

7 CELLULAR AND ANIMAL STUDIES

7.1 *In vitro* studies

Studies carried out at the cellular level are often used to investigate mechanisms of interaction with EMFs, but these are not generally taken alone as evidence of *in vivo* effects. There are a number of reasons for this. Cells in culture are removed from the normal constraints of *in vivo* growth, and quite often the cell lines used are derived from various types of cancer because of their ability to grow for long periods in culture. AGNIR (2001) noted that cellular studies are often used as a pre-screen to identify agents that are relatively inexpensive and rapid and are thus suitable for entry into long-term testing on animals or in human studies.

The studies reviewed in this section concern static magnetic field effects, including, for completeness, studies carried out in combination with time varying magnetic fields. Static electric fields generate a surface electric charge (see chapter 5, introduction) and are not appropriately studied *in vitro*.

7.1.1 Cell free systems

The number of options available for biological systems to detect magnetic fields increases with larger, more complex structures and the consequent greater possible specialization of putative transduction processes. As sub-systems of cells are addressed, or even cell-free biochemical reactions, it is necessary to consider strictly molecular-based interaction mechanisms, particularly in experiments conducted in suspension or in solution.

7.1.1.1 Membrane structure

Liburdy et al. (1986) studied lipid membrane breakdown as a function of temperature and magnetic field exposure. They found that a threshold field of 15 mT was able to shift the phase transition point to slightly lower temperatures, but the study is weakened by the lack of sham exposure. A direct application of a model based on diamagnetic anisotropy suggests that this threshold is about two magnitudes too low for overcoming the thermal noise. A theoretical paper (Tenforde & Liburdy, 1988) suggested a catastrophic model of membrane breakdown at close to the lipid phase transition temperature that accounts for this sensitivity to the magnetic field.

7.1.1.2 Enzyme activity

The possibility that magnetic field exposure might directly affect enzyme kinetics has long been of interest, but such studies are fraught with difficulty. The dependence of Ca²⁺-calmodulin-dependent myosin

phosphorylation on calcium concentration and various magnetic field characteristics was extensively studied by Markov and co-workers (1992; 1993) in a model originally developed by Shuvalova et al. (1991). The study by Bull et al. supported the observation that static magnetic fields affect Ca^{2+} -calmodulin-dependent reactions (Bull et al., 1993). A replication of some of these results was attempted (Coulton et al., 2000), but was not successful. The causes of the failure to reproduce the magnetic field effect are not known. It is possible that the calcium concentration in the reaction is a confounding variable between experiments, but other explanations are also possible. Engström et al. (2002) used this assay and found that a combination of static magnetic field intensity and gradient was able to influence the rate of phosphorylation.

A related experiment investigating cyclic nucleotide phosphodiesterase (Liboff et al., 2003) also appeared to be sensitive to very low magnetic fields, although this report may have used inappropriate statistical methods for evaluating exposure/control measurements.

Nossol et al. (1993) studied effects of static magnetic fields in the range of 50 μT - 100 mT on the redox activity of cytochrome-C oxidase. Static magnetic fields resulted in significant changes of up to 90% of overall activity only at 300 μT and 10 mT. No effects were observed at other flux densities. The observed effects were reversible. These data suggested that effects of static magnetic fields might be observed only at specific 'flux density windows'.

Taraban and Leshina (1997) investigated the reduction of hydrogen peroxide by horseradish peroxidase. Changes in catalytic rates of up to 30% were found for fields up to 0.3 T.

Small changes were also observed in plasmin activity at fields of 8 T, with a threshold of around 4 T for the magnetic field effects (Iwasaka et al., 1994).

7.1.1.3 Radical pair chemistry

Some clear demonstrations are available of how enzyme-catalysed metabolic reactions that involve a short-lived radical pair as an intermediate can be modulated with magnetic fields (Harkins & Grissom, 1994; Mohtat et al., 1998). (See section 5.3.) Demonstrations of the field effect of free radical recombination rates have also been reported by Eveson et al. (2000). Two of these studies used laser flash photolysis to show consistency with free radical theory for the field effect at approximately 2 mT (Eveson et al., 2000) and for hyperfine interactions in the 100 mT range (Mohtat et al., 1998).

Radical chemistry also offers clear mechanistic options as demonstrated in B₁₂ ethanolamine ammonia lyase by Harkins and Grissom (1994). The basic result was replicated, but two other coenzyme B₁₂-dependent enzymes (human and bacterial-derived, respectively) were found not to be magnetic field sensitive (Taoka et al., 1997) despite broad similarities between the chemical structures.

7.1.1.4 Crystallization of biologically relevant molecules

Some clear demonstrations are available that static magnetic fields, in the range of 0.1 - 10 T, affect crystallization of proteins and cholesterol (Sato et al., 2000; Sundaram et al., 2002). It is important to note that the crystallisation may be relevant to biology because some cellular structures such as complexes of DNA-protein-RNA in nuclei may possess the properties of liquid crystals.

Table 7. Cell free systems					
Authors	System	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Liburdy et al., 1986)	Liposome vesicles	Permeability	0.01 - 7.5 T 15 min	Increased permeability ED ₅₀ = 15 mT. Threshold two orders of magnitude too low for a mechanism based on diamagnetism.	
(Markov et al., 1993) (Markov et al., 1992)	Myosin light chain kinase and calmodulin isolated from turkey gizzard	Myosin phosphorylation	0 - 200 μ T 2 - 15 min	Differential response for controls, AC, DC, and AC+DC exposure. Dose response to fine scan of vertical DC field.	Assay of interest, because Ca ²⁺ - calmodulin binding is a probable field target.
(Coulton et al., 2000)	Myosin	Radiolabeled ATP into 20 kDa light chain myosin. Myosin phosphorylation	0 - 400 μ T 5 or 10 min	No effect of magnetic fields on rate of phosphorylation	Not clear what calcium concentration was required to observe the phosphorylation effect.

(Engström et al., 2002)	Myosin light chain (and kinase) from turkey gizzard	Myosin phosphorylation	0.7 - 86 mT 5 min	Increased phosphorylation, but not fully explained by SMF.	Magnetic field gradients played a specific role in the outcome of this experiment.
(Liboff et al., 2003)	Chemicals from commercial providers	Calmodulin-dependent cyclic nucleotide phosphodiesterase activity	17 - 24 μ T 30 min	Calmodulin-dependent cyclic nucleotide phosphodiesterase activity is activated by 19.8 μ T SMF. Field and Ca ²⁺ concentration consistent with previous experiments.	Data are very noisy. Incorrect statistical analysis used to test data.
(Nossol et al., 1993)	Cytochrome-C oxidase isolated from beef heart	Cytochrome-C oxidase activity	50 μ T - 100 mT up to 100 sec	Significant changes up to 90% of overall activity only at 300 μ T or 10 mT.	Effects observed in specific 'windows' of magnetic intensity around 0.3 and 10 mT.
(Taraban & Leshina, 1997)	Horseradish peroxidase	H ₂ O ₂ reduction	up to 0.3 T	Changes in catalytic rates of up to 30%.	
(Iwasaka et al., 1994)	Plasmin	Enzymatic activity	0 - 8 T 5 - 80 min	Slight changes (5 -10%) in plasmin activity. Threshold of observed effects at 4 T.	Neither statistical analysis, nor amount of independent experiments provided.
(Harkins & Grissom, 1994)	B ₁₂ ethanol-amine ammonia lyase	Free radical recombination rates, enzyme kinetics	0.1 - 0.15 T	~25% decrease of V _{max} /K _m for B ₁₂ ethanol-amine ammonia lyase around 0.1 T with unlabelled ethanol-amine; decrease ~60% around 0.15 T with perdeuterated ethanolamine.	Neither statistical analysis, nor amount of independent experiments provided.

(Mohtat et al., 1998)	Human and bovine serum albumin, calf thymus DNA	Benzophenone recombination, ketyl radicals	up to 150 mT up to 10 μ s	Change in lifetime of radicals. Consistent with theory of free radical recombination in containment.	
(Eveson et al., 2000)	Purely chemical experiment	Benzophenone ketyl radical concentration after flash stimulation	0 - 11 mT 9 μ sec	Observation of predicted low field effect in free radical recombination rates. Maximum response at 2 mT.	A pulsed magnetic field was applied.
(Taoka et al., 1997)	Coenzyme B ₁₂ – dependent enzymes	Coenzyme B ₁₂ -dependent rearrangement reactions catalysed by bacterial enzyme, ethanolamine ammonia lyase and human enzyme, methylmalonyl-CoA mutase	0 - 250 mT up to 5 h	No effect on enzyme kinetics greater than about 15%. B ₁₂ -dependent methyl-malonyl-CoA mutase not a likely transduction target for SMF in the studied field range.	Small differences between controls and SMF at some flux densities. However, no statistical analysis described and number of experiments not reported.
(Sato et al., 2000)	Chicken egg-white lysozyme	Crystallization	10 T 11 d	Enhancement in the perfection of lysozyme crystals.	No statistical analysis.
(Sundaram et al., 2002)	Anhydrous cholesterol	Cholesterol solubility and supersaturation in various solvents	100 mT up to 8 h	Induction time of cholesterol crystallization decreases in a magnetic field for all examined solvents. No changes in morphology due to field exposure.	Counter changes in induction period times did not result in change in the interfacial energy. Neither statistical analysis nor sham-exposure performed.
(Bull et al., 1993)	Calmodulin-dependent cyclic nucleotide phospho-diester-ase (PDE)	Activity of PDE	16.9 - 23.7 μ T 30 min	13% stimulation of PDE activity in samples exposed to SMF in the range from 19.2 to 20.4 μ T.	No statistical analysis described.
Studies considered to be uninformative					

(Chiles et al., 1989)

(Bras et al., 1998)

(Liu et al., 2005)

7.1.2 Magneto-mechanical effects on macromolecules and cells

Macromolecules and structurally ordered molecular assemblies with a high degree of magnetic anisotropy will experience a torque in a uniform magnetic field and they will rotate until they reach an equilibrium orientation that represents a minimum energy state (ICNIRP, 2003). Macromolecules that exhibit this property, such as DNA, generally have a cylindrical symmetry. Magneto-orientation occurs as a result of the anisotropy of the diamagnetic susceptibility tensor along the axial and radial coordinates. The extent to which these molecules orient is a function of their magnetic interaction energy relative to the Boltzmann thermal energy.

The extent of orientation of individual molecules in strong magnetic fields is very small for individual macromolecules. For example, optical birefringence measurements on calf thymus DNA in solution have demonstrated that a field of 13 T is required to produce orientation of 1% of the molecules (Maret et al., 1975). In contrast, there are several examples of molecular assemblies that can be completely oriented by fields on the order of 1 T (Tenforde, 1985). These assemblies behave as structurally coupled units in which the summed magnetic anisotropy is large, thus giving rise to a large magnetic interaction energy.

An example of an intact cell that can be oriented magnetically is the deoxygenated sickle erythrocyte. It has been shown that these cells, in which the deoxygenated haemoglobin is paramagnetic, will align in a 0.35 T static field with the long axis of the sickle cell oriented perpendicular to the magnetic flux lines (Murayama, 1965). In a follow-up to this experiment, Brody et al. (1985) investigated the magnetic resonance imaging of patients with sickle-cell disease. They studied flowing sickle erythrocytes in the presence and absence of a magnetic field of 0.38 T and found that sickle erythrocytes that were maintained under full deoxygenation exhibited a marked alignment, perpendicular to the magnetic field, even while flowing. It was suggested that orientated sickle erythrocytes could have difficulty negotiating capillary branch points.

In a series of experiments, Higashi and co-workers (1993; 1995; 1996) also studied the orientation of erythrocytes in the presence of a magnetic field of up to 8 T. They found that the erythrocytes were oriented with their disk plane parallel to the magnetic field direction.

These erythrocytes were even influenced by 1 T and almost 100% of them were oriented when exposed to 4 T. The degree of orientation was not influenced by the state of haemoglobin (oxygenated haemoglobin is diamagnetic; deoxygenated haemoglobin and methaemoglobin are paramagnetic). The data concurred with the theoretical equation for the magnetic orientation of diamagnetic substances. In contrast, glutaraldehyde-fixed erythrocytes were oriented perpendicular to the magnetic field. This was attributed to the paramagnetism of the membrane-bound methaemoglobin.

Emura et al. (2001) studied fixed bull sperm in a magnetic field of up to 1.7 T. The sperm became increasingly oriented perpendicular to the field, reaching 100% at about 1 T. Diamagnetic cell components (cell membrane, DNA in the head, microtubule in the tail) were thought to contribute to the orientation.

Hirose et al. (2003a) exposed human glioblastoma cells to a 10 T magnet in the presence and absence of collagen. Only cells embedded within the collagen gel and the collagen fibres were affected, and these were oriented perpendicular to the magnetic field. The effect was attributed to the arrangement of microtubules under the influence of magnetically oriented collagen fibres. The orientation of both rat Schwann cells and mouse osteoblasts was influenced by 8 T, in the presence as well as in the absence of collagen fibres (Eguchi et al., 2003). Without collagen fibres, it took 60 h to orient the cells parallel to the magnetic flux lines. Exposure in the presence of fibres resulted in perpendicular alignment after only 2 h. This resulted from perpendicular orientation of the collagen fibres, followed by growth of the cells along the fibres. This mechanism is thought to be potentially helpful in tissue regeneration.

Iwasaka et al. (2003) investigated the orientation of rat smooth muscle cells after exposure to 8 - 14 T for 60 h. Effects were seen for fields > 8 T, where circular spots of proliferating cells became elliptical. The same researchers (Iwasaka & Ueno, 2003) studied the effect on intracellular components in another study. A 2 - 3 h exposure to 14 T induced a change in transmission of polarized light, indicating an orientational effect on macromolecules in the cells. This agrees with the above-described observed effects on collagen molecules.

Okazaki and co-workers (1988; 1991) studied the flow and sedimentation rate of erythrocytes in the presence of a magnetic field. The presence of the magnetic field (up to 0.3 T) redistributed the flow of erythrocytes (depending on the magnetic properties of the solution) and caused an increase in the sedimentation rate of erythrocytes in a vertical cylinder.

Iino (1997; 2001) found an increased erythrocyte sedimentation rate specifically for anisotropic erythrocytes in the presence of a 6.3 T

field. This was attributed to an increased cell aggregation and was said to result from an increase in intermembrane adhesive area due to the magnetic orientation of the anisotropic erythrocytes.

Pacini et al. (1999b) investigated the effect of a 0.2 T static field on human neurons (FNC-B4), using human breast carcinoma cells (MCF-7) and murine leukaemia cells (WEHI-3) as non-neuron controls. Following a short exposure (0.25 h), the neuron cells became elongated and formed vortexes of cells. Exposed cells showed branched neurites and an increase of synaptic connections. Controls did not show any changes.

Exposure of human malignant melanoma cells (Short et al., 1992) to a 4.7 T superconducting magnet resulted in a decrease in adhesion of the cells. Normal human fibroblasts showed no effects. The magnetic field had no effect on cell numbers or viability of either the melanoma cells or the non-tumour controls. Danielyan et al. (1999) also investigated the effect of a magnetic field (0.2 T) on cancer tissue (breast cancer) and found that the magnetic field had a dehydrating effect on the cancer cells.

No changes in orientation, distribution or activity (alkaline phosphatase production) were observed by Papadopulos et al. (1992) in osteoblast cell cultures exposed to a static field of 178 mT.

Testorf et al. (2002) studied exposure of melanophores to both 8 and 14 T fields for times of up to 5 h. They found no statistically significant changes in aggregation.

