) *ELF electric and magnetic fields*

Cytogenetic effects, DNA breaks, DNA cross-links

The effect of long-term exposure to a magnetic field on subsequent cell proliferation and the frequency of SCE was examined in the peripheral lymphocytes of female Wistar rats following in-vivo exposure to a 50-Hz, 30-mT magnetic field for 7 or 28 days. As a positive control, another group of rats was treated with cyclophosphamide. The magnetic field influenced neither the frequency of SCE nor the proliferation characteristics of cultured peripheral lymphocytes (measured as mitotic indices and proliferation index) (Zwingelberg *et al.*, 1993).

The acute effect of magnetic fields on DNA integrity was examined in male Sprague-Dawley rats (age, 2–3 months; weight, 250–300 g), which were exposed in the Helmholtz coil system to a 60-Hz magnetic field at flux densities of 0.1, 0.25 and 0.5 mT for 2 h. Four hours after exposure, the rats were killed and DNA single-strand and double-strand breaks were assayed in brain cells by single-cell gel electrophoresis ('comet' assay) at neutral and alkaline pH. A significant increase in DNA single-strand breaks was observed in all cases, and the effect was dose-dependent. No significant effect on DNA double-strand breaks was observed after exposure to the 0.1-mT magnetic field, but a significant increase was seen at flux densities of 0.25 and 0.5 mT (Lai & Singh, 1997a).

Studies were conducted to determine whether treatment with melatonin and the spin-trap compound *N*-*t*-butyl-α-phenylnitrone could block the effect of magnetic fields on brain-cell DNA. Rats were injected with melatonin (1 mg/kg bw, subcutaneously) or *N*-*t*-butyl-α-phenylnitrone (100 mg/kg bw, intraperitoneally) immediately before and after two hours of exposure to a 60-Hz, 0.5-mT magnetic field. Brain cells were assayed by single-cell gel electrophoresis and both treatments were found to block induction by the magnetic field of DNA single- and double-strand breaks. Since melatonin and *N*-*t*-butyl-α-phenylnitrone are efficient scavengers of free radicals, the authors inferred that free radicals may play a role in DNA damage induced by magnetic fields (Lai & Singh, 1997b).

In the same laboratory, DNA–protein and DNA–DNA cross-links were studied by use of the single-cell gel electrophoresis assay. Male Sprague-Dawley rats (age, 2–3 months; weight, 250–300 g) were exposed in the Helmholtz coil system to a sinusoidal 60-Hz, 0.5-mT magnetic field for 2 h. Rats were killed 4 h after exposure. Most of the increase in DNA migration induced by the magnetic field was observed only after treatment with proteinase-K, suggesting the presence of DNA–protein cross-links. In addition, when brain cells from control rats were exposed to X-rays, an increase in DNA migration was observed, the extent of which was independent of treatment with proteinase-K. However, the X-ray-induced increase in DNA migration was retarded in cells from animals exposed to magnetic fields even after treatment with proteinase-K, suggesting that DNA–DNA cross-links were also induced by the field. The effects of magnetic fields were also compared with those of mitomycin C, a known inducer of

DNA cross-links. The pattern of effects was similar with the two agents (Singh & Lai, 1998).

Degradation of DNA was measured by single-cell gel electrophoresis in brain cells of CBA mice exposed *in vivo* continuously to 50-Hz, 0.5-mT magnetic fields for 2 h, 5 days or 14 days. No differences between the groups exposed for 2 h and 5 days and controls were observed. However, in the group exposed to the magnetic fields for 14 days, a significantly extended brain cell DNA migration was observed ($p < 0.05$) (Svedenstål *et al*, 1999a).

CBA mice were exposed outdoors to 50-Hz electromagnetic fields, with a flux density of about 8 μT, generated by a 220-kV transmission line. Possible genotoxic effects as well as effects on body weight, leukocyte and erythrocyte counts, and the level of ornithine decarboxylase activity in spleen and testis were determined after 11, 20 and 32 days of exposure. Ornithine decarboxylase is an enzyme involved in the synthesis of putrescine from ornithine, which is one of the polyamine synthesis pathways. DNA degradation was studied in brain cells by single-cell gel electrophoresis. After 32 days of exposure, a highly significant increase of the tail:head ratio of the comets was observed ($p < 0.001$), indicative of DNA damage. A decreased number of mononuclear leukocytes ($p < 0.05$) was observed in mice exposed for 20 days (Svedenstål *et al*, 1999b). [The Working Group noted that the single-cell gel electrophoresis assay following in-vivo exposure is particularly protocol-dependent, specifically with respect to the method of killing the animals and the treatment of tissue samples between exposure of the animal and analysis of the tissues.]

Dominant lethal mutations

Sexually mature, male C3H/He mice, aged 8–10 weeks at the beginning of a study to determine the induction of dominant lethal mutations, were exposed continuously for two weeks to a vertical, 50-Hz, 20-kV/m electric field or sham-exposed. Current densities induced in the testes were estimated to be approximately 100 $\mu A/m^2$. After exposure, each male was mated weekly with two different female mice for eight weeks. In this way, female mice were inseminated with sperm that had been exposed to the electric field at different stages of the spermatogenic cycle. Another group of male mice was exposed to 169-keV X-rays for about 150 min and served as a positive control. In this group, the estimated dose to the testis was 1.5 Gy. Whereas the positive controls gave clear evidence of mutagenesis, no significant changes related to exposure to an electric field were observed in fertilization rates or in survival of embryos before or after implantation (Kowalczuk & Saunders, 1990).

In a second assay of dominant lethal mutation a total of 42 male mice were exposed for eight weeks to a 50-Hz, 10-mT sinusoidal magnetic field. A group of 47 males was used as simultaneous cage controls. Each male was subsequently mated with two females on weeks 1, 3, 5, 7 and 9 post-exposure. The numbers of gestating females, corpora lutea, and live and dead implants were recorded. Multiple logistic regression

analyses were used to examine the effects of exposure on fertilization rate, pre-implantation survival and post-implantation survival. There were no differences in overall response between the exposed and control groups, nor was any significant effect of exposure seen at any number of weeks after exposure. Thus, exposure to power-frequency magnetic fields at 10 mT for the approximate period of spermatogenesis did not appear to induce dominant lethal mutation in the germ cells of male mice (Kowalczuk *et al*., 1995). [The Working Group noted that the dominant lethal mutation assay is not sufficiently sensitive to allow detection of weak mutagens.]

(*c*) *In-vitro studies*

(i) *Static magnetic fields*

Micronucleus formation

The formation of micronuclei occurs when a chromosome or a chromosome fragment is released from the nucleus as a result of strand breakage or disturbance of spindle function during mitosis.

The effects of exposure to a 4.7-T homogeneous static magnetic field on the frequency of micronuclei induced in cultured CHL/IU cells by mitomycin C were studied. Simultaneous exposure to the magnetic field and mitomycin C for 6 h significantly decreased the frequency of mitomycin C-induced micronucleated cells analysed after post-treatment culture periods of 18, 42, 54 and 66 h. In both field-exposed and control groups, the highest frequency of mitomycin C-induced micronucleated cells was observed 42 h after treatment; the frequency decreased gradually after this time (Okonogi *et al*., 1996).