Yano et al. (2001) exposed roots of radish seedlings to a static magnetic field from a small permanent magnet. The roots grew in a magnetic field gradient of 1.8 - 14.7 T m⁻¹, away from the magnet. The response to the south pole of the magnet was significant, while that to the north pole was not.

Table 8. Magneto-mechanical effects					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Murayama, 1965)	Erythrocytes	Orientation	0.35 T	Sickle erythrocytes orient perpendicular to the magnetic lines of force.	Number of independent experiments not described. No statistical analysis.

(Brody et al., 1985)	Erythrocytes	Orientation	0.38 T (flowing system: < 0.1 h)	Deoxygenated sickle erythrocytes in flowing suspension align perpendicular to a magnetic field.	SMF may affect capillary blood flow.
(Higashi et al., 1993)	Human erythrocytes	Orientation	1 - 8 T 0.5 - 2 h	Erythrocytes oriented with disk plane parallel to SMF direction. Influenced by a 1 T field and almost 100% oriented when exposed to 4 T.	
(Higashi et al., 1995)	Human erythrocytes	Orientation	0.5 - 8 T 1 h	Intact erythrocytes oriented within 5 s with disk planes parallel to SMF. Glutaraldehyde-fixed erythrocytes oriented perpendicular to SMF. State of haemoglobin had no effect on degree of orientation.	
(Higashi et al., 1996)	Glutaraldehyde-fixed erythrocytes	Orientation	1 - 8 T 1 hour	Orientation of glutaraldehyde-fixed erythrocytes with disk plane perpendicular to SMF. Effect depends on field intensity.	
(Emura et al., 2001)	Glutaraldehyde-fixed bull sperm	Orientation	1.7 T 10 min	Very strong orientation in comparison with erythrocytes or platelets. Orientation increased sigmoidally as function of SMF intensity and reached 100% at just below 1 T.	No statistical analysis, but effect very obvious. Number of experiments not indicated.

(Hirose et al., 2003a)	Human glioblastoma cells	Orientation, cell viability	10 T 1 h or 7 d	Cells embedded in collagen gel oriented perpendicular to direction of the SMF, due to arrangement of microtubules under influence of magnetically oriented collagen fibres. No specific orientation in cells not exposed or cultured in absence of collagen.	Number of experiments not described. No quantitative data nor statistical analysis. Descriptive study.
(Eguchi et al., 2003)	Rat Schwann cells	Orientation	8 T 2 or 60 h	Orientation parallel to 8 T SMF after 60 h. In collagen: after 2 h SMF exposure alignment perpendicular, along aligned collagen fibres.	
(Kotani et al., 2000)	Mouse osteoblasts	Cell orientation	8 T 60 min, 14 d	Collagen from osteoblasts aligns parallel to SMF; incubation of osteoblasts + collagen results in perpendicular orientation.	
(Iwasaka et al., 2003)	A7r5 rat smooth muscle cells	Orientation	8, 12, 14 T 60 h	Circular spots of proliferated cells became elliptical in presence of SMF. Ellipticity significant for fields > 8 T.	
(Iwasaka & Ueno, 2003)	Rat smooth muscle cells	Orientation of intracellular components	14 T 2 - 3 h	SMF induces change in polarized light intensity through lamellar cell assembly; corresponds to behavioural changes in cell components.	Descriptive study of limited value: only a single experiment with one Petri dish.

(Okazaki et al., 1988)	Human erythrocytes	Flow of erythrocyte suspension	110 - 300 mT	Inhomogeneous SMF redistributed flow of erythrocytes dependent on magnetic properties of erythrocytes and haematocrit concentration.	
(Okazaki et al., 1991)	Human erythrocytes	Sedimentation rate	110 - 300 mT up to 3 h	Higher sedimentation rate of paramagnetic erythrocytes with inhomogeneous SMF.	Number of independent experiments not described. No statistical analysis, but rate differences calculated
(Iino, 1997)	Human erythrocytes	Erythrocyte sedimentation rate (ESR) and aggregation	6.3 T vertical, exposures up to 1 h	SMF enhances ESR. Effect in plasma (> 20 min), not in saline solution. Increase in size of aggregates.	No statistical analysis for increase in size of aggregates.
(Iino & Okuda, 2001)	Human erythrocytes	Erythrocyte sedimentation rate (ESR) and aggregation	6.3 T up to 3 h	ESR only enhanced in anisotropic erythrocytes.	No statistical analysis for increase in size of aggregates.
(Pacini et al., 1999b)	Normal human neuronal cell culture (FNC-B4); mouse leukaemia; human breast carcinoma cells	Morphology, cell proliferation, production of endothelin-1, genome instability	0.2 T 5 - 15 min	No alterations in genome instability; dramatic changes of morphology only in neuronal cells. Significant decrease in cell proliferation, changes in production of endothelin-1.	
(Short et al., 1992)	Human melanoma cells; normal fibroblasts	Cell number, adhesion, and viability	0.5, 2, 4.7 T 12 - 72 h	Adhesion of melanoma cells diminished. No effect on normal fibroblasts.	No statistical analysis. Number of experiments not specified.

(Danielyan et al., 1999)	Human breast cancer and normal glandular tissues	Water content in tissues, ouabain ³ H binding	0.2 T 1 h	Decrease or increase of ouabain binding at low or high concentrations, respectively. Decrease in hydration of cancer tissues.	
(Papadopoulos et al., 1992)	Rat osteoblasts	Activity	178 mT 21 d	No effect.	
(Testorf et al., 2002)	Fish melanophores	Aggregation	8, 14 T up to 5 h	No effect on aggregation except for statistically significant irregularity in the speed of aggregation under exposure to 8 T.	8 T and 14 T experiments performed in different seasons. Significant difference between control groups for 8 T and 14 T.
(Yano et al., 2001)	Primary roots of radish (<i>Raphanus sativus</i>) seedlings	Tropism	13 - 68 mT 24 h	Roots responded tropically to SMF field gradient of 1.8 - 14.7 Tm ⁻¹ ; significant response to south pole of magnet.	
Studies considered to be uninformative					
(Hong et al., 1971) (Malinin et al., 1976) (Pate et al., 2003)					

7.1.3 Cellular metabolic activity

Effects on enzyme activity may well lead to changes in cellular metabolic activity. Lysozymal degranulation and cell migration in response to static magnetic field exposure at 0.1 T for 30 min were studied in human polymorphonuclear leucocytes (PMNs) (Papatheofanis, 1990). Time dependent effects on enzymatic activity, such as increased release of lysozyme and lactate dehydrogenase (degranulation), were observed. Static magnetic fields inhibited cell migration. The calcium channel antagonists diltiazem, nifedipine, and verapamil protected PMNs exposed to static magnetic fields. The results indicated that calcium channels might be involved in the effects of static magnetic fields on enzymatic activity.

Human peripheral blood mononuclear cells (PBMC) and Jurkat cells were exposed to 4.75 T for 1 h by Aldinucci et al. (2003b). Concentrations of interleukins (IL-1 β , -2, -6), interferon (IFN- γ), and tumour necrosis factor α (TNF α) in PBMC were not affected. On the other hand, static magnetic fields led to very low concentrations of IL-2 and Ca²⁺ in Jurkat cells. The data suggested that static magnetic fields might affect calcium transport in Jurkat cells, but not in normal PBMC. In contrast to this, Salerno et al. (1999) detected increased release of IFN- γ in normal PBMC after exposure to 0.5 T. They observed reduced expression of CD69 and increased release of IFN- γ and IL-4. A decrease in metabolic activity of human HL-60 promyelocytic cells in response to exposure for 72 h to 1 T was seen in a later study (Sabo et al., 2002). This decrease was also seen in the presence of the antineoplastic drugs 5-fluorouracil, cisplatin, doxorubicin, and vincristine. The data from these independent studies (Sabo et al., 2002; Aldinucci et al., 2003b) may indicate that normal and transformed cells might have different sensitivity to static magnetic fields.

Chignell and Sik (1995a; 1998a) studied effects of static magnetic fields on the photohaemolysis of human erythrocytes by ketoprofen and protoporphyrin IX. In these studies, application of a static magnetic field during UV-irradiation of ketoprofen and erythrocytes decreased the time required for photohaemolysis. This observation might be explained by increased concentration and/or lifetime of free radicals generated by the reduction of ketoprofen in its triplet excited state by erythrocyte membrane constituents, probably lipids. In contrast, no effects were observed on the protoporphyrin IX-induced photohaemolysis, which is initiated by singlet oxygen (Chignell & Sik, 1995b).

Heine et al. (1999) investigated the influence of magnetic fields emitted from a 1.5 T MRI device on human neutrophil function. Blood samples were obtained from 12 patients immediately before and after exposure, and then subjected to flow cytometric analysis of the induced respiratory burst by the intracellular oxidative transformation of dihydrorhodamine 123 to the fluorescent dye rhodamine 123. No significant differences were found between the percentage of superoxide-anion-producing neutrophils before and after MRI, suggesting short time exposure during MRI does not induce the respiratory burst of neutrophils in patients. Aldinucci et al. (2003a) exposed human lymphocytes and Jurkat cells using an NMR apparatus. They found an increase in intracellular free Ca²⁺ in lymphocytes, without changes in proliferation and cytokines. Ca²⁺ and proliferation decreased in Jurkat cells, also without cytokine activation. The effect in Jurkat cells is similar with or without RF (Aldinucci et al., 2003b), but combined exposure affected intracellular free Ca²⁺ in human lymphocytes.

Table 9. Cell metabolic activity					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Papatheofanis, 1990)	Human polymorpho-nuclear leucocytes (PMNs)	Lysozymal degranulation and cell migration	0.1 T 30 min	Increased release of lysozyme and lactate dehydrogenase; inhibition of cell migration; channel antagonists protected cells exposed to SMF.	Blood samples pooled from three donors. No significance levels provided for the reported effects. The electromagnetic could have produced alternating magnetic fields that were not controlled and might have produced the effects.
(Aldinucci et al., 2003b)	Human lymphocytes, Jurkat cells	Ca ²⁺ movement, cell proliferation, production of pro-inflammatory cytokines	4.75 T 1 h	No effects on human lymphocytes. In Jurkat cells, changed properties of cell membranes lead to decreased Ca ²⁺ transport and concentrations, and to decreased cell proliferation.	

(Salerno et al., 1999)	Human peripheral blood mononuclear cells (PBMC)	Expression of activation markers and interleukin release	0.5 T 2 h	Reduced expression of CD69; increased release of IFN- γ and IL-4; release of TNF- α , IL-6 and IL-10 not modified.	
(Sabo et al., 2002)	Human HL-60 promyelocytic cell line	Metabolic activity	1 T 72 h	Retardation of metabolic activity, including in the presence of antineoplastic drugs.	The custom-made DC power supply could have produced alternating magnetic fields, which were not controlled and might have produced the effects.
(Chignell & Sik, 1995d)	Human erythrocytes	UV-induced photohaemolysis by the phototoxic drug ketoprofen and the photodynamic agent protoporphyrin IX	335 mT 20 min or up to 150 min	Reduced time for 50% ketoprofen-induced haemolysis. Possibly by increased concentration and/or lifetime of free radicals. No effects on protoporphyrin IX-induced photohaemolysis. No difference between short and long exposure times.	The electromagnetic could have produced alternating magnetic fields, which were not controlled and might have contributed to the observed effects.

(Chignell & Sik, 1998b)	Human erythrocytes	UV-induced photohaemolysis by the phototoxic drug ketoprofen	10 - 150 mT 20 min	SMF decreased the time required for UV-photohaemolysis through increasing the concentration of radicals released during photolysis.	Unspecified source of SMF. Significance levels of effects and number of independent experiments not provided.
MRI or Combined Exposure Studies					
(Heine et al., 1999)	Human neutrophils	Intracellular oxidative transformation of dihydro-rhodamine 123 to the fluorescent dye rhodamine 123	1.5 T 27.6 (±11.4) min	No influence on the production of radical species in living neutrophils.	MRI exposure.
(Aldinucci et al., 2003a)	Human lymphocytes, Jurkat cells	Intracellular Ca ²⁺ concentration, cell proliferation, production of pro-inflammatory cytokines	4.75 T + pulse modulated RF 1 h	Increase in intracellular free Ca ²⁺ without changes in proliferation and cytokines.	
Studies considered to be uninformative					
(Jajte et al., 2003)					

7.1.4 Cell membrane physiology

The cell membrane represents an interface between the cellular components inside the cell and the extracellular environment. As such, it regulates the intracellular environment, maintains the negative (approximately 70 mV) potential of the interior to that of the exterior (the 'resting' potential), and regulates the flow of molecules (for example, through voltage-gated or ligand-gated ion channels and carrier proteins such as the Na-K-ATPases).

Most studies on the effects of static magnetic fields on membranes concern exposures of short duration and low flux densities. Rodent nerve preparations and hippocampal slices are frequently used, but snail ganglia and single snail neurons also serve as models because

the relatively large size of molluscan neurons makes them easy to manipulate.

Rosen performed studies with mouse phrenic nerve preparations. In his 1992 paper, nerve-diaphragm preparations were exposed to 120 mT for 50 seconds (Rosen, 1992). No change in postsynaptic membrane resting potential was observed. There was a modest increase in action potential firing frequency at ambient temperatures $< 35\text{ }^{\circ}\text{C}$, but a prominent decrease appeared at temperatures $> 35\text{ }^{\circ}\text{C}$. Since the effect did not appear in the absence of Ca^{2+} , it was suggested that static magnetic fields may influence the release of neurotransmitter by stimulating calcium influx into the nerve terminal. Using murine neuromuscular junction preparations, Rosen observed increased inhibition of miniature endplate potentials between 50 and 150 seconds of exposure to 123 mT (Rosen, 1993). Discontinuation of the field resulted in full recovery.

In cultured neuroblastoma cells, Sonnier et al. (2000) found no effect on the resting potential after 5 seconds of exposure to 0.1, 0.5, 5 or 7.5 mT. Santini et al. (1994) did not see any response of membrane conductivity in primary chick embryo myoblasts after exposure to 1, 3 or 5 mT for 1 h. Carson et al. (1990) exposed HL-60 cells to 150 mT for 23 min and observed no effect on cytosolic free Ca^{2+} . In contrast, Rosen (1996) saw small reversible effects on the activation of time constant of calcium ion channels in GH3 cells after 150 seconds of exposure to 120 mT. He suggested that this was probably due to conformational changes in the plasma membrane resulting from membrane deformation.

Yost and Liburdy (1992) investigated calcium signal transduction after mitogenic stimulation in lymphocytes following exposure to static and time-varying magnetic fields. The presence of static fields alone resulted in no change.

Exposures of human neuroblastoma cells to static magnetic fields of up to 7.5 mT did not result in changes in any of the studied parameters of the action potential (Sonnier et al., 2003). Results suggested that the cellular mechanism responsible for the action potential is not affected under the employed conditions. Opposite results were reported by Rosen (2003a), who saw a transient increase in the activation time-constant of the sodium channel component of the action potential. However, Rosen (2003) observed this effect only at temperatures above $35\text{ }^{\circ}\text{C}$, greater than the $25\text{ }^{\circ}\text{C}$ temperature used by Sonnier et al. (2003).

Miyamoto et al. (1996) did not observe any alterations in active and passive influxes of K^{+} ions in human HeLaS3 cells exposed to static magnetic fields of different flux densities (0.5, 1, 1.6 T). Increasing the temperature from 37.4 to $45.0\text{ }^{\circ}\text{C}$ did not change the outcome of the experiment.

McLean et al. (1995) exposed mouse dorsal root ganglion neurons to approximately 11 mT for 200 seconds. They observed a temporary reduction in the number of stimuli that elicited an action potential. The effect was maximal at 200 - 250 seconds after the start of exposure and returned to normal at 400 - 600 seconds. The effect was only found with magnet stacks of alternating polarity, not with single magnets. They speculated that there was a direct or indirect effect on action potential generating sodium channels.

Trabulsi et al. (1996) exposed mouse hippocampal slices to 2 - 3 or 8 - 10 mT for 20 min and measured excitatory postsynaptic potentials (EPSPs). They observed a biphasic effect of exposure in the 2 - 3 mT range (a small depression followed by a longer amplification) and a depression of EPSP in the 8 - 10 mT range. They suggested that changes in intracellular Ca^{2+} concentration were responsible for the effects.

The effect of exposure of isolated snail neurons to static magnetic fields was the object of three studies. Azanza (1989) exposed the cells to flux densities of 116 or 260 mT for 1 min and measured action potentials. She observed a Ca^{2+} -dependent effect, where 86% of the cells were excited and 14% were inhibited. Balaban et al. (1990) measured resting potential and input resistance of snail neurons during exposure to 23, 120 or 200 mT for 20 min. They observed a stimulus-dependent decrease in input resistance in normally silent cells, but an increase in resistance in spontaneously active cells. Exposure also resulted in changes in EPSPs. No effects were found after removal of glial cells surrounding the neuronal perikarya. The authors suggested that the observed effects were mediated by metabolic processes and that glial cells played a mediating role in these. Ayrapetyan et al. (1994) found that exposure to 2.3 - 350 mT for 3 - 5 min increased the firing of Ca^{2+} -dependent action potentials. They also exposed physiological solutions to static magnetic fields and found that the conduction of Ca^{2+} -containing solutions was altered. The activity of exposed neurons was further diminished when they were incubated in previously exposed Ca^{2+} -containing physiological solutions. The authors speculated that static magnetic field exposure might alter the hydration state of the Ca^{2+} ions and that this might have effects on their functionality.

Raybourn (1983) investigated the effect of exposure to 1 - 10 mT fields for up to 3 minutes on isolated turtle retinas by measuring the electroretinographic b-wave. The functional capacity of the retinas was assessed by using changes in sensitivity resulting from altered illumination conditions. A reduction of the response by static magnetic fields was found only at the transition from light to dark. No effect of dose was observed, which indicates a saturation effect occurred already at 1 mT. The sensitivity of the retinas was not altered.

Aoki et al. (1990) exposed human acute leukaemia-derived TALL-1 cells to 0.4 T for 15 minute, and observed an increased efflux of the drug adriamycin. They concluded that this was caused by alterations in the plasma membrane.

In the only study using plant material, Reina and Pascual (2001; 2001) studied water uptake through lettuce seed cell membranes. They exposed seeds to 0 - 10 mT for 10 minutes and observed a dose-dependent increase in water uptake. They explained the observations by (unspecified) alterations in cell membrane properties.

Højevik et al. (1995) studied cyclotron resonance effects in rat insulin-producing RINm5F cells. They exposed the cells to 20.9 μ T static fields in combination with 20.9 μ T_{peak} ELF fields, with frequency varying from 12 - 60 Hz. No cyclotron resonance effects were observed.

Table 10. Cell membrane physiology					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Rosen, 1992)	Murine phrenic nerve-diaphragm preparation	Miniature end-plate potentials	120 mT 50 s	No change in postsynaptic membrane resting potential. Modest frequency increase < 35 °C; prominent decrease > 35 °C; no effect in absence of Ca ²⁺ .	
(Rosen, 1993)	Isolated murine neuromuscular junction preparation	Miniature end-plate potentials. Reversible alteration in presynaptic membrane function	123 mT 150 s	Increasing inhibition between 50 and 150 s; recovery time constant at 135 s.	
(Sonnier et al., 2000)	Human neuroblastoma cells	Resting potential	0.1, 0.5, 5, 7.5 mT 5 s	No effect.	

(Santini et al., 1994)	Primary chick embryo myoblasts	Membrane conductivity and permittivity	1, 3, 5 mT 1 h	No effect.	Lack of statistical methods (only means and SD given), but absence of changes was clear.
(Carson et al., 1990)	Human HL-60 promyelocytic cell line	Ca ²⁺ concentration	0.15 T 23 min	No effect.	Number of independent experiments not reported.
(Rosen, 1996)	GH3 cells	Calcium channel activation	120 mT 150 s	Small reversible changes in calcium channels, probably due to membrane deformation of the intramembrane part.	
(Yost & Liburdy, 1992)	Rat thymic lymphocytes	Calcium signal transduction	23.5 μ T 1 h	No effect of SMF alone, only in combination with ELF field.	
(Sonnier et al., 2003)	Human SH-SY5Y neuroblastoma cell	Action potentials	0.1, 0.5, 7.5 mT	No detectable change in any of the studied parameters of the action potential.	
(Miyamoto et al., 1996)	Human HeLa S3 cells	Active and passive influx of K ⁺	0 - 2 T 15 min	No effect of SMF on total K ⁺ channels and different channel subtypes.	

(Rosen, 2003a)	GH3 cells	Kinetics of voltage activated Na ⁺ channels	125 mT 150 s	Slight shift in current-voltage relationship; < 5% reduction in peak current. Increase in activation time constant during and following exposure > 35 °C.	
(McLean et al., 1995)	Adult mouse dorsal root ganglion neurons	Action potentials (AP)	approx. 11 mT 200 sec	Reduction in number of stimuli that elicit AP; maximal at 200 - 250 s, returned to normal at 400 - 600 s; effect only seen with stacks of alternating polarity, not with single magnet.	
(Trabulsi et al., 1996)	Mouse hippocampal slices	Excitatory postsynaptic potential (EPSP)	2 - 3, 8 - 10 mT 20 min	Biphasic effect in 2 - 3 mT range; depression of EPSP in 8 - 10 mT range.	No statistics, only examples.
(Azanza, 1989)	Isolated snail neurons	Action potentials	116 or 260 mT 1 min	Ca ²⁺ dependent inhibition or excitement; 86% of cells excited, 14% inhibited.	No statistics, no effect of flux density described.

(Balaban et al., 1990)	Snail neurons	Resting potential, input resistance	23, 120, 200 mT 20 min	Strength-dependent decreased input resistance in normally silent cells, increased in spontaneously active cells; changes in excitatory postsynaptic potentials; no effect after glia removal.	
(Ayrapetyan et al., 1994)	Land snail neurons	Action potentials (AP)	2.3 - 350 mT 3 - 5 min	Increase of firing of Ca ²⁺ -dependent AP. Exposure of physiological solutions alters their properties, which leads to changes in neuron activity.	
(Raybourn, 1983)	Turtle retinas	Electroretinographic b-wave response	1 - 10 mT up to 3 min	Short-term reduction; only shortly, after lights off; no dose effect. No reduction in retinal sensitivity. Brief suppressive effect on extracellularly monitored light-elicited ionic current fluxes.	
(Aoki et al., 1990)	Human acute leukaemia-derived TALL-1 cell line	Accumulation and efflux of adriamycin	0.4 T 15 min	Increased efflux of adriamycin.	

(Reina & Pascual, 2001)	Lettuce seed cell membrane	Water uptake	0 - 10 mT (1 mT steps) 10 min	Dose-dependent increase in water uptake, induced by alterations in cell membranes.	Strong point is the backup by theoretical explanation .
MRI or combined exposure studies					
(Höjevik et al., 1995)	Rat insulin-producing RINm5F cells	Ca ²⁺ transmembrane transport	20.9 μ T SMF, 20.9 μ T _{peak} ELF fields, f = 12 - 60 Hz 100, 500 ms	No cyclotron resonance effects observed.	
Studies considered to be uninformative					
(Bellossi, 1986b) (Rosen, 1994) (Cavopol et al., 1995) (Osuga & Tatsuoka, 1999) (Wieraszko, 2000)					

7.1.5 Gene expression

The cell membrane is also a prime site for receiving external physical or biochemical ‘signals’, and can, through a signal transduction process acting via specialized receptor molecules, activate intracellular metabolic signalling pathways that result in the expression of specific genes. These in turn lead to the expression of specific proteins that initiate appropriate cellular responses.

One study focused on protein expression in cultured rat primary cortical and hippocampal cells in response to short, 15 min, exposure to 100 mT (Hirai et al., 2002). This study analysed DNA binding activator protein-1 (AP1), neuronal marker protein MAP2, neuronal differentiation marker proteins GAP-43, Fos-family proteins c-Fos, Fos-B, Fra-2 and Jun-family proteins c-Jun, Jun-B, and Jun-D, along with cytoplasmic Ca²⁺ and LDH activity. The authors found increased AP1 DNA binding through expression of Fra-2, c-Jun, and Jun-D proteins in immature hippocampal neurons. The results suggested that the short exposure to weak static magnetic fields may have lead to desensitisation of N-methyl-d-aspartate (NMDA) receptor channels through modulation of *de novo* synthesis of particular inducible target proteins at the level of gene transcription by the AP1 complex in immune hippocampal neurons. The

effects were not observed in mature hippocampal neurons or in cortical neurons.

Hirose et al. (2003b) explored the effects of static magnetic fields on the expression of proto-oncogenes, including c-Jun, c-Myc, and c-Fos proteins, by exposing HL-60 cells to spatially homogeneous 10 T or inhomogeneous 6 T static magnetic field (with a 41.7 T m⁻¹ gradient) for up to 72 h. The data revealed that c-Jun was expressed after exposure to the inhomogeneous 6 T for 24, 36, 48, and 72 h with increased protein amount and phosphorylation level, but that no response was found with shorter exposure duration and after exposure to a homogeneous 10 T field. C-Myc and c-Fos did not respond to either field. The authors concluded that a static magnetic field gradient has significant biological effects and needs to be taken into consideration.

Human L-132 cells were exposed to 1.5 T for 240 min (Guisasola et al., 2002a). Heat shock proteins hsp70, hsp27, and corresponding mRNAs, were measured, along with cAMP and Ca²⁺ ions. No effects of static magnetic fields were observed.

Table 11. Gene expression					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Hirai et al., 2002)	Rat neuronal cells: primary cortical and hippocampal cell cultures.	DNA binding activator protein-1 AP1; neuronal marker protein MAP2; neuronal differentiation marker protein GAP-43; c-Fos, Fos-B, Fra-2; c-Jun, Jun-B, Jun-D. Cytoplasmic Ca ²⁺ . LDH activity	100 mT 15 min	Increased AP1 DNA binding through expression of Fra-2, c-Jun, and Jun-D proteins in immature hippocampal neurons. Suggestive of desensitization of NMDA receptor channels at the level of gene transcription by the AP1 complex.	

(Hirose et al., 2003b)	Human HL-60 cells	c-Myc, c-Fos, c-Jun expression	6 or 10 T 1 - 72 h	No effect on c-Myc, c-Fos and c-Jun by homogeneous 10 T SMF. SMF gradient field induced c-Jun expression.	6 T field included exposure to gradient field.
(Guisasola et al., 2002b)	Human L-132 cells	hsp70, hsp27, and corresponding mRNAs; cAMP, Ca ²⁺	1.5 T 240 min	No effect of SMF. SMF exposure during MRI procedures does not induce any cellular stress response.	Number of independent experiments not reported.
Studies considered to be uninformative					
(Cohly et al., 2003) (Mnaimneh et al., 1996) (Hiraoka et al., 1992) (Richardson et al., 1992) (Schneeweiss et al., 1995)					

7.1.6 Cell growth, proliferation and apoptosis

Both proliferative and apoptotic responses, often the result of the activation of appropriate signalling pathways and the consequent expression of relevant genes, may be involved in carcinogenic processes, if activated inappropriately. A proliferative stimulus to a cell carrying an oncogene or having lost a tumour suppressor gene through mutation, or inhibition of apoptosis in damaged cells, may initiate the clonal expansion of a colony of such cells, thereby paving the way for the growth of a tumour.

A number of investigators have studied the effect of static magnetic fields exposure on *in vitro* cell growth and proliferation. Most studies have been designed to determine the effects of long-lasting, continuous exposure.

No effects were observed in a number of different cell types using a variety of exposure conditions. Rockwell (1977) did not find any effects after exposure of EMT6 mouse mammary tumour cells to 0.148 T for 48 h. Chinese hamster V79 cells were not influenced by several hours of exposure to 0.75 T (Ngo et al., 1987). Considerably higher flux densities of 1.5 T applied for 1 h or 7.05 T for 4 or 24 h did not influence the cell cycle progression of human HL60 and EA2 tumour cells. Exposure for 48

and 96 h to 1.5 T did not alter the growth rate of human HeLa and Gin-1 (gingival fibroblast) cells (Sato et al., 1992). Yamaguchi et al. (1993) also exposed Gin-1 cells, but to 0.2 T and for 6 - 8 months without an effect on cell growth. In yeast cells, 1.5 T applied for 15 h did not result in growth changes (Malko et al., 1994). No effect was observed of exposure to fields as high as 7 T for up to 8 days in P388 mouse leukaemia cells and V79 Chinese hamster fibroblasts (Sakurai et al., 1999), nor of 10-T fields applied for 4 days to Chinese hamster CHO-K1 cells (Nakahara et al., 2002). Finally, repetitive exposure to 1.5 T (3 times 1 h per week for 3 weeks) did not result in alterations of proliferation and clonogenicity of human fetal lung fibroblasts (Wiskirchen et al., 1999). Aldinucci et al. exposed human peripheral blood mononuclear cells (PBMC) and Jurkat cells to 4.75 T for 1 h (Aldinucci et al., 2003b). No proliferative effects of static magnetic fields were observed in the human lymphocytes, either quiescent or activated by phytohaemagglutinin (PHA).

In contrast, a number of studies with a variety of mammalian cells of different origin did find effects. Buemi et al. (2001) exposed rat renal VERO cells and cortical astrocytes to 0.2 T for up to 6 days. They observed an influence of exposure on the balance between cell proliferation and death. Linder-Aronson and Lindskog (1995) observed impaired attachment and growth of human periodontal fibroblasts during exposure to 0.1 - 0.2 T for up to 5 weeks. Exposure to 0.2 T for up to 3 hours led to decreased ³H thymidine incorporation in the human MCF-7 breast cancer and NC-B4 neurons cell lines (Pacini et al., 1999a). No effect was found in murine leukaemia WEHI-3 cells, nor was there an effect on colony formation in any of the three cells lines.

Pacini et al. (1999b) also investigated cell proliferation and signalling following the exposure of human neurons (FNC-B4) to a 0.2 T static field. Cell proliferation, assessed by ³H thymidine incorporation was inhibited in the neurons but not in the controls. Further tests indicated that the field did not affect twelve DNA microsatellites, indicating that no genomic instability was induced by static magnetic field exposure.

The viability of lymphocyte cells was followed after exposure to a 10 T field (Onodera et al., 2003). Without lymphocyte stimulation, there were no significant differences in the viability of exposed and unexposed T cells (CD4+ and CD8+), B cells and NK cells. Stimulated T cells had reduced viability following exposure. A 10 T static magnetic field had acute effects on immune cells during cell division, but a minimal effect on cells in a nondividing phase.

Raylman applied flux densities of 7 T to human lymphoma (Raji) cells for 18 hours and observed an 11% reduction in the number of viable cells and a reduced growth rate (Raylman et al., 1997). Exposure to 7 T for 64 hours reduced the viable cell number in human melanoma (HTB 63) cells, human ovarian carcinoma (HTB 77 IP3) cells and human

lymphoma (Raji; CCL 86) cells (Raylman et al., 1996). The authors attributed the effect to growth retardation, although the cell cycle was unaltered. No increased number of DNA breaks was found, which is in agreement with the lack of effect on viability.