Mutation

The possible mutagenic and co-mutagenic effects of strong static magnetic fields were examined in a bacterial mutagenicity test. A super-conducting magnet was used to generate a homogeneous static magnetic field with a flux density of up to 5 T. Exposure to this field produced no mutations in four strains of *Salmonella typhimurium* (TA100, TA1535, TA1537 and TA98) or in *Escherichia coli WP2 uvrA* either using the pre-incubation method or in the plate-incorporation assay. In the co-mutagenicity test, *E. coli WP2 uvrA* cells were treated with various chemical mutagens and simultaneously exposed to a 2-T or a 5-T static magnetic field. The mutation rate in the group exposed to the magnetic field was significantly higher than in the unexposed group when cells were treated with six different alkylating agents. Exposure to the magnetic field did not affect the mutagenicity of 2-aminoanthracene, 9-aminoacridine, N^4-aminocytidine or 2-acetoamidofluorene (Ikehata *et al*., 1999).

(ii) *ELF electric and magnetic fields*

Chromosomal aberrations and sister chromatid exchange

Many researchers have determined the frequency of chromosomal aberrations and sister chromatid exchange in response to exposure to ELF electric and magnetic fields. Several studies with peripheral human blood lymphocytes have been reported in which exposure *in vitro* to 50- or 60-Hz magnetic fields (30 μT–7.5 mT) caused no increase in the frequency of chromosomal aberrations or sister chromatid exchanges (Cohen *et al.*, 1986a,b; Rosenthal & Obe, 1989; Antonopoulos *et al.*, 1995; Paile *et al.*, 1995). However, 72 hours of continuous in-vitro exposure to 10-ms pulses of a magnetic field (50 Hz, 1.05 mT) caused a significant increase in the frequency of chromosomal aberrations and sister chromatid exchanges in the lymphocytes from three male donors (Khalil & Qassem, 1991).

In-vitro exposure of human peripheral lymphocytes to a 50-Hz electric current with a current density of 1 mA/cm^2 did not induce any chromosome damage. Exposure to 10 spark discharge pulses (duration, 3 μs) with a peak field strength in the samples of 3.5 kV/cm, however, resulted in chromosome breaks at a frequency similar to that induced in lymphocytes *in vitro* by 0.75 Gy ionizing radiation (Nordenson *et al.*, 1984).

Lymphocytes from human peripheral blood were exposed for 48 h to 50-Hz magnetic fields (62.8, 80, 88.4, 504, 1061, 1750 and 2500 μT) and examined for cytogenetic effects. No significant changes in chromosomal aberration or sister chromatid exchange frequencies were observed. Combined treatments with mutagens (mitomycin C or X-rays) and 50-Hz magnetic fields did not reveal any significant synergistic, potentiating or antagonistic effects between magnetic fields and these mutagens (Maes *et al.*, 2000).

Exposure of Chinese hamster V-79 cells to 25-μs pulses of a magnetic field (0.18–2.5 mT) repeated at 100 Hz for 24 h did not increase sister chromatid exchanges (Takahashi *et al.*, 1987).

A significant increase in sister chromatid exchanges was found in mouse m5S cells exposed for 42 h to a strong ELF magnetic field (50 Hz, 400 mT) (Yaguchi *et al.*, 1999). In contrast, exposure to magnetic fields ≤ 50 mT did not cause any increase in the frequency of sister chromatid exchange in these cells. Chromosomal aberration analysis revealed an increased frequency of chromatid-type aberrations such as gaps in response to exposure to magnetic fields with flux densities of 50 mT and higher (Yaguchi *et al.*, 2000). Intermittent (15 s on, 15 s off) exposure of human amniotic cells to a 50-Hz, 30-μT magnetic field over 72 h doubled the frequency of chromosomal aberrations including gaps ($p < 0.05$). However, increased frequencies of chromosomal aberrations were not observed after continuous exposure to a 300-μT magnetic field (Nordenson *et al.*, 1994). When mouse m5S cells were exposed to a 60-Hz, 50-mT or a 50-Hz, 400-mT magnetic field after pre-exposure to X-rays (3 Gy) or mitomycin C (1 μM), chromatid-type aberrations were enhanced by the ELF magnetic field (Yaguchi *et al.*, 2000). Combined exposure of lymphocytes from human peripheral blood to an ELF

magnetic field (60 Hz, 1.4 mT) and ionizing radiation (3 Gy) resulted in a higher frequency of tetraploid cells than that produced by ionizing radiation alone (Hintenlang, 1993).

DNA strand breaks

Various studies in which cultured mammalian cells were exposed to ELF magnetic fields (0.2 μT–5 mT) found no induction of DNA single-strand breaks (Reese *et al*., 1988; Fiorani *et al*., 1992; Fairbairn & O'Neill, 1994; Cantoni *et al*., 1996). In contrast to the results of an in-vivo exposure study in rats (Lai & Singh, 1997a), there was no significant increase in DNA strand breaks, as measured by single-cell gel electrophoresis, in cultured MO54 human brain tumour cells exposed for 30 min to strong 50- or 60-Hz fields (5–400 mT) (Miyakoshi *et al*., 2000a).

In a study examining the combined effects of ELF electric and magnetic fields and oxidative stress, human Raji cells were treated with hydrogen peroxide and simultaneously exposed to a pulsed field with a peak amplitude of 5 mT (pulse duration, 3 ms; pulse frequency, 50 Hz). Analysis of these cells by the single-cell gel electrophoresis assay showed no effect of the electric and magnetic field on the number of DNA single-strand breaks induced by hydrogen peroxide (Fairbairn & O'Neill, 1994). Similarly, exposure to 50-Hz fields (20-kV/m electric, 0.2-mT magnetic, or a combination of these) had no influence on DNA single-strand breaks (measured by alkaline elution) in Chinese hamster ovary, CCRF-CEM and McCoy's cells pre-treated with methylmethane sulfonate, potassium chromate, ultraviolet radiation or hydrogen peroxide (Cantoni *et al*., 1995, 1996).

Three studies reported on the modifying effects of exposure to ELF electric and magnetic fields on the repair of DNA damage induced in human lymphocytes by ionizing radiation. Two of the studies found no inhibition of the repair of DNA damage induced by ionizing radiation (100 Gy or 5 Gy) after post-irradiation exposure of the cells to pulsed magnetic fields (repetition rate, 50 Hz; peak intensity, 2.5 mT) or to 60-Hz magnetic or electric fields, as judged by indices of DNA rejoining and unscheduled DNA synthesis (Cossarizza *et al*., 1989a; Frazier *et al*., 1990). However, when human glioma MO54 cells were exposed to 50- and 400-mT magnetic fields for 30 min after X-ray irradiation at 4 °C (to inhibit enzymatic strand rejoining), a slight but significant increase in the number of DNA strand breaks was observed (Miyakoshi *et al*., 2000a).