Norimura et al. (1993) exposed human T-lymphocytes to 2 - 6.3 T for up to 3 days. They observed inhibition of growth by field strengths of 4 - 6.3 T in cells stimulated by phytohaemagglutinin, but not in unstimulated cells. The radiosensitivity of the cells increased and the repair capacity decreased after exposure to 6.3 T. The authors concluded that temporary physiological alterations were induced by exposure to static magnetic fields of 4 T and higher. Apparently, these alterations are only observed when the cells are challenged.

Human and mammalian cells in culture were used in a few studies devoted to functional activity in response to long static magnetic field exposure. Flipo et al. (1998) analysed the mitogen response to concanavalin A, phagocytosis, Ca^{2+} influx, and apoptosis in C57BI/6 murine macrophages, spleen lymphocytes, and thymic cells exposed to static magnetic fields at 2.5 - 150 mT for 24 h. Static magnetic fields altered several functional parameters in all cell types. No intensity threshold was observed in this study. Exposure of murine immune cells to static magnetic field decreased macrophage phagocytosis, enhanced apoptosis of thymocytes, and inhibited the response of lymphocytes to the mitogen concanavalin A in association with an increased Ca^{2+} influx. Fanelli et al. (1999) analysed apoptosis and Ca^{2+} influx in human U937 and CEM cells. Exposure to 0.6 - 6 mT for 4 h increased cell survival by inhibiting apoptosis induced by several agents in an intensity-dependent fashion. Protective effects on apoptosis could be attributed to increased Ca^{2+} influx. However, this effect was not seen in rat cells where apoptosis was induced by Ca^{2+} influx.

Teodori et al. (2002) followed the occurrence of apoptosis in human HL60 cells exposed to a 6 mT static magnetic alone (18 hours) or in combination with the known apoptosis-inducer camptothecin (5 hours). They used two approaches (flow cytometry and laser scanning cytometry) and found no effect of static magnetic fields, alone or in combined treatment, on overall apoptosis. However, the authors observed a different distribution of early versus late apoptotic cell populations in the co-exposure treatment, suggesting a premature shift of cells into late apoptosis. It is unclear whether this could lead to inflammatory events.

Several authors used bacterial strains and plant cells to study the effects of static magnetic field exposure. Stansell et al. (2001) exposed *E. coli* to 8 - 60 mT for 19.5 h and studied the effect of the antibiotic piperacillin. Exposure increased the resistance against the antibiotic. In contrast, Benson et al. (1994) observed enhancement of the effect of the antibiotic gentamicin to *Pseudomonas aeruginosa* exposed to 0.5 - 2 mT

for 20 min. It cannot be excluded that the decreased growth might have been influenced by different light conditions.

When grown in a mixed culture in stationary phase, the *E. coli* strains ZK126Nalr and ZK126Smr have different death rates, which lead to a growth advantage for ZK126Smr. When grown under continuous exposure to inhomogeneous 5.2 - 6.1 T fields, or 7 T homogeneous fields, the death rate of the ZK126Nalr cells decreases and the growth advantage of the other strain disappears (Okuno et al., 2001). The effect is stronger with the inhomogeneous fields.

Stasiuk (1974) exposed *Mycobacterium tuberculosis* to static magnetic fields (180 mT) and found that exposures of up to 1 h did not change the bacterial growth, but that longer exposure (2 h and more) resulted in significant inhibition. No changes were observed in sensitivity to antibiotics or growth in tissues of infected animals. Khar'kova et al. (1976) studied the effect of long exposure of *Staphylococcus* to a 5 mT field. Permanent changes in colour of colonies (after 16 days), and changes in morphology and in fermentation of proteins and hydrocarbons, were observed by 18 months. By 8 months, the sensitivity to antibiotics increased significantly. The lethality increased in mice upon infection with static magnetic field-exposed *Staphylococcus*. These results could have implications for human health, but they must first be independently replicated.

The growth and viability of the plant-growing bacteria *Serratia marcescens* and of callus cells of *Hordeum vulgare* (barley) and *Rubus fruticosus* (blackberry) was investigated after exposure to an inhomogeneous field varying between 6 and 10 mT for 24 or 48 h (Piatti et al., 2002). The number of bacterial cells was reduced, while both the number and viability of *H. vulgare* cells were lower after exposure. *R. fruticosus* cells were not influenced.

Several studies looked at the effects of MRI exposures on *in vitro* cell growth. Schiffer et al. (2003) used a clinical MR scanner to investigate the effects of signals relevant to MRI exposure on the cell cycle of two human cell lines. Appropriate controls were included (positive control for cell cycle alteration, as well as temperature and vibration controls). Combining static magnetic fields (1.5 or 7.05 T) with either a time-varying bipolar gradient field for 1, 2 or 24 hours or a pulsed radiofrequency field did not alter the cell cycle distribution, nor did a one-hour exposure to the MRI signal.

To elucidate the effect of MRI exposure on early murine embryo development, two-cell embryos were treated for various lengths of time by MRI using pulse sequences employed in current clinical imaging (Chew et al., 2001). There were no significant differences detected in the rate of blastocyst formation between control and exposed groups.

Tofani et al. (2001) exposed human colon adenocarcinoma (WiDr), breast adenocarcinoma (MCF-7), and embryonal lung fibroblasts (MRC-5) cells to either a static magnetic fields alone (1 - 30 mT) or in combination with ELF (3 mT at 16, 50 or 100 Hz). They observed apoptosis in WiDr and MCF-7 cells after exposure to static magnetic fields only with flux densities > 1 mT. The effect increased with simultaneous 50 Hz ELF exposure, but only for latency times up to 24 h.

Table 12. Cell growth, proliferation and apoptosis					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Rockwell, 1977)	Mouse mammary tumour cells (EMT6)	Viability or growth	140 mT 48 h	No effect.	Lack of statistical information, but absence of effect was clear.
(Ngo et al., 1987)	Chinese hamster fibroblast cells (V79)	DNA synthesis, survival	0.75 T several h or 1 h following neutrons	No effect. Data indicated that SMF exposure alone does not exert any changes in survival and DNA synthesis in comparison with the effects from neutron radiation.	Results clear cut and experiments seem well-performed; however, no information on statistical analysis.
(Sato et al., 1992)	Human HeLa cells, human gingival fibroblast cells (Gin-1)	Growth, DNA content, DNA synthesis, labelling index	> 1.5 T 48, 96 h	No effect.	
(Yamaguchi et al., 1993)	Human gingival fibroblast cells (Gin-1)	Growth, DNA content, rate of lactate production, glucose consumption, ATP content and cell morphology	0.2 T 6, 8 mo + 1 or 2 wk; Sm-Co magnets	No influence on growth, morphology and glycolytic activity.	
(Malko et al., 1994)	Yeast cells	Growth	1.5 T 15 h	No effect.	

(Sakurai et al., 1999)	Mouse leukaemia cells (P388), Chinese hamster fibroblast cells (V79)	Growth pattern of cells, DNA distribution and sensitivity to bleomycin	7 T 3 h - 8 d	No effect.	Only one experiment per endpoint.
(Nakahara et al., 2002)	Chinese hamster ovary cells (CHO-K1)	Growth, cycle distribution, micronucleus (MN) formation	10 T 4 d	No effects of SMF alone, but enhancement of 4-Gy-induced MN-formation. SMF exposure may have been a co-factor.	
(Wiskirchen et al., 1999)	Human fetal lung fibroblast	Proliferation, clonogenic assay	1.5 T 1 h, 3x/wk, 3 wk	No significant effects.	
(Aldinucci et al., 2003b)	Human lymphocytes, Jurkat cells	Ca ²⁺ movement, cell proliferation, production of pro-inflammatory cytokines	4.75 T 1 h	No effects on lymphocytes. In Jurkat cells, changed properties of cell membranes lead to decreased Ca ²⁺ transport and concentrations, and to decreased cell proliferation.	
(Buemi et al., 2001)	Rat renal cells (VERO); cortical astrocytes	Cell proliferation, cell death balance	0.2 T 2, 4, 6 d	Influence of SMF on cell proliferation / death balance; differs between cell lines. This could have been partly due to astrocytes being primary cells and renal cells being immortalized. SMF exposure may have interacted with apoptosis.	

(Linder-Aronson & Lindskog, 1995)	Human periodontal fibroblasts	Attachment and growth	107 - 230 mT (intradisk variation) 1 - 5 wk	Impaired attachment and growth.	With increasing passage number, there was also decrease in attachment & growth in controls, but difference with exposed cells was clearly significant.
(Pacini et al., 1999a)	Human breast cancer cell line (MCF-7); neurons (NC-B4); murine leukaemia cells (WEHI-3)	Cell damage and proliferation	0.2 T 5 min - 3 h	Decreased ³ H thymidine incorporation in human cells, not in murine; no effect on formation of cell colonies. Effect of vitamin D permanent, that of SMF temporary.	
(Pacini et al., 1999b)	Normal human neuronal cells (FNC-B4); mouse leukaemia cells; human breast carcinoma cells	Morphology, cell proliferation, production of endothelin-1, genome instability	0.2 T 5 - 15 min	No alterations in genome instability; dramatic changes of morphology in neuronal cells only. Significant decrease in cell proliferation, changes in production of endothelin-1.	
(Onodera et al., 2003)	Human peripheral blood mononuclear cells (PBMC)	Viability, apoptosis, lymphocyte subpopulations	field increase 0 - 10 T over 0.5 h, constant for 3 h, decrease to 0 T over 0.5 h	Without lymphocyte stimulation, no significant differences in viability of exposed and unexposed T cells (CD4 ⁺ and CD8 ⁺), B cells and NK cells. Stimulated T cells had reduced viability following exposure.	

(Raylman et al., 1997)	Human lymphoma cells (Raji)	Viable cell number	7 T 18 h	11% reduction of number of viable cells due to exposure; growth rate retarded.	
(Raylman et al., 1996)	Human melanoma cells (HTB 63), ovarian carcinoma cells (HTB 77 IP3), and lymphoma cells (CCL 86, Raji)	Cell viability; flow cytometry	7 T 64 h	Reduction in viable cell number – HTB63 19%, HTB77 22%, Raji 41%; cell cycle unaltered; no increase in DNA breaks, growth after exposure slowed for 1 d. Cell growth recovered when SMF exposure was halted.	Sensitive testing method (cell cycle analysis).
(Norimura et al., 1993)	Human T-lymphocytes	Cell growth and radiation response	2 - 6.3 T up to 3 d	No effect in normal cells; growth inhibition in stimulated cells only at >4 T; radiosensitivity up, repair decreased after 6.3 T. Stimulated or stressed cells possibly more susceptible to SMF than normal growing cells.	Peculiar way of determining cloning efficiency.
(Flipo et al., 1998)	Murine macrophages (C57Bl/6), spleen lymphocytes, and thymic cells	Mitogen response to concanavalin A, phagocytosis, Ca ²⁺ influx, apoptosis	2.5 - 150 mT 24 h	Decreased macrophage phagocytosis, enhanced apoptosis of thymocytes, and inhibited response of lymphocytes to Con A in association with an increased Ca ²⁺ influx.	Data support conclusion about non-linear dependence of effects.

(Fanelli et al., 1999)	Human U937 and CEM cells; human peripheral blood leucocytes; rat thymocytes	Apoptosis, Ca ²⁺ influx	0.6 - 6 mT 4 h	Protective effect on apoptosis was due to increase of Ca ²⁺ influx. Effect not seen in rat cells where apoptosis was induced by Ca ²⁺ influx.	
(Teodori et al., 2002)	Human HL-60 cells	Apoptosis	6 mT 18 h	No effect of SMF alone, slight acceleration of apoptosis in camptothecin-treated cells.	
(Stansell et al., 2001)	Bacteria (<i>E. coli</i>)	Number of cells with piperacillin treatment	8 - 60 mT 19.5 h	Increased number of cells.	
(Benson et al., 1994)	Bacteria (<i>Pseudomonas aeruginosa</i>)	Number of cells with gentamicin treatment; autoradiograms from ¹¹¹ In uptake	0.5 - 2 mT 20 min	Decreased number of cells due to SMF enhancement of antibiotic activity.	No sham controls; growth difference may have been influenced by different light conditions.
(Okuno et al., 2001)	Bacteria (<i>E. coli</i> ZK126Nalr and ZK126Smr)	Differential cell growth	5.2 - 6.1 T, inhomogeneous (maximum gradient 23 T/m) 7 T homog.	Growth advantage in stationary phase disappears; effect stronger with inhomogeneous field.	
(Stasiuk, 1974)	<i>Mycobacterium tuberculosis</i>	Cell growth in culture, resistance to antibiotics, growth of bacteria from infected guinea pigs	180 mT 1, 5, 30, 60, 120 min and 24 h	No effects at 1, 5, 30 and 60 min exposures. Significant inhibition of cell growth at 120 min and almost complete blockage at 24 h exposure. No changes in sensitivity to antibiotics. No effects on growth in tissues from exposed animals.	Amount of independent exposures not reported.

(Khar'kova et al., 1976)	Haemolytic <i>Staphylococcus</i> , strain 209-P	Colour and morphology of colonies, fermentation of proteins and hydrocarbons sensitivity to antibiotics. Pathogenic properties upon infection of mice.	5 mT up to 18 months	Permanent changes in colour of colonies by 16 d exposure, changes in morphology and fermentation of proteins and hydrocarbons by 18 months. Sensitivity to antibiotics increased after 8 months. Increased lethality of mice after infection with SMF-exposed <i>Staphylococcus</i> .	Amount of independent exposures and number of measurements not reported.
(Okuno et al., 2001)	Bacteria (<i>E. coli</i> ZK126Nalr and ZK126Smr)	Differential cell growth	5.2 - 6.1 T, inhomogeneous, 7 T homogeneous	Growth advantage in stationary phase disappears; effect stronger with inhomogeneous field.	
(Piatti et al., 2002)	Bacteria, plant cell cultures	Growth, viability	6 - 10 mT 24 or 48 h; magnetic disks	Reduction in number of bacterial cells; reduction in number and viability of <i>H. vulgare</i> cells; no effect on <i>R. fucticosus</i> .	
MRI or combined exposure studies					
(Schiffer et al., 2003)	Human HL60 and EA2 tumour cells	Cell cycle distribution	1.5 T, 2 h 7.05 T, 4 or 24 h 7.05 T + gradient field, 2 or 24 h 1.5 T + RF, 1 h 1.5 T + gradient + RF, 1 h	No effect.	

(Chew et al., 2001)	Two-cell murine embryos	Rate of blastocyst formation	8 pulse sequences employed in current clinical imaging 1.5 T 2.27 - 6.11 min	No effect; development rates 70 - 75%. MR imaging sequences did not exert harmful effects to blastocyst formation.	Used standard MRI imaging sequences.
(Tofani et al., 2001)	Human colon adenocarcinoma cells (WiDr), breast adenocarcinoma cells (MCF-7), embryonal lung fibroblasts (MRC-5)	Growth, apoptosis	1, 3, 10, 30 mT SMF, 3 mT ELF at 16, 50 100Hz 20 min	Apoptosis observed in WiDr and MCF-7 cells only after > 1 mT SMF; increased with simultaneous 50 Hz ELF. Only for latency times up to 24 h.	
Studies considered to be uninformative					
(Esformes et al., 1981) (McDonald, 1993) (Tsuchiya et al., 1999) (Stepanian et al., 2000) (Horiuchi et al., 2001) (Ishizaki et al., 2001) (Mariani et al., 2001) (Horiuchi et al., 2002) (Jajte et al., 2002) (Zhang et al., 2002)					

7.1.7 Genotoxic effects

The genotoxicity of an agent is an indication of its potential to damage DNA, and is therefore implicated in the process of mutagenesis. As a result of this, stable and heritable genetic mutations are generated, often through misrepair. Mutations leading to the activation of oncogenes, or the inactivation of tumour suppressor genes, are early events in the formation of cancers.

Tests of genotoxicity typically include chromosomal aberrations, sister chromatid exchange, micronucleus formation, different assays for mutagenesis, and measures of DNA damage (such as the comet assay).

Cooke and Morris (1981) analysed chromosomal aberrations and sister chromatid exchanges following 1-h exposure of human lymphocytes to static magnetic fields. Even while there was a trend for an increase in number of chromosome lesions and proportion of cells with lesions (50 - 80%) at both employed field intensities, (0.5 and 1 T), these increases were not statistically significant. The authors concluded that static magnetic fields had no significant effect on any of the measured parameters.

Effects of static magnetic fields on conformation of chromatin/nucleoids were studied in human and bacterial cells (Matronchik et al., 1996; Belyaev et al., 1997; Binhi et al., 2001). In these studies, transient condensation or decondensation of chromatin was observed at specific 'intensity windows' in the range of 0 - 110 μ T. The authors attributed these transient changes to adaptation of cells to the changes in the static magnetic field. A possible interplay of these changes in chromatin conformation with genotoxic effects was suggested. The observed effects were explained on the basis of phase modulation of hypothetical natural high-frequency oscillations.

A number of studies have been designed to determine effects of long lasting continuous exposure in mammalian cells. Okonogi et al. (1996) did not find effects of a 6 h exposure to 4.7 T on the background level of micronuclei in Chinese hamster CHL/IU cells. On the other hand, a decrease in the formation of micronuclei induced by concomitant exposure to mitomycin C was observed. The authors suggested that static magnetic field exposure might have a protective effect on DNA damage produced by mitomycin C.

Exposure of rat lymphocytes to the much lower flux density of 7 mT for 3 h did not increase the number of cells with DNA damage, as measured with the comet assay (Zmyslony et al., 2000). The amount of damage was significantly increased when the FeCl₂-incubated cells were simultaneously exposed to static magnetic fields. However, the same exposure to static magnetic fields did not modify H₂O₂-induced DNA damage. According to the authors, the low level of FeCl₂ did not induce any noxious effects on lymphocytes. They hypothesized that the static magnetic field might have had an effect on radical pairs, leading to an increased number of free radicals generated in the cells by iron cations, but they also felt that reasonable explanations for their results were lacking. Nakahara et al. (2002) investigated the formation of micronuclei in Chinese hamster ovary CHO-K1 cells. Exposure to 1 or 10 T alone for 4 days did not result in induction of micronuclei. No synergistic effects were observed in combined treatments with X-irradiation, except for exposure to 10 T and 4 Gy. In those cases, static magnetic field exposure increased the formation of X-ray-induced micronuclei by 10%.

A similar variability in results was seen in studies with bacterial cells. Ikehata et al. used the Ames test to study effects of exposure to 2 - 5 T for 20 minutes up to 48 h (Ikehata et al., 1999). They observed no direct effect of static magnetic field exposure on mutagenicity or growth rate. Mutation rate in the static magnetic field-exposed groups was significantly higher than in the non-exposed groups when cells were treated with several (but not all tested) chemical mutagens. No dose-response for mutagenic effects was observed in this study. Mutagenic effects were also not detected with the Ames test at the higher magnetic fields of 5 and 7.2 T (Teichmann et al., 2000).

Zhang et al. analysed mutagenic effects of 24-h exposure to 5 or 9 T in *Escherichia coli* cells (Zhang et al., 2003). They observed no effects on survival and mutation rate in wild type, or in strains defective in DNA repair enzymes or redox-regulating enzymes. Mutation frequency increased in *soxR* and *sodA**sodB* mutants (which are defective in defence mechanisms against oxidative stress). The expression of superoxide-inducible genes was stimulated. The data suggested that static magnetic fields above 5 T induce mutations through elevated production of intracellular superoxide radicals in *E. coli* (Zhang et al., 2003).

Baum and Nauman used plant cells to study mutagenic effects of static magnetic field exposure (Baum & Nauman, 1984). They analysed micronuclei and pink mutations in *Tradescantia*. Magnetic field at intensities of about 0.16 to 0.78 T applied continuously over 6 or 7 days did not induce genotoxic effects in *Tradescantia* cells.

Wolff et al. (1985) exposed human lymphocytes and Chinese Ovary cells to a 2.35 T static magnetic field combined with radiofrequency fields for 12.5 h. Sister chromatid exchange and chromosomal aberrations were assessed. No effects on these cytogenetic endpoints were found.

Table 13. Genotoxic effects					
Authors	Cells	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Cooke & Morris, 1981)	Human lymphocytes	Frequency of gross chromosome lesions, sister chromatid exchanges and proportion of amodal cells.	0.5 T, 1.0 T 1 h	No effect.	Trend for increased chromosomal lesions and increased proportion of cells with lesions.
(Matronchik et al., 1996)	Bacterial <i>E. coli</i> cells	Conformation of nucleoids	0 - 110 μ T 15 min	Changes on conformation of nucleoids dependent on SMF in specific 'intensity windows'. Condensation or decondensation of nucleoids was observed in specific windows.	
(Belyaev et al., 1997)	Normal human fibroblasts and lymphocytes	Conformation of chromatin	zeroed natural magnetic field 10 - 120 min	Time-dependent transient condensation of chromatin characteristic of stress response with maximum at 40 - 80 min. Effects reproduced in experiments with lymphocytes from the same donor.	

(Binhi et al., 2001)	Bacterial <i>E. coli</i> cells	Conformation of nucleoids	0 - 110 μ T 15 min	Changes in conformation of nucleoids dependent on 'SMF intensity windows' with extrema at 0, 26, 43, 61, 72, 83 and 105 μ T. Explained by phase modulation of ion interference in rotations of DNA-protein complexes.	
(Okonogi et al., 1996)	Chinese hamster cells (CHL/IU)	Micronuclei (MN) formation	4.7 T 6 h	No effects of SMF on the background level of MN. Decrease in formation of MN induced by Mitomycin C. Possibly influence on cell cycle.	
(Zmyslony et al., 2000)	Rat lymphocytes	DNA damage including single strand breaks and alkali labile sites	7 mT 3 h	No effect of SMF only. Enhancement of FeCl ₂ -induced DNA-damage by SMF. No effects on H ₂ O ₂ -induced damage.	
(Nakahara et al., 2002)	Chinese hamster ovary cells (CHO-K1)	Growth, cell cycle distribution, micronucleus (MN) formation	1, 10 T 4 d	No effects of SMF alone, but enhancement of 4-Gy-induced MN-formation. SMF exposure may have been a co-factor.	Enhancement was at the border of statistical significance

(Ikehata et al., 1999)	Bacterial strains from the Ames test	Mutagenicity and co-mutagenicity (Ames test), cytotoxicity	2, 5 T 20 min, 1.5, 3, 6, 15, 24, 48 h	Mutagenicity of several (but not all) tested compounds increased; no dose-response; no direct effect of SMF on mutagenicity or growth rate. Possibly influence of SMF on reaction intermediates.	
(Teichmann et al., 2000)	Bacteria from the Ames test	Mutagenicity (Ames test)	0.5, 7.2 T 1 h, 24 h	No effect.	Although statistics are lacking, it was clear from comparison of the means \pm SD that there was no effect of SMF.

(Zhang et al., 2003)	<i>Escherichia coli</i> wildtype, soxR, sodAsodB mutants	Induction of mutations, clonogenic survival	5 or 9 T 24 h	No effects on survival and mutation rate in wild-type, or strains defective in DNA repair enzymes or redox-regulating enzymes. Mutation frequency increased in soxR and sodAsodB mutants (defective in defence mechanisms against oxidative stress). Expression of superoxide inducible gene was stimulated.	Changes larger than SD, however, statistical analysis was not performed.
(Baum & Nauman, 1984)	<i>Tradescantia</i> clones 4430 and 02	Micronuclei, pink mutations	0.16, 0.76 - 0.78 T 6 - 11 d	No effect of SMF applied continuously over 6 or 7 days on <i>Tradescantia</i> pollen mother cells.	Field variations of $\pm 25\%$.
MRI or combined exposure studies					
(Wolff et al., 1985)	Chinese hamster ovary cells; human lymphocytes	Chromosomal aberrations (CA), sister chromatid exchanges (SCE).	2.35 T + RF 12.5 h	MR imaging conditions did not cause cytogenetic damage.	

Studies considered to be uninformative
(Rossner & Matejka, 1977) (Wolff et al., 1980) (Geard et al., 1984) (Takatsuji et al., 1989) (Yamazaki et al., 1993) (Mahdi et al., 1994) (Schreiber et al., 2001)

7.1.8 Conclusions

The results of *in vitro* studies are useful for elucidating interaction mechanisms and for indicating the sorts of effects that might be investigated *in vivo*, but are not sufficient to identify health effects without corroborating evidence from *in vivo* studies.

A number of different biological effects of static magnetic fields have been studied *in vitro*. The observed effects are rather diverse and were found after exposure to a wide range of magnetic flux densities.

Despite some inconsistencies, the studies on cell free systems show that biologically relevant biochemical reactions can be affected by static magnetic fields in the millitesla to tesla range.

The body of reviewed data on magneto-mechanical effects showed that static magnetic fields can affect orientation of cells. These effects were observed under relatively high field intensities of more than 1 T, and for different exposure times that ranged from minutes to hours.

The studies on metabolic activity suggest that it may be affected by static magnetic fields, dependent on cell type or whether or not the cells are transformed. The data obtained from the 'cell-free system' studies indicated that radicals and calcium metabolism might be primary targets for effects of static magnetic fields. This is also the case for combined exposures to static magnetic fields and RF fields. It should be mentioned that most of the *in vitro* studies involved electromagnets that might create alternating fields, which were usually not measured.

The studies on membrane effects show that exposure to static magnetic fields in the millitesla range is able to change membrane properties in isolated systems and cultured cells, possibly through changes in (calcium) ion channel structure and/or activity. These changes may lead to changes in neuronal functioning, such as changes in action potential generation and, consequently, neurotransmitter release. However, most of the effects seem to be reversible.

Few studies have been performed on gene expression. The data show that static magnetic fields can affect the expression of specific genes in human and mammalian cells. These effects may depend on exposure duration and field gradients.

There are many studies on cell growth, with contrasting results. The occurrence of effects in mammalian cells appears highly cell-type dependent, since several cell lines showed no effects on growth from fields up to 10 T. When an effect was found, it was generally growth retardation. The extent of any effect was usually dependent on exposure time and field strength (in the millitesla to tesla range). However, the dependence on field strength was addressed only in two studies and non-linear responses have been observed.

The few studies dealing with apoptosis in mammalian cells and those on growth of bacteria also show contrasting results. The effects in bacteria appear strongly strain-dependent.

Only a few studies on genotoxicity have been performed. No genotoxic effects of static magnetic fields up to 9 T have been shown, except for one study with repair-deficient bacterial strains. The studies with combined exposure to mutagens and static magnetic fields indicated modification of the effects of some of the tested mutagens. The effects may be strain/cell type dependent, but there are no indications for dose-dependence.

There are positive and negative findings regarding *in vitro* effects. There is evidence that static magnetic fields can affect several endpoints at intensities lower than 1 T, in the millitesla range. However, most data, both negative and positive, have not been replicated. Biological variables such as cell type, cell activation, and other physiological conditions during exposure were shown to be of importance. Thresholds for some of the effects were reported, but other studies indicated non-linear response without clear threshold values and even 'flux-density' windows were reported. The mechanisms for these effects are not known, but effects on radicals and ions may be involved. So far, dosimetric issues have not been studied and it is not clear whether the equivalent of 'dose' equals 'time of exposure' times 'intensity' is applicable for quantification of those effects and use in health risk assessments. In addition, there is no consistent knowledge regarding how prolonged or interrupted exposure would affect biological systems *in vitro*. Besides possible complicated dependence on physical parameters such as intensity, duration, recurrence and gradients of exposure, biological variables appear to be important for the effects of static magnetic fields. Finally, combined effects of static magnetic fields with other agents, such as genotoxic chemicals, seem to produce synergistic effects, both protective and stimulating. Further studies should clarify these issues.

7.2 Static field effects *in vivo*

This section discusses static field effects on *in vivo* biological systems, which are based on animal studies, in relation to possible health effects in humans. These kinds of investigation elucidate some *in vivo* mechanisms that cannot be investigated directly in humans, but are very important for understanding the mechanisms of static field interactions with complex living organisms. These investigations thus enable a more precise estimation of the Environmental Health Criteria and their applications.

Static electric fields induce a surface charge on animals and humans (see chapter 5). These can be perceived via surface charge effects such as hair movement. A few studies, mostly relating to perceptual effects, have been carried out and are briefly reviewed below. Most of the reviewed studies concern the effects of static magnetic fields; these can interact with living organisms in a number of different ways (see chapter 5 for a full discussion). This includes possible interactions with certain types of metabolic reactions and physiological processes, particularly those that involve the exchange of charged particles (ion fluxes) across cell membranes and the consequent generation of transmembrane electrical potentials (gradients of ion concentration).

7.2.1 Static electric fields

A review by Kowalczyk et al. (1991) concluded that the few published animal studies of static electric field effects provided no evidence of any adverse health effect. There have been few subsequent studies, and these have mostly examined surface charge effects. Rats showed aversive behaviour in an electric field of 55 kV m^{-1} and above, but not to fields of 42.5 kV m^{-1} or less (Creim et al., 1993). Exposure to 75 kV m^{-1} did, however, not induce taste aversion learning (Creim et al., 1995). The results of an earlier study suggested that locomotor and rearing activity was not significantly modified by exposure to up to 12 kV m^{-1} (Bailey & Charry, 1986).

Table 13. Static electric field effects					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static electric field effects					
(Creim et al., 1993)	Rats	Avoidance behaviour using a shuttle-box	Up to 80 kV m ⁻¹ for 1 h with constant or varied air ion concentration	Exposure to fields of 55 or 80 kV m ⁻¹ resulted in significant avoidance compared to sham exposed controls.	Air ion concentration, which simulates conditions under HVDC overhead lines, had no effect on avoidance behaviour.
(Creim et al., 1995)	Rats	Taste-aversion learning using a saccharin-flavoured drink	75 kV m ⁻¹ for 4 h at a constant air ion concentration	Exposure to 75 kV m ⁻¹ did not induce taste aversion.	Experimental procedure validated using a positive control.
(Bailey & Charry, 1986)	Rats	Locomotor and rearing activity; food and water intake	3 kV m ⁻¹ for 2, 18 or 66 h; 12 kV m ⁻¹ for 18 h.	Exposure even at 12 kV m ⁻¹ did not affect activity, or food and water intake.	
Studies considered to be uninformative					
(Möse & Fischer, 1970)					
(Fam, 1981)					

7.2.2 Static magnetic fields

Throughout life, all living organisms interact with geomagnetic fields in different ways. Some are thought to have developed specific sense organs with high magnetic sensitivity for the purposes of spatial orientation and directional cues during migration. Others, including humans, are not only constantly exposed to the action of geomagnetic fields, but are also occasionally exposed to much larger static fields produced by artificial sources.