Lymphocytes from male Wistar rats were exposed for 3 h to either static or 50-Hz magnetic fields at 7 mT. In some cases, hydrogen peroxide or $FeCl_2$ was added to the medium. DNA damage (single-strand breaks and alkali-labile sites) were detected using the single-cell gel electrophoresis assay. Exposure to the static or 50-Hz fields did not produce any detectable DNA damage, nor did hydrogen peroxide or $FeCl_2$ alone. However, when lymphocytes were incubated with $FeCl_2$ and simultaneously exposed to 7-mT magnetic fields, the number of damaged cells was significantly

increased and reached about 20% after exposure to static and 15% after exposure to 50-Hz magnetic fields (Zmyslony *et al*., 2000).

Micronucleus formation

Micronucleus formation was examined in human lymphocytes exposed to 50-Hz sinusoidal electric fields at 0.5, 2, 5 and 10 kV/m in air. No difference was found between the frequency of micronuclei in cultures exposed to the electric fields at any of the intensities tested and that in unexposed control cultures (Scarfi *et al*., 1993). When mitomycin C was added to the cultures, the frequency of micronuclei increased significantly, but no difference was found between field-exposed and unexposed cultures. Many studies have found no effect of sinusoidal and pulsed power-frequency fields of 30 μT to 2.5 mT on micronucleus formation (Saalman *et al*., 1991; Scarfi *et al*., 1991, 1994; Lagroye & Poncy, 1997; Scarfi *et al*., 1999). In contrast, two studies have shown positive effects. A statistically significant increase in the frequency of micronuclei in human squamous-cell carcinoma SCL II cells was observed after continuous exposure to 50-Hz magnetic fields (0.8 and 1.0 mT) for 48 h and 72 h (Simkó *et al*., 1998a). However, in a non-transformed cell line cultured from human amniotic fluid used in the same study, there was no increase in the number of micronuclei induced by similar exposure to an ELF magnetic field. Another group reported an increase in the number of micronuclei in the same cell line after horizontal exposure to a 50-Hz, 1-mT magnetic field (Simkó *et al*., 1998b). Exposure to a 50-Hz, 100-μT magnetic field for 24 h after 6 Gy γ-radiation caused a significant increase in the number of binucleated cells with micronuclei compared to exposure to γ-radiation alone (Lagroye & Poncy, 1997).

Scarfi *et al.* (1997) reported an increase in the number of micronuclei in human lymphocytes from donors with Turner syndrome when the cells were exposed for 72 h to magnetic fields pulsed at 50 Hz (peak flux density was 2.5 mT, rise time 1.2 ms, pulse width ~ 2 ms, rate of change of 1.0 T/s, and the induced electric field was estimated to be 0.05 V/m). However, they observed no change in the number of micronuclei seen in lymphocytes from either normal donors or those with Turner syndrome when these cells were exposed to sinusoidal 50-Hz magnetic fields at 1 mT for 72 h (Scarfi *et al.*, 1996).

Mutation

The Ames assay using different strains of *Salmonella typhimurium* (TA100, TA98, TA97a and TA1102) revealed no effect of exposure to ELF magnetic fields (60, 600 and 6000 Hz; 0.3 mT, for 48 h) on mutation frequency (Morandi *et al*., 1996). Juutilainen and Liimatainen (1986) found no increase of mutation in *S. typhimurium* strains TA100 and TA98 exposed to 100-Hz magnetic fields (0.13, 1.3 or 130 μT), alone or in combination with the chemical mutagens 4-nitro-*ortho*-phenylenediamine or sodium azide. Exposure of Chinese hamster cells to ELF magnetic fields for seven

days (1 μT; 50 Hz) did not cause a significant increase in the mutation frequency of the *Hprt* gene encoding the enzyme hypoxanthine-guanine phosphoribosyl transferase (Nafziger *et al*., 1993). However, exposure of human melanoma MeWo cells to a strong magnetic field (50 Hz, 400 mT) resulted in an increase in the number of mutations of this gene. When the MeWo cells were exposed to the magnetic field in an annular culture plate (diameter, 15 cm), the frequency of *HPRT* mutations increased from the centre of the plate towards the edge, indicating increased mutation frequency with increasing current density. Under conditions of inhibited DNA synthesis, no induction of mutation was observed. Specifically, there was increased mutation induction during the S phase of the cell cycle (Miyakoshi *et al*., 1996a, 1997). In a study of exposure of *Drosophila melanogaster* larvae to an ELF magnetic field (20 mT) using an annular plate, the frequency of somatic mutations increased as a function of induced current (Koana *et al*., 2001). In a direct examination of the effects of electric fields, a 10-h exposure to a 60-Hz, 10-V/m electric field induced about twice as many *Hprt* gene mutations as in sham-exposed larvae (Ding *et al*., 2001).

Exposure to a strong magnetic field (50 Hz, 400 mT) induced mutations in the *HPRT* gene of *p53*-deficient human osteosarcoma cells. These mutations were suppressed by expression of the wild-type *p53* gene introduced on a plasmid (Miyakoshi *et al*., 1998a).

In a study of the mutagenic effects of ELF fields, continuous exposure to a 60-Hz, 5-mT magnetic field for six weeks did not significantly increase the frequency of *Hprt* mutations in CHO-K1 cells (Miyakoshi *et al*., 1999).

Concomitant exposure to an ELF magnetic field (60 Hz, 3 mT) and menadione, a compound that induces the formation of free radicals, or *N*-methyl-*N*-nitrosourea, an alkylating agent, did not influence the mutation rate in the *E. coli lacI* target gene in *lacI*-transgenic rat embryo fibroblasts (Suri *et al*., 1996).

Two studies on mutation induction by combined exposure to ionizing radiation and EMF fields showed that the frequency of mutations in the *Hprt* gene induced by X-rays (3 Gy) in CHO-K1 cells was significantly increased by exposure to 5-mT ELF magnetic fields during 1–6 weeks following X-ray irradiation (Miyakoshi *et al*., 1999; Walleczek *et al*., 1999). In a third study, human glioma cells were exposed for eight days to a 60-Hz, 5-mT magnetic field following X-ray irradiation (4 Gy). The frequency of *HPRT* gene mutation was increased approximately fourfold, compared to that induced by X-rays alone (Ding *et al*., 2001). These results show that ELF electric and magnetic fields can modulate the effects of ionizing radiation.

4.4.2 *Effects relevant to non-genotoxic carcinogenesis*

(*a*) *In-vivo studies*

(i) *ELF electric and magnetic fields*

The influence of a 50-Hz magnetic field and simulated solar radiation on ornithine decarboxylase (ODC) activity and polyamines was studied in mouse epidermis. Chronic exposure of mice to combined magnetic fields and simulated solar radiation

had no persistent effects on ODC activity or polyamines in comparison with animals exposed to ultraviolet radiation alone, although the same magnetic field treatment had previously been found to accelerate skin tumour development (Kumlin *et al*, 1998b). In an acute 24-h experiment, elevation of putrescine and down-regulation of ODC activity were observed in animals exposed to a 50-Hz, 100-μT magnetic field. No effect was seen 24 h after exposure to simulated solar radiation alone (Kumlin *et al*, 1998a).