To investigate the complex nature of static magnetic field effects on biological systems *in vivo*, a detailed analysis of the effects at different levels of organization is necessary. Animals, particularly in animal models of specific conditions, are very useful for investigating and interpreting the effects of static magnetic field exposure that are often

difficult, or impossible, to investigate in humans. They provide qualitative information about possible human responses, but are unlikely to provide quantitative information (since there are many differences in metabolism, physiology, lifetime etc.).

This review has been structured according to basic principles of anatomical and physiological organization, with the objective of identifying probable static magnetic field effects in animals that could also occur in humans. The principal areas covered include neurobehavioural studies, the musculo-skeletal system, the cardiovascular system and haematology, the endocrine system, reproduction and development, and genotoxicity and cancer.

7.2.2.1 Neurobehavioural studies

The nervous system enables animals and humans to respond to their environment and communicate with each other, as well as to maintain their internal physiological state. It comprises the brain and spinal cord of the central nervous system, the motor and sensory nerves of the peripheral nervous system, and the sympathetic and parasympathetic nerves and ganglia of the autonomic nervous system. Individual nerve cells receive and transmit information along their axons by the propagation of electrical impulses (action potentials). Information transmission at synapses (junctions) with other nerve cells takes place via the release of specialized neurotransmitter substances. In addition, specialized neurosecretory cells in the hypothalamus, pituitary gland and pineal organ of the brain release hormones that exert control over more peripheral endocrine organs, such as the thyroid and gonads.

7.2.2.1.1 Neurophysiological studies

The Lorentz force that acts on moving charge carriers, such as ionic currents, might be expected to affect ion channel conduction properties, thereby affecting nervous system function. However, calculation by Wikswo and Barach (1980) suggested that a field of ~ 24 T would be required to produce a 10% change in Na^+ or K^+ ion channel conductivity, with larger fields required for heavier ions.

Support for this view comes from the work of three groups. Schwartz (1978; 1979) found no effect of a 1.2 T field on action potential conduction velocity and ion channel currents in lobster giant axons. Similarly, Tenforde and colleagues found no effect of a 2 T field on conduction velocity, refractory period and excitation threshold in excised frog sciatic nerve preparations (Gaffey & Tenforde, 1983). Hong et al. (1986) and Hong (1987) found no effect of exposure to a static field of up to 1.2 T or to 1 T on nerve conduction velocity in anaesthetised rats nor in (awake) human subjects, respectively. In addition, a 1.5 T field had no effect on the amplitude or latency of somatosensory evoked potentials in volunteers (Hong & Shellock, 1990). (See chapter 8.8.1).

Rosen and colleagues have carried out a number of electrophysiological studies over the past 10-20 years, including both *in vitro* (previous chapter) as well as *in vivo* studies (see Rosen, 2003b for a review). With regard to the *in vivo* studies, Rosen and Lubowsky (1987) studied the effects of a pure static magnetic field of 120 mT on the excitability of striate cortex in three adult cats. To avoid effects from the anaesthesia, the animals were decerebrated (immobilised through surgical transection of the mid-brain). All animals subjected to a 120 mT field showed a gradual decrease in the maximum amplitude of the visual evoked potential, as well as a reduction in its variability. This change began 50 to 95 seconds after the field was turned on and persisted for 200 to 285 seconds after the field was turned off, with maximum effect evident at 100 to 175 seconds. At 80 mT, an effect was found in only one animal but not in the two others. Because the effects developed slowly and persisted for some time after the field was turned off, the authors suggested that the field alters the ionic environment or neurotransmitter availability at synapses, rather than having an effect on axonal conduction.

The same conclusion was drawn based on the results of the second study (Rosen & Lubowsky, 1990). The effects of 123 mT on spontaneous discharge frequency and discharge pattern of principle cells in the lateral geniculate body of five adult cats were examined. Effects on the lateral geniculate body would result in altered visual evoked potential, as was observed earlier. This experiment revealed that 45% of studied cells showed a decrease in frequency after the field was turned on. Once more, the effect developed slowly (75 seconds after field activation) and returned to baseline 250 seconds after the field was turned off.

These results appear consistent with other studies by Rosen (1996; 2003a) on cultured neuroblastoma cells, in which patch-clamp techniques revealed slow, long-lasting increases in the activation time-constants of the voltage-gated Na⁺ and Ca²⁺ ion channels. These results are also consistent with studies by Wieraszko and colleagues (Trabulsi et al., 1996; Wieraszko, 2000) on the modulation of synaptic excitability by static fields of 2 - 3 mT (see Table 10).

Two Russian studies investigated the effect of a 10 to 30 min static field exposure on evoked potentials in rats (Klimovskaia & Smirnova, 1976; Smirnova et al., 1982). The first study found increased amplitudes and additional waves in the evoked potentials after 0.4 T exposure. The effects were reversible. In the second study, different exposure levels between 0.1 and 1.6 T had been applied. An intensity-dependent increase in the amplitude of the potentials as well as modified shapes were observed. Effects were stronger in the hippocampus than in the cortex.

Cordeiro et al. (1989) exposed rats that had been subjected to sciatic nerve transection to a static magnetic field of 1 T for 12 h per day for 4 weeks. Exposure, when compared to sham exposure, had no effect on the speed of nerve regeneration.

Several animal species use geomagnetic fields for their orientation and navigation. This means that these animals are very sensitive to static magnetic field changes. Lohmann et al. (1991) subjected the marine mollusc *Tritonia diomedea* to alterations in geomagnetic field orientation at a 1 minute-on, 1 minute-off schedule for 26 min, and observed increased action potential firing in two specific brain neurons.

It is known that the electro-fish *Apteronotus* emits weak sinusoidal electric signals. Stojan et al. (1990) observed a change in the amplitude of the electric signal if a fish was exposed to a static magnetic field up to 10 T during 20 h. This effect was observed above a threshold of 1 T. The change in the amplitude was 8 to 10 mV for field strengths between 2 and 10 T. The effect was observed immediately after the field was turned on. The effect in the 2 T condition disappeared after 5 to 10 h, whereas the effect persisted almost unchanged until the field was turned off in the 10 T condition.

Table 14. Neurophysiological studies					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static Magnetic Field Effects					
(Schwartz, 1978)	Lobster	Nerve conduction velocity in giant axon.	1.2 T SMF parallel or perpendicular to axon 20 - 30 min	No effect on nerve conduction velocity	

(Schwartz, 1979)	Lobster	Resting potential, membrane action potential and transmembrane currents in giant axon.	1.2 T SMF parallel or perpendicular to axon < 5 min or 20 - 30 min	No effects on membrane potential or transmembrane currents	The preparation viability declined after about 10 - 15 min.
(Gaffey & Tenforde, 1983)	Frog	Action potential amplitude, conduction velocity, absolute and relative refractory periods in sciatic nerve	Up to 2.0 T field parallel to or perpendicular to nerve 4 - 17 h	No significant effect of 2 T field on action potential amplitude, conduction velocity, absolute and relative refractory periods. No effect of 1 T field on threshold	
(Hong et al., 1986)	Adult Sprague-Dawley albino rat	Motor nerve conduction and excitability	0.3 - 1.2 T 15, 30 or 60 sec	No effect on nerve conduction. Increased motor nerve excitability at > 0.5 T and t>30 sec. Effect disappeared 1 minute after termination of the field exposure. Effects were dose related.	Anaesthetized with intraperitoneal chloral hydrate.
(Rosen & Lubowsky, 1987)	Cat	Visual evoked response	120 mT + 80 mT 100 sec	At 120 mT decrease in amplitude, variability of a visual evoked response; starts 50 sec after start of exposure, lasts 285 sec after termination.	Increased amplitude of the visual evoked response at 80 mT in one of three animals (no effect in the others).

(Rosen & Lubowsky, 1990)	Cat	Principle cells in the lateral geniculate body	123 mT 100 sec	Decrease of discharge frequency in 45% of the cells studied, starting 75 sec after field activation and returning to baseline 250 sec after field was turned off.	Decerebration before the experiment instead of anaesthesia. This paper documents a spontaneous change of discharge frequency in the same order as the magnetic field-induced ones.
(Klimovskaia & Smirnova, 1976)	Albino rats	Cerebral and cerebellar cortex potentials evoked by sciatic stimulation	0.05 - 0.4 T 10 - 20 min	SMF at 0.4 T increased the amplitude of evoked potentials. Appearance of additional waves in evoked potentials. Effects of SMF were reversible.	
(Smirnova et al., 1982)	White rats	Somatosensory potentials in brain cortex and hypothalamus	0.1 - 1.6 T 15, 30 min	Intensity-dependent changes in potential in both cortex and hippocampus, with stronger response in hippocampus. Effects of 1.6 T were the same for different electromagnets. One of those magnets had a weak component, 1.8%, of alternating field, 100 Hz.	
(Cordeiro et al., 1989)	Rat	Nerve regeneration	1 T 12 h/d, 4 wk	No effect on nerve regeneration.	

(Lohmann et al., 1991)	Marine mollusc (<i>Tritonia diomedea</i>)	Action potentials	Alterations in geomagnetic field orientation, 1min on, 1 min off, for 26 min	Increased action potential firing in response to changes in ambient earth-strength SMF.	
(Stojan et al., 1990)	Electric fish <i>Apteronotus</i>	Natural electrical activity	0.5, 1, 1.5 2, 4 and 10 T 20 h	Prompt increase in amplitude of electric signals when field was turned on; threshold was 1 T. Amplitude decreased, but only marginally, after 2 - 5 h in a 10 T field.	
Studies considered to be uninformative					
(Tkach et al., 1987) (Kloiber et al., 1990)					

7.2.2.1.2 Sensory receptors: the eye and the ear

The eye and the ear are both very closely associated with the central nervous system. In fact the retina comprises, in addition to the photoreceptors, complex neural circuitry that is derived embryologically as an outgrowth of the forebrain. The hair cell receptors of the inner ear project via sensory nerve fibres to parts of the brain, and receive neural input projected from these brain nuclei.

Specialized nerve cells in the retina relay diurnal day/night information to the pineal, thereby affecting the release and production of melatonin and so affecting diurnal behaviour (see section 7.2.2.4.1). The retina is also thought to play a key role in orientation and migratory behaviour through detection of changes in the natural geomagnetic field. Although the mechanism for this is unknown, the participation of radical pairs from light sensitive molecules, e.g. cryptochromes, in magnetic field perception has been recently suggested (Mouritsen et al., 2004). Olcese et al. published two studies of the effect of artificial changes in the geomagnetic field on catecholamines in the neuroretina (Olcese et al., 1988; Olcese & Hurlbut, 1989). They observed a reduction in retinal nocturnal dopamine and norepinephrine levels in rats with no effect on melatonin synthesis in the retina itself. Further study with both nocturnal and diurnal animals revealed different reactions for cone-dominant and rod-dominant retina. An intact retina was essential for any effect to be detected. The mechanism and implications are unknown, although the authors speculated that the effect might influence diurnal rhythms.

Only one study has investigated the effects of exposure to static magnetic fields on hearing. Tausch-Treml et al. (1989) studied the acoustic action potentials of the cochlea in guinea pigs after exposure for up to 3 h to 8.5 T SMF. No effects were observed. Possible effects on the vestibular system of the inner ear are described in section 7.2.2.1.4 on animal behaviour.

Table 15. Sensory receptors: the eye and the ear					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Olcese et al., 1988)	Rat	Retinal melatonin synthesis and catecholamine contents	Artificial geomagnetic field strength Duration?	Alteration of ambient SMF reduced retinal nocturnal dopamine and norepinephrine levels; no effect on melatonin synthesis.	Exact mechanism and implications not known.
(Olcese & Hurlbut, 1989)	Rat, hamster, ground squirrel	Retinal catecholamine contents	Artificial geomagnetic field strength SMF < 60 mT Duration?	Retinal dopaminergic system differentially responsive to SMF. Dopamine levels can be reduced by SMF. Different reaction for cone-dominant retina as compared to rod-dominant retina.	Exact mechanism and implications not known.
(Tausch-Treml et al., 1989)	Guinea pig	Acoustic action potential	8.5 T 3 h	No effect.	Non-quantitative analysis of ECGs.
Studies considered to be uninformative					
(Sacks et al., 1986)					

7.2.2.1.3 Analgesia

The effect of a hypogeomagnetic field on stress-induced analgesia in mice was investigated in two studies. The experimental procedure in

the first study (Del Seppia et al., 2000) consisted of firstly maintaining the mice under various magnetic exposure conditions for 90 minutes, secondly immobilising the animals in a tube for 30 minutes and thirdly recording the nociceptive responses of the restraint-stressed mice as the latency of front paw lifting to an aversive thermal stimulus. Two experiments were conducted with 3 and 5 groups of mice. One group was exposed to a hypogeomagnetic field with a flux density of 4 μ T and another group was exposed to an ambient geomagnetic field of 46 μ T. The latency of foot-lifting responses in both experiments was significantly reduced in the animal group exposed to a hypogeomagnetic field compared to the group exposed to a normal geomagnetic field. The observed effect strength was comparable with the effect of an injection of 1.0 mg kg⁻¹ of the specific opiate antagonist naloxone hydrochloride. Those effects were more systematically investigated in the second study (Choleris et al., 2002). A pre-stress exposure was necessary for the anti-analgesic effects to occur. An anti-analgesic effect was observed if the ambient field was strongly reduced by shielding during 2 h, but no effect was observed in two near-zero magnetic conditions. The authors suggested that the elimination of the extremely weak time-varying component of the magnetic environment, which was only achieved by shielding, may have been responsible for the observed effects.

Exposure to MRI fields where the static field component was 0.15 T was reported to alter morphine-induced analgesia to a heat stimulus in mice (Ossenkopp et al., 1985). The authors suggested that exposure resulted in alterations in neuronal calcium binding and/or alterations in nocturnal pineal gland activity. It is not possible, however, to determine whether exposure to the static magnetic field affected this response.

Table 16. Analgesia					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Del Seppia et al., 2000)	Male C57 mice	Analgesia	Hypogeo-magnetic field using magnetic shield; SMF = 4 μ T. Oscillating field: f < 0.1 Hz: 20-70 μ T; 40 Hz: 80 μ T 2 h	Reduction of the response latency, if Earth's natural field was shielded. This effect was similar with oscillating magnetic field (f < 0.1 Hz).	Effects are comparable with the effect of an injection of 1.0 mg kg ⁻¹ of the specific opiate antagonist naloxone hydrochloride.
(Choleris et al., 2002)	Female CD1 mice	Analgesia	< 0.1 μ T 2 h	Reduction of analgesia by strong reduction of ambient magnetic field. However, no effect with zeroed field.	Zeroed field contained time-varying components (f < 0.1 Hz). Shielded field did not contain time varying components
MRI or combined exposure studies					
(Ossenkopp et al., 1985)	Mice	Analgesia	0.15 T 2x22.5 min	Alterations in day- and night-time responses to morphine.	Well-designed experiment. Exposure to SMF and RF fields (simulation of diagnostic procedure).

7.2.2.1.4 Behaviour

Behaviour is chiefly controlled by the central nervous system acting on the body's musculature via the peripheral nervous system and may be either spontaneous or a response to an environmental stimulus. Behaviour may be either innate or learned.

A number of studies have investigated the effects of static magnetic fields on the behaviour of animals. A majority of the papers have involved rodents (mice, rats, and mole rats), but studies of paramecium, turtles and mosquitoes have also been reported.

Davis et al. (1984) studied the behaviour of adult mice continuously exposed for up to 72 hours to 1.5 T. Three types of behavioural tests were employed, namely memory of electroshock,

general locomotor activity and sensitivity to a seizure-inducing neuropharmacological agent (pentylentetrazole or PTZ). No behavioural alterations were found in the exposed mice when compared to the controls in any of the experiments. Hong et al. (1988) exposed infant rats to 0.5 T for 14 postnatal days. Following a one-month rest period, there was no significant difference in learning ability (escape avoidance of a mild foot shock) between the control and exposed groups.

Nakagawa and Matsuda (1988) also exposed rats, but for a longer period. Rats were trained and observed for Sidman avoidance (SA) and for discriminative avoidance (DA) for 7 and 14 weeks, respectively. Prior to the completion of avoidance conditioning, rats in the SA group were exposed for 0.6 T for 16 hours/day for 4 days and rats in the DA group were exposed for 0.6 T for 6 hours/day for 4 days. Both exposed groups showed a diminished performance of avoidance responses. Trzeciak et al. (1993) exposed male, as well as pregnant and non-pregnant female, rats to 0.49 T for 2 hours per day for 20 consecutive days. Exposure had no effect on open-field behaviour and locomotor activity for either the male or female rats. However, a decrease in the 'irritability' of the rats, i.e. their responsiveness to being touched, was noted.

Weiss et al. (1992) investigated the aversive response of rats at static magnetic field levels of 0, 1.5 and 4 T using a simple T-maze with one arm extended into the bore of the magnet. No behavioural differences were found for 0 and 1.5 T. However, at 4 T it was found that the rats would not enter the exposed arm of the maze in 97% of the trials, and that this effect persisted for a short while when the exposed and sham exposed arms were reversed. The authors proposed that the aversive response was due to magnetic induction effects caused by motion in the strong magnetic field gradient. A similar experiment by Nolte et al. (1998), in which rats were given a conditioned stimulus (a taste solution) followed by exposure for 30 min to a 9.4 T magnetic field, showed a conditioned taste aversion that lasted for up to 8 days after the cessation of field exposure.

These behavioural effects of exposure to intense static fields were further explored in a series of recent studies by Houpt and co-workers (Lockwood et al., 2003; Houpt et al., 2003), who exposed restrained rats, and unrestrained and restrained mice, to static magnetic fields of up to 14 T. Exposure to a 9.4 T magnetic field for the same duration used by Nolte et al. (1998) resulted in increased c-Fos expression, taken as an index of neural activity, in the vestibular and visceral nuclei of the brain, suggesting activation of the vestibular and visceral neural pathways (Snyder et al., 2000). These authors noted that such effects were consistent with the suggestion by Schenck (2000; 2005) that small head movements in a strong static field could induce vestibular stimulation through the action of magnetohydrodynamic forces on the fluid within the semicircular canals. Further study by these authors (Lockwood et al.,

2003; Houpt et al., 2003) found that exposure of rats to either 7 or 14 T suppressed rearing as well as inducing tight circling, the direction of which was related to orientation within the field. Conditioned taste aversion was induced after exposure to either field strengths. Very similar results were obtained with mice following exposure to a 14.1 T static field. All mice developed a conditioned taste aversion and a significant number displayed tight circling and a suppression of rearing. Unrestrained mice exhibited larger effects than those that were restrained, supporting the view that these responses are consistent with vestibular stimulation as well as the reports of vertigo and nausea in people during movement in very strong static magnetic fields.

Tsuji et al. (1996) reported decreased food and water intake in mice exposed in a 5 T magnetic field for 24 or 48 h. The authors raise a number of possible explanations, including the suggestion that this was caused by discomfort experienced in the 5 T field.

A number of investigators have studied magnetic fields as navigational markers and their effects on spatial discrimination. Kimchi and Terkel (2001) used the blind mole rat in an eight-arm maze under natural and artificial magnetic fields to show that the rodent was able to perceive and use the Earth's magnetic field to orient in space. Lohmann et al. (2001) found that hatchling loggerhead sea turtles, when exposed to magnetic fields found in widely separated oceanic regions, swam in the direction that would keep them within the currents that facilitate their migratory pathway.

Strickman et al. (2000) found that mosquitoes placed in a 0.1 mT magnetic field moved until they were orientated parallel to the field. Mosquitoes were tested for the presence of a remnant ferromagnetic material indicative of a biological compass, but it was found that the external surface appeared to have an affinity for ferromagnetic particles.

Rosen and Rosen (1990) studied the motility of *Paramecium* following exposure to a 126 mT magnetic field. They observed a reduction in swimming velocity and a disorganization of movement pattern. The authors speculated that a realignment of anisotropic molecules within the membrane could have been responsible for the observation. This work was supported by a more recent study by Nakaoka et al. (2002), in which they found that the *Paramecium* swam perpendicular to 0.78 T.

Levine and Bluni (1994) and Levine et al. (1995) investigated whether a decrement in spatial discrimination learning in mice following exposure to a static magnetic field was due to an interaction between the field and physiological ferromagnetic material (magnetite) or instead to an effect on circulating melatonin levels. These authors exposed mice to static magnetic fields of up to 2 T for up to 100 min. The data partially supported learning interference following magnetic field exposure, but no

consistent effect on levels of circulating melatonin was found. However, the exposure was reported as having duplicated a 'spin-echo' MRI sequence, and so the possibility that pulsed gradient fields were also present cannot be ruled out.

Two studies by Ossenkopp and colleagues (Innis et al., 1986; Ossenkopp et al., 1986) examined the effects of exposure of rats to MRI fields for about 23 min upon the performance of several behavioural tests (namely spatial memory, open field behaviour and passive avoidance learning). The MRI exposure had no effect on task performance by exposed animals compared to those that were sham exposed. Similarly, Messmer et al. (1987) found that the exposure of rats for 30 min to MRI fields where the static field component was 1.9 T did not induce taste aversion. In this test, taste aversion to e.g. saccharin is induced by an associated exposure to a stimulus that was perceived as unpleasant.

Table 17. Behaviour					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Davis et al., 1984)	Mouse	Behaviour: passive avoidance trials, locomotor activity, PTZ seizure threshold	1.5 T 72 h	No effect on behaviour.	
(Hong et al., 1988)	Rat	Learning ability (escape-avoidance of mild foot shock)	0.5 T 14 d, 3x5 min d ⁻¹ (postnatal)	No effect of SMF on learning.	
(Nakagawa & Matsuda, 1988)	Rat	Behaviour	0.6 T 6 or 16 h d ⁻¹ , 4 d	Performance of avoidance responses inhibited, staying low for 2 - 3 wk after exposure.	
(Trzeciak et al., 1993)	Rat	Behaviour	0.49 T 2 h d ⁻¹ , 20 d	Decrease in irritability; no effect on open-field behaviour, locomotor activity.	

(Weiss et al., 1992)	Rat	Behaviour: walking into or avoiding magnetic field	0, 1.5, 4 T 4 min	Behaviour disruption at 4 T: initially 4 T is avoided; trained animals abruptly stop upon encountering a 4 T field.	Consistency with other high field experiments affecting vestibular region. Not clear if it is induced field or a direct effect of SMF exposure.
(Nolte et al., 1998)	Rat	Aversion to a conditioned taste stimulus	9.4 T 30 min	With one SMF conditioning day, approximately half of the rats showed conditioned taste aversion; with three conditioning days, 7/8 rats showed taste aversion lasting up to 8 days.	Consistent with other high field experiments affecting vestibular region. Not clear if it is induced fields or a direct effect of SMF exposure.
(Snyder et al., 2000)	Rat	c-Fos induction in visceral and vestibular nuclei of the rat brain stem	9.4 T 30 min	More c-Fos-positive cells in brainstem: neural activation in visceral and vestibular nuclei. Counterclockwise turning behaviour after exposure in 4/6 rats.	Consistency with other high field experiments affecting vestibular region. Not clear if it is induced fields or a direct effect of SMF exposure.

(Lockwood et al., 2003)	Mice	Behaviour: locomotor activity, conditioned taste aversion (CTA)	14 T 30 min	All restrained and unrestrained mice developed CTA; a significant number displayed tight circling and suppression of rearing. Effects larger in unrestrained mice.	Restraint stress might suppress effect somewhat, or effect might be enlarged due to movement of unrestrained animals
(Houpt et al., 2003)	Rat	Behaviour: locomotor activity, conditioned taste aversion (CTA)	7 or 14 T > 30 min	Exposure suppressed rearing and induced tight circling and CTA acquisition; strongest effect with 14 T.	Follow up of experiments of Nolte et al. 1998. Influence of restraint stress unknown.
(Tsuji et al., 1996)	Mouse	Food and water intake	5 T 24, 48 h	Exposure-dependent decreased food, water intake, body weight; no effect on organ weight.	
(Kimchi & Terkel, 2001)	Mole rat	Eight-armed maze test	Artificial geomagnetic field strength 2 d	Significant directional preference shown independent of light stimulation; decrease in performance under shifted SMF.	Relevance to human health unclear.
(Lohmann et al., 2001)	Sea turtles (hatchling)	Appropriate migratory direction	Artificial geomagnetic field strength Duration not reported	Significant directional preference shown.	Relevance to human health unclear.

(Strickman et al., 2000)	Mosquito	Blood-sucking	0.1 mT 5 min	Oriented parallel to SF; two of 3 species took fewer blood meals in a rotating MF (90° each 15 sec) than in the Earth's normal MF.	Advantage of orientation not clear; disrupted feeding possibly because of unnatural field conditions.
(Rosen & Rosen, 1990)	Paramecium	Motility	126 mT 48 h	Reduction in velocity, disorganization of movement.	Low numbers of organisms.
(Nakaoka et al., 2002)	Paramecium	Swimming orientation	0.68 T 0.5 - 1 sec	<i>Paramecium</i> swam perpendicular to SMF; no effect in AC MF (60 Hz, 0.65 T).	Lack of statistics, but effect clear. A result of diamagnetic anisotropy of cilia and trichocysts?

MRI or combined exposure studies					
(Levine & Bluni, 1994)	Mouse	Left-right discrimination learning ability and serum melatonin levels	0.3 T 30 min	Significant interference with spatial discrimination learning; variable melatonin levels.	MRI fields present. Animals transported 12 miles between MRI unit and laboratory.
(Levine et al., 1995)	Mouse	Left-right discrimination learning ability and serum melatonin levels	2.0 T 100 min	Significant interference with spatial discrimination learning; no changes in serum melatonin levels.	Possible confounding with other MRI magnetic fields.
(Innis et al., 1986)	Rat	Spatial memory test	0.15 T 23 min	No effect on spatial memory.	
(Ossenkopp et al., 1986)	Rat	Open field behaviour and passive avoidance test	0.15 T 22.5, 23.3 min, 5 d	No effect.	
(Messmer et al., 1987)	Rat	Taste aversion paradigm	1.89 T 30 min	No effect.	Positive control group (injected with 0.15 M lithium chloride) developed taste aversion.
Studies considered to be uninformative					
(Grzesik et al., 1988) (Nikolskaia et al., 1999) (Niko'skaia et al., 2000) (Phillips et al., 2001) (Nikolskaia & Echenko, 2002) (Wiltschko et al., 2002)					

7.2.2.2 Musculoskeletal system

The body musculature is, by and large, an effector organ of the nervous system. The skeletal system of animals provides an articulated frame against which the muscles of the body act to produce movement. In most animals, muscle contraction can be rapid, and results from the inherent excitability of muscle fibres. Muscles of the gastrointestinal tract and cardiovascular system are also under nervous system control, but have more of a 'housekeeping' regulatory role.

The bones of the skeletal system are relatively stable, but undergo constant remodelling in response to mechanical stresses. Piezoelectric effects within the bone are thought to have a role in this remodelling process, through the generation of electric fields.

7.2.2.2.1 Muscles

The biomechanical activity of muscle results from its chemical, electrical and mechanical excitability. The release of neurotransmitter from a motor nerve terminal results in an action potential in the muscle fibre that is transmitted along the cell membrane, activating a contractile mechanism. *In vivo* regulation of the biomechanical activity of the muscles is a complicated and integrated process. Animal models are very useful for investigation of the processes of *in vivo* effects of static magnetic field action on the processes of regulation of muscle activity.

The effects of chronic application of 0.02 T on specific ATPase activities, and bioelectrical and biomechanical responses, in isolated rat diaphragm muscle have been reported by Itegin et al. (1995). The mean activities of Na⁺-K⁺ ATPase and Ca²⁺ ATPase determined from diaphragm homogenates were significantly higher in the magnetic field exposed group, but that of Mg²⁺ ATPase was not significantly lower compared to the control group.

Static magnetic field effects on motor nerve conduction and resting membrane potential on muscle have been reported by Hong et al. (1986) and Itegin et al. (1995). The resting membrane potential, amplitude of muscle action potential, and overshoot values have been investigated. The latency was found to increase in the experimental group, and all the above-mentioned bioelectrical differences between the groups were statistically significant. Force of muscle twitch was found to decrease significantly in the magnetic field-exposed group. This finding was attributed to the augmenting effect of magnetic field on Ca²⁺ ATPase activity. According to Itegin et al. (1995), these results suggest that static magnetic field exposure changes specific ATPase activities and, hence, bioelectrical and biomechanical properties in the rat diaphragm muscle. Hong et al. (1986) described measurements of motor nerve conduction and excitability on the tail nerve of anaesthetized rats before and after nerve exposure to a static magnetic field of various intensities and

durations. No significant change was found in either the distal latencies or the amplitudes of the compound muscle action potential (CMAP) measured from stimulating the tail nerve after it was exposed to 1.2 T for 60 seconds. However, the nerve excitability expressed as changes of the amplitudes of the submaximally evoked CMAP increased significantly when the tail nerve was exposed to a static magnetic field with a magnetic flux density higher than 0.5 T for more than 30 seconds.

Table 18. Muscles					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Itegin et al., 1995)	Rat diaphragm muscle	ATPase activities and bioelectrical and biomechanical responses.	0.2 mT 4 h d ⁻¹ , 19 wk	Na ⁺ -K ⁺ ATPase and Ca ²⁺ ATPase activity increased; Mg ²⁺ ATPase not increased; resting membrane potential, amplitude of muscle action potential, and overshoot values lowered.	
(Hong et al., 1986)	Adult Sprague-Dawley albino rat	Motor nerve conduction and excitability	0.3 - 1.2 T 15, 30 or 60 sec	No effect on nerve conduction. Increased motor nerve excitability at > 0.5 T and t > 30 sec. Effect disappeared 1 min after termination of exposure. Effects dose related.	Anaesthetized with intraperitoneal chloral hydrate.
Studies considered to be uninformative					
(Gorczyńska & Wegrzynowicz, 1986b) (Tamaki et al., 1987) (Takeshige & Sato, 1996) (Satow et al., 2001)					

7.2.2.2.2 Bone growth

Osteoblasts and osteoclasts cells are dynamic components in this stable and slow-changing system. Environmental factors that have direct or indirect action on these two types of cells in bone tissue can play a very important role in processes of remodelling and re-shaping of the bones, as well as bone healing processes. The long bones are first modelled in cartilage and transformed into bone by ossification that begins in the shaft of the bone (enchondral bone formation). Osteoblast precursors secrete factors that affect osteoclast development, an observation that is not surprising given the necessity of maintaining a balance between resorption and formation. The influence of static magnetic fields on the growth of rodent bones has been studied using various methods. In general, low field strengths and long exposure times were employed.