A biomarker study was conducted during an ongoing initiation–promotion assay with SENCAR mice (see section 3, Sasser *et al.*, 1998). The study focused on early biochemical changes in epidermal cells associated with skin tumour promotion, including labelling index, ODC activity, protein kinase C activity and epidermal thickness, which were obtained from animals that had been DMBA-initiated and (12-*O*-tetradecanoylphorbol 13-acetate)-promoted and subsequently exposed to a 60-Hz, 2-mT magnetic field for 6 h per day for 5 days per week. No differences were reported for ODC activity or epidermal thickness, but a significant increase in down-regulation of the activity of protein kinase C was seen in the field-exposed mice at certain times during the promotion phase (DiGiovanni *et al*., 1999). [The Working Group noted that ambient levels of protein kinase C activity in the pilot experiment were substantially lower (by a factor of 10).] The authors concluded that the inability of the 60-Hz, 2-mT magnetic field to alter early biomarkers provides further evidence for the lack of a promotional or co-promotional effect of magnetic fields seen in the tumour development assay (Sasser *et al*., 1998).

Female rats were exposed to a 50-Hz, 50-μT magnetic field for a period of six weeks, in combination with oral administration of the chemical carcinogen DMBA. In control rats, exposure to the magnetic field alone resulted in an approximate doubling of ODC activity in mammary tissue, and a significant increase in ODC activity in the spleen, but not in the liver, small intestine, bone marrow or ear skin. Combined treatment with the magnetic field and DMBA was not more effective in increasing ODC activity than treatment with DMBA alone, except in liver tissue (Mevissen *et al*., 1995).

(*b*) *In-vitro studies*

(i) *Static magnetic fields*

Cell proliferation

Fibroblasts from fetal human lung were exposed to static magnetic fields of 0.2 T, 1.0 T and 1.5 T for 1 h per day on five consecutive days. Cell cycle analyses of synchronously and non-synchronously growing cells were conducted and population doublings (parameters used to describe cell growth) were calculated. The proliferation kinetics of the cells were analysed for 21 days to rule out mid-term effects. No statistically significant differences between exposed and sham-exposed cells were observed. The calculations of population doublings did not reveal any modulation of cell growth during

exposure. Proliferation kinetics did not provide evidence of any mid-term growth modulation effects of repeated exposure to magnetic fields (Wiskirchen *et al.*, 2000).

Fibroblasts from fetal human lung were exposed to a static 1.5-T magnetic field for 1 h three times a week for three weeks. Population doublings and cumulative population doublings were calculated weekly to detect treatment-related differences in overall cell growth. No significant differences between groups were found. Clonogenic activity, DNA synthesis, cell cycle and cell proliferation kinetics were not altered by exposure to the magnetic field (Wiskirchen *et al.*, 1999).

MCF-7 human breast cancer cells were exposed for different lengths of time (5–180 min) to the static magnetic field generated by a 0.2-T magnetic resonance tomograph. This treatment significantly decreased the incorporation of [^{3}H]thymidine into DNA in these cells (Pacini *et al.*, 1999a).

Gene expression

Exposure to a static magnetic field of 0.18–0.2 T for 1–6 days did not affect the growth of HeLaS3 cells. The effects of X-rays or heat treatment, which caused a transient delay in cell growth were not enhanced by subsequent exposure to the static magnetic field. Expression of the *c-fos* oncogene was measured in the HeLaS3 cells after exposure to the magnetic field for 2–24 h. No *c-fos* mRNA was detectable in unexposed cells, but it was expressed following incubation at a temperature of 45 °C for 10 and 15 min, and the expression was further enhanced by subsequent exposure of the cells to the magnetic field for 4 h (Hiraoka *et al.*, 1992).

Signal transduction

The effects of a static magnetic field generated by a 0.2-T magnetic resonance tomograph on cultured human neuronal cells (FNC-B4) were examined. Examination of the cells by scanning electron microscopy immediately after 15 min of exposure showed a significant change in cell morphology. At the same time, thymidine incorporation and inositol lipid signalling were significantly reduced. Sham-exposed control cells or non-neuronal cells (mouse leukaemia cells, human breast carcinoma cells) were unaltered. The release of endothelin-1 from FNC-B4 cells was much reduced after exposure to the magnetic field for only 5 min. However, no field-related alterations were found in 12 different DNA microsatellite sequences selected as indicators of genome instability (Pacini *et al.*, 1999b).

Apoptosis

Static magnetic fields generated by permanent magnetic discs showed an inhibitory effect on apoptotic cell death induced by various agents such as hydrogen peroxide, heat shock, ageing in culture and dexamethasone treatment in mitogen-stimulated human lymphocytes and certain human cell lines (U937, CEM and Burkitt

lymphoma cells). For U937 cells, the reduction in apoptosis was first evident at flux densities of 0.6 mT and increased in a dose-dependent fashion until 6 mT, at which a plateau was reached that extended to 66 mT. Similar results were seen for CEM cells, but there was no anti-apoptotic effect of exposure to static magnetic fields on human lymphocytes or Burkitt lymphoma cells. Studies to test the involvement of calcium ion influx suggested that the inhibitory effect of magnetic fields on apoptosis is mediated by an enhanced influx of calcium ions from the extracellular medium. Thus, this effect would be limited to cells in which calcium influx has an anti-apoptotic effect (Fanelli *et al.*, 1999).

Exposure of the human leukaemic cell line HL60 to a 50-Hz, 45-mT magnetic field for a minimum of 1 h induced an increase in the number of apoptotic cells, but this effect was not observed in lymphocytes from human peripheral blood (Hisamitsu *et al.*, 1997; Narita *et al.*, 1997). In mouse haematopoietic progenitor cells (FDCP-mix (A4)), no alteration in the frequency of apoptosis was detected after exposure to 50-Hz magnetic fields at 6 μT, 1 mT or 2 mT for various lengths of time up to seven days (Reipert *et al.*, 1997). Rat tendon fibroblasts and rat bone-marrow osteoprogenitor cells were exposed to static magnetic fields and 60- or 1000-Hz alternating fields at flux densities of up to 0.25 mT. Various combinations of field strengths and frequencies resulted in increased apoptosis and detachment of the cells from the substratum, or in failure to attach (Blumenthal *et al.*, 1997). An increased frequency of apoptotic cells was found in a transformed human squamous-cell carcinoma line (SCL-II), but not in a non-transformed human amniotic fluid cell line after exposure to a 50-Hz field with a flux density of about 1.0 mT (Simkó *et al.*, 1998b).

Ismael *et al.* (1998) examined spontaneous and dexamethasone-induced apoptosis in thymocytes and spleen cells from mice exposed to 60-Hz magnetic fields at 0.4–1 μT or static magnetic fields of 8–20 μT. The animals were exposed continuously (24 h per day) for 12 months. The relative weights of the thymus and the spleen did not differ between control and exposed groups. Cells were isolated from both these organs, incubated with or without dexamethasone (10^{-7} M) and examined for apoptosis. Spontaneous apoptosis was not different between groups. Statistically significant increases were observed in dexamethasone-induced apoptosis only in thymocytes from animals exposed to 60-Hz, 0.4–1.0-μT magnetic fields.

The results reviewed above may suggest an increase in apoptosis in some cell types under certain experimental conditions of exposure to electric or magnetic fields, but further studies would be useful.

(ii) *ELF electric and magnetic fields*

Cell proliferation

No significant change was observed in the proliferation characteristics of Chinese hamster ovary cells exposed to magnetic fields at either 220 μT or 5 mT (Livingston *et al.*, 1991; Miyakoshi *et al.*, 1996b). Oscillatory, time-dependent changes in cell proliferation, however, have been found in SV40-3T3 mouse fibroblasts after a single

1 h exposure to a 50-Hz, 2-mT magnetic field (Schimmelpfeng & Dertinger, 1997). In another study, a 30-min exposure to magnetic fields (50 Hz, 80 μT) caused an increase in the rate of proliferation of human epithelial amnion cells, but no effects were seen with other combinations of flux density and exposure duration (Kwee & Raskmark, 1995). SV40-transformed cells derived from a healthy donor and from an ataxia telangiectasia patient were exposed to an ELF magnetic field (50 Hz, 400 mT) for 2 h after pretreatment with 6 and 4 Gy X-rays, respectively. The magnetic field had no effect on the X-ray-induced reduction of survival of either cell line (Miyakoshi *et al*., 1994). When human myelogenous HL60 cells were exposed to an ELF magnetic field (45 mT) for 1 h, apoptotic cells were observed, but the same study found no apoptosis in human peripheral lymphocytes exposed under the same conditions (Narita *et al.*, 1997).

The effects of ELF pulsed fields on cell proliferation were studied in cultured human lymphocytes from 24 young and 24 old donors (mean ages, 24 and 86 years, respectively). The pulse duration of the fields was about 2 ms and the repetition rate was 50 Hz, yielding a duty cycle of 1/10. The intensity of the magnetic field was 2.5 mT, and its average time variation of the order of 1 T/s. The maximum induced electric field was estimated to be 0.02 mV/cm. The cultures were exposed for 24–66 h, and then incubated for a further 6 h to allow incorporation of [^{3}H]thymidine. The exposure to the pulsed fields had no effect on control lymphocytes but increased the phytohaemagglutinin-induced proliferation of the lymphocytes from the two donor groups. The effect was stronger in lymphocytes from old people. These cells normally show a reduced proliferative capability, but after exposure to the pulsed magnetic field, the incorporation of [^{3}H]thymidine was similar to that observed in lymphocytes from young subjects (Cossarizza *et al*., 1989b).

The effects of rapidly changing magnetic gradient fields were examined in fetal human lung fibroblasts exposed for 2–24 h to trapezoid-shaped waveforms of 500- and 75-Hz base frequency and an amplitude of 2 mT. Proliferation of the cells was monitored for three weeks after exposure. Cell cycle analysis was carried out until 24 h after cessation of exposure to detect alterations in cell division. No differences in proliferation or cell cycle distribution between exposed and unexposed cell cultures were observed (Rodegerdts *et al.*, 2000).

A study using human breast cancer MCF-7 cells (provided by D. Blask, Cooperstown, NY) reported the effects of exposure to ELF electric and magnetic fields on cell proliferation. When these cells were exposed to a sinusoidal electric and magnetic field (60 Hz, 1.2 μT) with concomitant melatonin treatment (10^{-9} M) the proliferation-inhibiting effect of this compound was reduced (Liburdy *et al.*, 1993). The same series of studies demonstrated that exposure to ELF sinusoidal, but not full-wave rectified, electric and magnetic fields reduced the ability of tamoxifen, an agent used clinically for the treatment of breast cancer, to inhibit cell proliferation (Harland & Liburdy, 1997; Harland *et al.*, 1999). These results, with respect to both melatonin and tamoxifen, have been independently replicated by Blackman *et al.* (2001) using the same cells, provided by the Liburdy laboratory. In a study with melatonin-

insensitive MCF-7 cells, obtained from the Japanese cell bank, no effect of exposure to fields of 60 Hz, 5 mT was observed on cell growth either in the presence or absence of melatonin (Tachiiri *et al.*, 1999). [The Working Group noted that some but not all MCF-7 cell lines are responsive to melatonin; responsiveness may depend on the presence of estrogen receptors in these cells.]

Exposure of the yeast *Saccharomyces cerevisiae* to a 50-Hz, 120-μT magnetic field delayed recovery from the growth inhibition induced by ultraviolet B (UVB) radiation. The progression of the cell cycle after UVB exposure was also modified by the magnetic field (Markkanen *et al.*, 2001).

The effects of exposure to pulsed electric and magnetic fields were studied in lymphocytes from the peripheral blood of 25 patients with Down syndrome, a disorder in which premature ageing is characterized by precocious immune system derangement, including age-related defective proliferative capability of lymphocytes. After exposure to the pulsed fields, a significant increase in phytohaemagglutinin-induced cell proliferation was observed in cells from children and young adults with Down syndrome, but this phenomenon was much more evident in lymphocytes from older Down syndrome patients (Cossarizza *et al.*, 1991).

In studies that have used incorporation of [^{3}H]thymidine into nuclear DNA as an indicator of cell proliferation, the effects of exposure to electric and magnetic fields on cell growth *in vitro* have been mixed. Human fibroblasts exposed to various frequencies (15–4000 Hz) of magnetic fields (2.3–560 μT) showed enhanced DNA synthesis (Liboff *et al.*, 1984). Exposures to 0.18–2.5-mT pulsed electromagnetic fields, at specific ranges of pulse width, pulse height and pulse repetition rate, also stimulated DNA synthesis in Chinese hamster V79 cells (Takahashi *et al.*, 1986). In contrast, exposure to ELF electric and magnetic fields (1–200 Hz, 230–650 μT) inhibited DNA synthesis in phytohaemagglutinin-induced human lymphocytes (Conti *et al.*, 1983; Mooney *et al.*, 1986), whereas no significant effect on DNA synthesis was seen in HL-19 normal human fibroblasts exposed for 30 h to 50-Hz magnetic fields over a wide range of flux densities (20 μT–20 mT) (Cridland *et al.*, 1996).

Gene expression

The transcription of the oncogene *c-myc* in human leukaemia HL60 cells was enhanced by a 20-min exposure to an ELF magnetic field (150–15 Hz: maximum expression at 45 Hz, 200 μT–2.3 mT) (Goodman & Shirley-Henderson, 1991; Goodman *et al*, 1994). It was also reported that chloramphenicol transferase expression was enhanced when a specific DNA region upstream of the *c-myc* gene was transfected into human HeLa cells as a chloramphenicol transferase construct, followed by a 20-min exposure to a 60-Hz, 8-μT magnetic field (Lin *et al.*, 1994). Other studies, however, have failed to reproduce the enhancement of *c-myc* expression by exposure to ELF electric and magnetic fields (Lacy-Hulbert *et al.*, 1995; Saffer & Thurston, 1995; Balcer-Kubiczek *et al.*, 1996; Miyakoshi *et al.*, 1996b). Exposure to an ELF magnetic field

(60 Hz, 1 mT, for 75 min) caused no change in transcription rate of *c-myc* or the β-actin gene in HL60 cells, but it enhanced transcription of 45S ribosomal-RNA (Greene *et al.*, 1993). [The Working Group noted that HL60 cells display a high endogenous level of *c-myc* expression.]

In human T-lymphoblastoid cells (CEM-CM3), the transcriptional activities of *c-fos*, *c-myc* and the protein kinase C gene, which are associated with signal transduction, were increased by exposure to a 60-Hz, 100-μT magnetic field although this effect was strongly dependent on the duration of exposure and the cell density during the assay (Phillips *et al.*, 1992).

A chloramphenicol transferase gene construct containing the upstream regulatory region of the *c-fos* gene (from base pair –700 to +42) was transfected into HeLa cells. The cells were then exposed to a 60-Hz, 6-μT magnetic field for up to 40 min. An approximately 1.2-fold increase in chloramphenicol transferase-protein expression was seen 60 min after a 20-min exposure (Rao & Henderson, 1996). [The Working Group noted that the chloramphenicol transferase activity appeared to be very low, suggesting that the response of the construct was poor under the conditions tested.]

Primary and immortalized rat tracheal epithelial cells exposed for 30 min to a 50-Hz, 100-μT magnetic field displayed an approximately 3-fold enhancement of *c-jun* protein expression. In the same study, however, *c-fos* expression was decreased to approximately 70% of control levels following a 5-h exposure to the magnetic field (Lagroye & Poncy, 1998). Exposure to 60-Hz magnetic fields at 5.7 or 570 μT for 10–40 min caused no change in *c-fos* mRNA expression in HL60 cells (Balcer-Kubiczek *et al.*, 1996).

Transcription of the gene encoding the heat shock protein hsp70 was increased by about 1.8-fold in HL60 cells exposed for 20 min to a 60-Hz, 8-μT magnetic field. Increased transcription of the heat shock gene *SSA1* in the yeast *Saccharomyces cerevisiae* was observed under the same conditions (Goodman *et al.*, 1994). By means of the chloramphenicol transferase assay, the increased expression of hsp70 in HL60 cells, induced by exposure to ELF magnetic fields (60 Hz, 8 μT), was shown to be caused by enhanced binding of the *c-myc* protein to sites within the heat shock protein promoter region (Lin *et al.*, 1998a,b). However, in C3H mouse mammary carcinoma-derived 34i cells, a 20-min exposure to magnetic fields (50 Hz, 1.5 and 3 mT) had no effect on the expression of hsp70 or hsp90 (Kang *et al.*, 1998). Likewise, 2–20 h of exposure to a 50-Hz, 50-mT magnetic field had no influence on the expression of hsp70 protein in HL60RG cells. In this study, however, hsp70 expression induced by mild heat treatment (40 °C or 42 °C) could be suppressed by simultaneous application of the magnetic field (Miyakoshi *et al.*, 2000b).

The exposure of Chinese hamster ovary cells to a 50-Hz, 400-mT magnetic field caused a transient increase (maximum approximately 6 h) in the expression of the gene encoding the neuron-derived orphan receptor 1 (NOR-1); exposure to a 5-mT field had no effect (Miyakoshi *et al.*, 1998b).

Signal transduction

The focus of several investigations on the effect of electric and magnetic fields on cellular signal transduction has been the role of calcium, since it is intimately involved in the regulation of many signal transduction pathways.

Mononuclear blood cells from healthy adult volunteers were stimulated with phytohaemagglutinin and exposed to a squared waveform field (3 Hz, 6 mT). The uptake of Ca^{2+} was lower than in cells treated with phytohaemagglutinin alone (Conti *et al.*, 1985). Conversely, exposure of rat thymocytes for 60 min to an induced 60-Hz electric field of 1.0 mV/cm produced an average 2.7-fold increase in concanavalin A-dependent Ca^{2+}-uptake compared to that in unexposed, isothermal control cells (Walleczek & Liburdy, 1990). Oscillatory increases in the concentration of intracellular calcium were induced in human Jurkat cells exposed to a 50-Hz, 0.1-mT magnetic field (Lindström *et al.*, 1993). In a further study, the same cells displayed oscillations in intracellular Ca^{2+} when exposed to magnetic fields with a wide frequency range (5–100 Hz), the strongest effect being seen at 50 Hz. At this frequency, the response showed a no-effect threshold at 0.04 mT, and a plateau at 0.15 mT (Lindström *et al.*, 1995a, 1996).

Oscillations of free intracellular calcium were seen in individual Jurkat cells in response to exposure to a 50-Hz, 0.15-mT magnetic field. In contrast, a CD45-deficient Jurkat cell line did not respond to stimulation by a magnetic field. The phosphatase activity of CD45 may regulate the activity of p56lck tyrosine kinase by removing an inhibitory phosphate. By using Jurkat cells that expressed a chimeric molecule, comprising the cytoplasmic phosphatase domain of CD45, the field-induced calcium response could be restored (Lindström *et al.*, 1995b).

Exposure to magnetic fields (50 Hz, 0.1 mT) also resulted in a significant increase in the concentration of inositol 1,4,5-trisphosphate in Jurkat cells. This effect was not inhibited by chelation of intracellular calcium ions, which implies that the oscillations in calcium concentration induced by the magnetic fields were not due to direct stimulation of the calcium-dependent phospholipase C-γ1, an enzyme involved in the formation of inositol 1,4,5-triphosphate (Korzh-Sleptsova *et al.*, 1995).

A later study reported that exposure to magnetic fields (60 Hz, 0.15 mT) had no effect on intracellular calcium signalling in Jurkat E6-1 cells (Lyle *et al.*, 1997).

Exposure of HL60 cells to an electric field (60 Hz, 10–100 V/m) for 1 h significantly decreased the activity of cytosolic protein kinase C. However, no concomitant rise in membrane-bound protein kinase C activity was observed, indicating that the electric field promotes down-regulation of cytosolic protein kinase C activity (Holian *et al.*, 1996).

Changes in signal transduction events as a result of exposure to magnetic fields have been described in a number of studies with human B-lineage lymphoid cells and chicken lymphoma B-cells (DT40). Other investigators, however, have failed to replicate these findings. Some of the studies are summarized below.

Exposure of human B lymphoid cells to a magnetic field (60 Hz, 0.1 mT) stimulated various tyrosine kinases, which resulted in tyrosine phosphorylation of many proteins and subsequent activation of phosphokinase C in a time-dependent manner. Analysis of various steps in the signal transduction pathway led the authors to conclude that the growth regulation of B lymphoid cells may be altered by the activation of a specific tyrosine kinase (Lyn) by the magnetic field (Uckun *et al.*, 1995).

Exposure of DT40 chicken lymphoma B cells to a vertical magnetic field (60 Hz, 0.1 mT) resulted in the activation of phospholipase C-γ2, leading to increased turnover of inositol phospholipids. This activation is mediated by Bruton's tyrosine kinase (BTK), which was shown to be the responsive target for interaction with the magnetic field (Kristupaitis *et al.*, 1998).

In an attempt to replicate the findings described above, Miller & Furniss (1998) examined the effects of magnetic fields on wildtype DT40 cells, on BTK-deficient DT40 cells and on BTK-deficient cells that had been reconstituted with the human *BTK* gene. The cells were all obtained from the Uckun laboratory. No effects were seen on production of inositol-1,4,5-trisphosphate, BTK-activation or tyrosine phosphorylation after exposure of these cells to 60-Hz, 0.1-mT magnetic fields. The authors suggest that the conflicting results may be due to some critical parameter in the exposure environment that is different between laboratories.

In a further study aimed at replication of previous findings, Woods *et al*. (2000) exposed human B lymphoid cells (obtained from Uckun's laboratory) and chicken lymphoma DT40 cells (obtained from Miller's laboratory, but originally from Uckun) to a 60-Hz, 0.1-mT magnetic field, with or without a parallel, static magnetic field of 0.046 mT. No significant changes were detected in tyrosine phosphorylation or in activation of Lyn and Syk tyrosine kinases in either cell line.

Exposure of β-galactosidase-transfected PC12-VG cells stimulated by forskolin to a 400-mT magnetic field for 4 h enhanced β-galactosidase expression. This enhanced expression was significantly inhibited by calcium entry blockers and almost completely suppressed by concomitant treatment with calphostin C, a protein kinase C inhibitor (Ohtsu *et al.*, 1995). The induction of expression of the neuron-derived orphan receptor (NOR-1) gene by exposure to a 50-Hz, 400-mT magnetic field was also inhibited by treatment with various Ca^{2+} influx inhibitors (Miyakoshi *et al.*, 1998b).

Ornithine decarboxylase activity is controlled by a signal transduction pathway associated with cell proliferation. In a study using human lymphoblastoid cells (CEM), mouse myeloma cells (P3) and rat hepatoma cells (Reuber H35), exposure to 60-Hz electric fields (10–1000 V/m) caused a transient, several-fold increase in the activity of ornithine decarboxylase (Byus *et al.*, 1987). A twofold increase in the activity of this enzyme was also seen in mouse L929 cells exposed to a 60-Hz magnetic field (1–100 μT) (Litovitz *et al.*, 1991). The same group also showed a dose–response relationship with a consistently elevated activity of ornithine decarboxylase at flux densities > 4 μT (Mullins *et al.*, 1999). However, two studies designed to replicate this result with L929 cells from the same or a different source, failed to find a significant

change in the activity of ornithine decarboxylase as a result of exposure to electric or magnetic fields (Azadniv *et al.*, 1995; Cress *et al.*, 1999).

Three mammalian tumour cell lines (human promyelocytic leukaemia HL60 cells, mouse ascites tumour ELD cells and mouse teratocarcinoma F9 cells) were used to determine the effects of exposure to magnetic fields on ornithine decarboxylase gene expression. All cell lines showed elevated levels of activity of the enzyme when exposed during culture to a 50-Hz, 30-μT vertical sinusoidal magnetic field for 24, 48 or 72 h. The increase ranged from about 20% in HL60 cells to up to five- to sixfold in ELD cells compared to the controls. The effect was stronger at later stages of growth, when the inherent activity of ornithine decarboxylase is lower (Mattsson & Rehnholm, 1993).

Two lymphoblastic leukaemia cell lines of human origin, Jurkat cells and CEM-CM3 cells, were exposed to horizontal or vertical magnetic fields (50 Hz, 0.10 mT). Exposure to the vertical magnetic field for 3 h or 3 days increased the activity of ornithine decarboxylase in the Jurkat cells by 77% and 47%, respectively. Only a small effect of exposure to the horizontal magnetic field was seen, perhaps due to the lower intensity of the induced electric field. However, the CEM-CM3 cells did not respond to either type of exposure (Valtersson *et al.*, 1997).

Growth factors and differentiation

The effects of pulsed electric and magnetic fields on mitogen-stimulated lymphocytes from aged human volunteers (mean age, 88 years) were studied by measuring the production of interleukin-2 and the expression of interleukin-2 receptor in these cells. The pulse duration of the magnetic field was about 2 ms, the repetition rate 50 Hz, the intensity 2.5 mT, and the average time variation was of the order of 1 T/s. The maximum induced electric field was estimated to be 0.02 mV/cm. Control cultures were maintained in the same incubator in a position where no electric or magnetic field was detectable. [The Working Group noted the close proximity of the control and the exposed samples.] Cultures were exposed for 18 h for evaluation of interleukin-2 receptor-positive cells and percentage of T-activated lymphocytes, and for 24 and 48 h for examining the production of interleukin-2. In exposed cultures that showed increased [^{3}H]thymidine incorporation compared with unexposed controls, the production of interleukin-2 was lower, but the percentages of interleukin-2 receptor-positive cells and of T-activated lymphocytes were increased (Cossarizza *et al.*, 1989c).

A study of the ability of nerve growth factor-stimulated PC-12 cells, derived from the rat adrenal medulla, to produce neurites under a variety of conditions of exposure to magnetic fields, was designed to establish those field parameters critical for production of biological effects. Twenty-three hours of exposure both to sub-optimal concentrations of nerve growth factor and to a flux density series of vertical, 45-Hz magnetic fields demonstrated reduced neurite outgrowth at flux densities between 5 and 10 μT, where the inhibition reached a plateau. The cell response at the periphery

of culture dishes of different diameter was identical to that at the centre of the dishes indicating that the induced electric current was not responsible for the effect (Blackman *et al*., 1993). The frequency response, which was tested from 15 Hz–70 Hz for each of six flux densities (3.5–9.0 μT), displayed frequency-specific profiles of inhibition of neurite outgrowth (Blackman *et al*., 1995). Trillo *et al*. (1996) showed that different specific flux densities of static and alternating magnetic fields (at 30 Hz, 0.79–2.05 μT alternating and 1.97 μT static; at 45 Hz, 0.29–4.11 μT alternating and 2.96 μT static) could produce a characteristic, but slightly different inhibition response, in which a narrow flux density region around the value that had produced maximal inhibition, displayed no inhibition. The effects observed using 45-Hz fields were not seen when the static field was reduced to 1.97 μT. Blackman *et al*. (1999) tested the frequency dependence of the findings of Trillo *et al*. (1996) using flux densities for maximum effects at 45 Hz and observed a maximal inhibition at 45 Hz with lesser inhibition at 42.5 and 47.5 Hz, and no inhibition at 40 and 50 Hz. Blackman *et al*. (1996) showed that the neurite outgrowth response changed from field-induced inhibition to enhancement when the static magnetic field was changed through a series of angles from parallel to perpendicular to the alternating magnetic field. In a study based on the work of Blackman and colleagues, McFarlane *et al.* (2000) observed a field-induced (50 Hz, 4–8 μT) inhibition (~ 22%) of neurite outgrowth in PC-12 cells cultured in 15% serum (weakly differentiating conditions) and enhancement (~ 17%) of the outgrowth in cells cultured in 4% serum (strongly differentiating conditions). No significant changes were observed at higher or lower flux densities.

A Friend erythroleukaemia cell line that can be chemically induced to differentiate was used to determine whether magnetic fields could alter cell proliferation and differentiation in a manner similar to that of a chemical tumour promoter. Exposure of this cell line to 60-Hz fields resulted in a dose-dependent inhibition of differentiation, with a maximal inhibition of 40% at 4 μT. Exposure at 2.5 μT caused a 20% inhibition while a 1-μT field was ineffective. At flux densities in the range of 0.1–1 mT, cell proliferation was stimulated up to 50% above that of sham-treated cells. The activity of telomerase, a marker of undifferentiated cells, decreased 100-fold when the cells were induced to differentiate under sham conditions, but only 10-fold when the cells were exposed to a 50-μT magnetic field. In summary, exposure to ELF electric and magnetic fields appears to partially block the differentiation of Friend erythroleukaemia cells, and this results in a larger population of cells remaining in the undifferentiated, proliferative state, which is similar to results obtained with chemical tumour promoters (Chen *et al*., 2000).

Intercellular communication

Ubeda *et al*. (1995) observed that the increased gap-junctional intercellular communication induced in C3H10T1/2 mouse embryo cells by physiological concentrations of melatonin, could be completely eliminated when the cells were exposed for 1 h to vertical, 50-Hz, sinusoidal magnetic fields at 1.6 mT.

Li *et al.* (1999) exposed Chinese hamster lung cells to the tumour promoter TPA alone or in combination with a 50-Hz magnetic field. Combined treatment with 5 ng/mL TPA for the last hour of a 24-h period of exposure to magnetic flux densities of 0.2, 0.4 or 0.8 mT, significantly inhibited gap-junctional intercellular communication compared with TPA treatment alone. The inhibition was dependent on the flux density.

Gap-junctional intercellular communication was also studied in clone 9 cells treated with 2.5 mM chloral hydrate for 24 h prior to exposure to a 45-Hz, 23.8-μT magnetic field, in parallel with a 36.6-μT static magnetic field for 40–45 min. There was no statistically significant effect of exposure to the magnetic field on gap-junctional intercellular communication (Griffin *et al.*, 2000).

Cell transformation

In a study using anchorage-independent growth as an index, mouse epidermal JB6 cells (clone 41) were exposed to magnetic fields (60 Hz, 1, 10 and 100 μT) for eight or 14 days, resulting in a 1.2–3.2-fold increase in colony-forming efficiency of transformants (West *et al.*, 1996). In contrast, in a co-culture of C3H10T1/2 mouse fibroblasts and mutant daughter 10e cells, intermittent exposure to an ELF magnetic field (60 Hz, 100 μT, 1 h, four times a day) for 28 days caused no increase in focus formation. In the same culture system, however, concomitant exposure to the magnetic field and treatment with TPA (10–100 ng/mL) caused a significant increase in focus formation (by an average of 150%) compared with that in cell cultures treated with TPA alone (Cain *et al.*, 1993).

There are reports suggesting that ELF electric and magnetic fields have no effect on cell transformation. In a soft-agar assay, 60-Hz magnetic fields of 0.01, 0.1, 1.0 or 1.1 mT flux density did not induce anchorage-independent growth of mouse epidermal JB6 cells, enhance TPA-induced transformation, increase the maximum number of transformed colonies or produce a shift in the dose–response curve (Saffer *et al.*, 1997). Similarly, in another study, continuous exposure to a magnetic field of 60 Hz, 200 μT for 24 h showed no effect in two transformation systems (Syrian hamster embryo cells and CH310T1/2 clone 8) with or without post-treatment with TPA (Balcer-Kubiczek *et al.*, 1996).

Cultures of primary Syrian hamster dermal cells were continuously exposed to power-line frequency magnetic fields of 10, 100 and 1000 μT for 60 h, with or without prior exposure to an immortalizing dose (1.5 Gy) or a non-immortalizing dose (0.5 Gy) of ionizing radiation. Exposure to the magnetic field alone did not immortalize these cells at a detectable frequency (1×10^{-7} or higher) or enhance the frequency of immortalization induced by ionizing radiation (Gamble *et al*, 1999). The lack of cell-transforming activity of pulsed electric and electric and magnetic fields had previously been shown in a BALB/3T3 cell transformation assay (Jacobson-Kram *et al.*, 1997).

CH310T1/2 clone 8 cells were exposed for 24 h to strong magnetic fields (5–400 mT, 60 Hz) to investigate a change in transformation frequency as analysed by

focus formation. No significant increase in transformation frequency was seen after exposure to the magnetic fields alone, but exposure to 3 Gy X-rays followed by exposure to the magnetic fields for 24 h decreased the transformation frequency in comparison with exposure to X-rays alone. In addition, long-term exposure for six weeks at 60 Hz, 5 mT significantly suppressed both spontaneous and X-ray-induced transformation (Miyakoshi *et al.*, 2000c).

4.5 Mechanistic considerations

Limited data are available on the effects of static fields alone. Therefore, the following considerations of a possible mechanism will address primarily ELF electric and magnetic fields.

It is widely agreed that certain alterations in the genetic structure of the cell are causally related to cancer. There is little experimental or theoretical evidence that mutations could be directly caused by ELF magnetic fields. The results of most genetic toxicology studies of ELF magnetic fields have been negative. However, a single laboratory has reported that exposure of human cells to extremely high ELF flux densities ($\geq$ 400 mT), which far exceeds the field intensities encountered in residential or occupational environments, induces sister chromatid exchange, chromatid-type aberrations and mutation in the *HPRT* gene.

It is also relevant to ask whether ELF electric and magnetic fields have effects similar to those of known 'non-genotoxic' carcinogens, 'tumour promoters' or 'co-carcinogens', i.e. agents that seem to enhance cancer by a mechanism other than that of direct DNA damage.

There is little evidence that ELF electric or magnetic fields can cause malignant transformation of cells in culture. There have been relatively few studies of the effects of ELF electric and magnetic fields on DNA repair or genomic stability in mammalian cells, and the results are inconclusive. There is some evidence for an effect of magnetic fields on cellular kinetics: few studies using in-vitro systems have shown enhancement of apoptosis. The results of studies on cell proliferation using a variety of exposure conditions and cell types have varied from inhibition to enhancement. The cell-proliferation response to physical and chemical factors has also been reported to be altered by exposure to ELF magnetic fields. The available experimental evidence suggests that ELF electric or magnetic fields are not cytotoxic.

The effects of ELF electric and magnetic fields on signal transduction have been reported to include changes in intracellular calcium levels and protein phosphorylation, but a number of studies have reported negative findings. These results cannot be used to identify plausible cancer-related pathways.

Several research groups have reported changes in gene expression resulting from exposure to ELF magnetic fields. However, other studies have failed to replicate many of these results.

In relation to both the genotoxic and non-genotoxic cellular and molecular end-points that have been studied, many of the data concern changes evoked following a combined exposure: that is, experiments involving (electric or) magnetic fields together with other agents. There is only weak evidence that ELF magnetic fields potentiate the effects of chemical agents, or ionizing or ultraviolet radiation.

The risk for cancer can also be enhanced through systemic effects in humans or animals. For example, it has been suggested that the hormone melatonin may suppress mammary cancer through hormonal mechanisms; anticarcinogenic effects through free-radical scavenging have also been proposed. Several human and animal studies have investigated possible suppressing effects of ELF magnetic fields on melatonin, but the results are equivocal. Although most experimental studies of the possible immunotoxicity of exposure to magnetic fields have yielded negative results, effects on T-cell proliferation capacity in animals have been reported. However, the effects are inconsistent.