Yan et al. (1998) implanted magnetic rods in rat femurs. The flux density close to the magnet was approximately 180 mT at most, while at 5 mm from the rod it was practically zero. The authors claimed to have observed a decrease in bone mineral density and calcium content in femurs implanted with non-magnetized rods at 12 weeks after the procedure. The authors concluded that normal bone mineral density and calcium levels were observed when the rods were magnetized. However, no significant differences seemed to be present in the data. It is likely that the statistical analysis of the data was incorrect. A further indication for this might be that the authors also claimed to observe changes in bone mineral density and calcium in the areas of the femur where the flux density was zero.

The same group also studied the effect of the same procedure in rat femurs that were made ischaemic (Xu et al. (2001). In this case, exposure lasted for 3 weeks. The authors observed significant differences between magnetized and unmagnetized rods only in the parts of the bone where the flux density was zero. Again, the statistical analysis of the data seems problematic. The authors speculated that exposure to the static magnetic field from magnetized rods resulted in an improvement in the blood circulation, leading to an improvement of bone growth. It is difficult to envisage that this would have an effect only in the unexposed areas. These experiments seem well-performed, but the analysis of the data is flawed. There is no effect of the implanted magnetic rods on bone growth.

Camilleri and McDonald (1993) used permanent magnets to apply 100 mT for 1 to 10 days to the sagittal sutures of rats. They observed a transient reduction in thymidine uptake at 3 days after the start of exposure, but no difference in tetracycline-incorporation. No effect on bone growth and inhibition of cell division could be demonstrated.

Fracture healing in a rabbit radius was studied by Bruce et al. (1987). They applied 22 - 26 mT for 4 weeks, using permanent magnets, and determined the force needed to break bone units consisting of ulna

and radius, which could not be separated without damaging the healing callus. Greater forces were needed to break the static magnetic field-exposed units. The authors suggested that static magnetic fields might accelerate the maturation of tissues, leading to thicker trabecular bone and thereby increasing the strength of a callus. However, no histological differences were detected.

The presumed effect of static magnetic field exposure on bone remodelling has led to the exploration of the use of permanent magnets in orthodontics to accelerate the repositioning of teeth. Tengku et al. (2000) studied the effect of such orthodontic magnets on the movement of the rat tooth. The magnets resulted in maximum flux densities of 10 - 17 mT and remained in position for 1 - 14 days. Tooth movement was identical in the animals treated with magnets and those treated with equal seized unmagnetized weights. However, a transient greater root resorption, an increased width of the periodontal ligament space and greater activity of clastic cells that are needed for bone remodelling were observed at 7 days in the animals with magnets. The force exerted by the device was reduced to zero by day 7 and remained so until day 14. It is possible that only force-induced alterations are influenced by the static magnetic field, but the overall effect on tooth movement is nil.

The influence of much higher fields on bone formation has also been studied. Kotani et al. (2002) implanted mice with pellets containing bone morphogenetic protein (BMP) 2 and exposed them for 60 hours to an 8-T field. Bone growth in and around the pellets was significantly higher in the exposed animals compared to the unexposed controls. The orientation of bone formation was parallel to the static magnetic field. The authors speculated upon several mechanisms, varying from alterations in membrane phospholipids to static magnetic field-induced mechanical stress, both leading to osteoblast differentiation. Exposure to a strong static magnetic field in combination with bone morphogenetic protein might be a clinically viable option for improving bone healing.

Kwong-Hing et al. (1989) used autoradiography and liquid scintillation to examine acute exposure effects of MRI on dentin and bone formation in mice. They found that exposure to a standard 23.2 min clinical multislice MRI (0.05 T) procedure caused a significant increase in the synthesis of the collagenous matrix of dentin in the incisors. The results suggest that the magnetic fields associated with MRI can affect cell activity. The mechanisms that may alter the incorporation of ³H-proline into the predentin layer remain largely speculative. The relative role of the three magnetic fields (static, radiofrequency, and time-varying) associated with MRI on the increase in the incorporation of ³H-proline still need to be examined.

Table 19. Bone growth					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Yan et al., 1998)	Rat	Bone formation	Implanted magnetic rods ~ 180 mT 12 wk	Authors reported a decrease in bone mineral density (BMD) and Ca content in femurs implanted with non-magnetized rods and normal levels in femurs implanted with magnetized ones.	No effect was observed.
(Xu et al., 2001)	Rat with ischaemic bone model	Bone formation	Implanted magnetic rods ~ 50 mT 3 wk	Bone mineral density lower in ligated + unmagnetized rods at 3 wk ; strongest in proximal and distal parts. Enhancement of femoral bone formation.	Stated flux density of 180 mT not in bone; 50 mT max, but at 5 mm from rod practically zero (i.e. in proximal and distal parts).
(Camilleri & McDonald, 1993)	Rat	Bone growth	100 mT 1 - 10 d	Transient reduction in thymidine uptake at 3 d. No difference in tetracycline-incorporation. No effect on bone growth.	
(Bruce et al., 1987)	Rabbit	Fracture healing	22 - 26 mT 4 wk	Greater forces needed for breaking bone units exposed to SMF; no difference in histopathologic comparison.	Change in breaking force not supported by histology.
(Tengku et al., 2000)	Rat	Orthodontic tooth movement	10 - 17 mT 1 - 14 d	Greater root resorption, increased width of periodontal ligament space and greater tartrate-resistant acid phosphatase activity at 7 d. SMF did not enhance tooth movement.	No description of magnetic material.

(Kotani et al., 2002)	Mouse	Bone formation (induced by implanted bone morphogenetic protein [BMP-2] – containing pellets).	8 T 60 h	Increase of ectopic bone formation in and around BMP-2-containing pellets, with orientation of bone formation parallel to SMF.	Orientation of bone formation parallel to SMF possibly due to diamagnetic anisotropy of membrane phospholipid in osteoblastic cells.
MRI or combined exposure studies					
(Kwong-Hing et al., 1989)	Mouse	Formation of dentin and bone	0.15 T 23.2 min	Increase in the synthesis of the collagenous matrix of dentin in the incisors.	
Studies considered to be uninformative					
(Linder-Aronson & Lindskog, 1991) (Linder-Aronson et al., 1992) (Darendeliler et al., 1995) (Linder-Aronson et al., 1995) (Linder-Aronson et al., 1996) (Darendeliler et al., 1997)					

7.2.2.3 Circulatory system

The circulatory system is the transport system of the organism that supplies O₂ and substances absorbed from the gastrointestinal tract to the tissues, and returns CO₂ to the lungs and other products of metabolism to the liver and kidneys. It also has a central role in the regulation of body temperature, and the distribution of the hormones and other agents that regulate tissue and cell function.

Blood, the carrier of these substances, is pumped through a closed system of blood vessels. The circulation is controlled by multiple regulatory systems, from blood pressure and heart rate controlling systems to those maintaining adequate capillary blood flow in all organs (but particularly in the heart and brain). The main components of the circulatory system are blood cells (erythrocytes, leucocytes and platelets), blood plasma (protein and non-protein component) and lymph. This section is concerned with static magnetic field effects on the circulatory system and on pharmacologically-induced changes to this system.

7.2.2.3.1 Cardiac function

The rhythmic beating of the heart, which maintains blood circulation, is driven by periodic waves of electrical excitation. These begin in the pacemaking region of the right atrium, spread through the right and left atria, triggering atrial contraction, then move through the atrio-ventricular node and conducting system of the bundle of His to the ventricular septum and walls, triggering ventricular contraction. This electro-mechanical process of wave propagation through the heart causes the pumping action of the heart and the circulation of blood.

Electrical potentials (flow potentials), generated across a blood vessel by the flow of blood in static magnetic field, have been recorded in a number of animal species exposed to magnetic fields greater than about 100 mT. While the physiological significance of these flow potentials remains unclear, they are of great importance in the light of the strong static fields used by MRI systems for clinical diagnostic analysis. They result from Lorentz forces acting on moving charges (see chapter 5) which are generally associated with ventricular contraction and the ejection of blood into the aorta. They appear superimposed on the T-wave of the ECG, which indicates the repolarisation of the ventricular heart muscle as electrical excitability gradually recovers following contraction.

Studies have been performed on a number of animal species. Briefly, flow potentials have been recorded in rats (Gaffey & Tenforde, 1981), rabbits (Togawa et al., 1967), dogs (Gaffey & Tenforde, 1979), monkeys (Beischer & Knepton, Jr., 1964; Beischer, 1969; Tenforde et al., 1983; Gorczyńska & Wegrzynowicz, 1989) and baboons (Gaffey et al., 1980). These and other studies are reviewed by Tenforde (2005). In large animal species, the flow potential can be detected in the ECG at magnetic field levels above approximately 0.1 T. The flow potential is a linear function of field strength up to 1.0 T. At higher field levels, the total electrical potential at the T-wave locus in the ECG increases more rapidly as a function of magnetic field strength, possibly as a result of the superposition of additional, weaker flow potentials. Total electrical potential also increases with body size; for example, the average increase in the T-wave signal amplitude in a 1.0 T field in rats is $\sim 75 \mu\text{V}$, whereas it is $\sim 175 \mu\text{V}$ in juvenile baboons. Similar alterations in the ECG have been observed in humans exposed to strong magnetic fields (see section 8.1.2.2.1).

Three studies have been performed on large mammals. Tenforde et al. (1983) exposed Macaca monkeys to 0.5 - 1.5 T fields. The authors observed an increase in the ECG signal amplitude with fields > 0.1 T but no magnetohydrodynamic effect on blood flow and no effect on blood pressure. They concluded that there was little or no cardiovascular stress. Kangarlu et al. (1999) performed cardiac and physiological safety studies in relation to magnetic resonance imaging in pigs. The animals were exposed in an MRI machine for 3 h to a field that had a flux density of 8 T at the location of the heart. This did not result in effects on heart rate,

blood pressure, cardiac output or several other vital parameters. Finally, Bourland et al. (1999) reported that exposure to a static magnetic field of 1.5 T had no effect on the cardiac ectopic beat threshold of anaesthetized dogs in which a temporary and reversible cardiac arrest had been induced by vagus nerve stimulation. The ectopic beat was induced by eddy currents resulting from rapidly switched gradient magnetic fields. A lack of effect on ectopic beat threshold was also seen in two dogs in which the heart was beating normally during stimulation.

As part of a study on potentially hazardous effects of magnetic field produced by MRI on cardiac function in the rat and guinea pig, Willis and Brooks (1984) exposed the animals to 0.16 T static magnetic fields only (5 min on, 5 min off, repeated four times). No changes were observed in blood pressure, heart rate, or ECG. No sham exposures were performed, but a comparison was instead made between the field on and field off periods.

Other studies have been more equivocal. Nakagawa (1984) reported that 600 mT fields for 33 min had no effects of various rabbit heart rate parameters, but that a transient effect was seen immediately after exposure to the field. A previous study by Nakagawa (1978) revealed that reserpine-treated rabbits exposed to 60 mT for 5 weeks had an increased in heart rate and blood flow when compared with the sham exposed animals.

Klimovskaia and Smirnova (1975) exposed rabbits to 0.45 T for 30 min or 3 h. They observed a transient hypotension, a decrease in respiratory rate and a trend towards brachycardia. They also subjected the animals to accelerations of 6 and 10 G. The compensatory reactions of the cardiovascular and respiratory system induced by such treatments were not influenced by SMF exposure.

In two papers, Gmitrov and Ohkubo (2002a; 2002b) reported the effects on cardiovascular regulation of exposure to an artificial SMF in relation to geomagnetic field disturbances. The baroreflex sensitivity (BRS), arterial pressure and heart rate were determined in rabbits before and after 40 min of local 0.35 T SMF exposure applied at the position of the sinocarotid baroreceptor. Increased geomagnetic field disturbances decreased baroreflex sensitivity, which lead to deregulated blood pressure. Administration of nitroprusside interrupted the baroreceptor reflex, and the static magnetic field antagonized this effect. Verapamil, a Ca^{2+} channel-blocking agent, antagonized the effects of applied static magnetic field and geomagnetic field disturbances. The authors speculated that exposure of the sinocarotid baroreceptor to static magnetic field, along with modification of the pharmacotherapy for hypertension, should be effective on days with intense geomagnetic disturbance, perhaps through modulation of Ca^{2+} channel permeability.

Table 20. Cardiac function					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Gaffey & Tenforde, 1981)	Rat	ECG, heart rate, breathing rate	up to 2.1 T 2 - 3 min (ECG, heart rate, breathing rate); 1.5 T 5 h (post-exposure ECG)	If SMF > 0.3 T, T-wave amplitude increased in a field strength-dependent manner; no changes in heart rate or breathing rate. No short-term or long-term effects of 5-h exposure.	Effect might be caused by field gradient.
(Tenforde et al., 1983)	Monkey	ECG, blood pressure	0.5 - 1.5 T	Increase in ECG signal amplitude in fields > 0.1 T; no effect on blood pressure; magnetohydrodynamic effect on blood flow. Little or no cardiovascular stress.	
(Kangarliu et al., 1999)	Swine	Cardiac function	8 T 3 h	No effect on body temperature, heart rate, ventricular pressure, and cardiac output	
(Bourland et al., 1999)	Dogs	Ectopic beat threshold in temporarily arrested and beating hearts	1.5 T Ectopic beats stimulated by rapidly changing gradient magnetic field	No difference in ectopic beat threshold in exposed and unexposed animals.	Dogs anaesthetised with pentobarbital
(Willis & Brooks, 1984)	Rat, guinea pig	ECG, blood pressure	0.16 T 5 min on, 5 min off, x 4	No effect on ECG and blood pressure.	

(Nakagawa, 1984)	Rabbit	Electrophysiological responses	600 mT 33 min	Transient decrease in heart rate after exposure.	No description of methods for statistical analysis.
(Nakagawa, 1978)	Rabbit	Cardiovascular system	60 mT 5 wk	Increase in heart rate (at 3 and 5 weeks) and blood volume of a central artery of an ear lobe (2 - 5 weeks) as compared with reserpine treated animals.	
(Klimovskaia & Smirnova, 1975)	Rabbit	EEG, arterial pressure, heart rate, respiratory rate, reactions to adrenalin, and electrical stimulation of brain	0.45 T 30 min and 3 h	Transient hypotension; decrease in respiratory rate; bradycardia. No decrease of compensatory reactions in response to accelerations of 6 and 10 G.	
(Gmitrov & Ohkubo, 2002a)	Rabbit	Baroreflex sensitivity (BRS), arterial pressure, heart rate	0.35 T 40 min	Increase in BRS by SMF for nitroprusside depressor test; SMF antagonized decrease in BRS by geomagnetic disturbance.	
(Gmitrov & Ohkubo, 2002b)	Rabbit	Baroreflex sensitivity (BRS), arterial pressure and heart rate	0.35 T 40 min	SMF and geomagnetic effect on BRS antagonized by Ca ²⁺ channel blocker.	
Studies considered to be uninformative					
(Reno & Beischer, 1966)					
(Behari & Mathur, 1997)					

7.2.2.3.2 Blood pressure

The regulation of blood pressure by the cardiovascular system during circulatory changes faced normally in everyday life and

abnormally in disease illustrates the integrated operation of the cardiovascular regulatory mechanisms.

Hypertension is sustained elevation of the systemic arterial pressure. The arterial pressure is determined by the cardiac output and the peripheral resistance (pressure = flow \times resistance). The peripheral resistance is determined by the viscosity of the blood and, more importantly, by the calibre of the resistance vessels. Hypertension can be produced by elevating the cardiac output, but sustained hypertension is usually due to increased peripheral resistance.

Meszaros (1991) investigated effects of 40 mT and platelet activating factor on blood pressure in rats. Transient hypotension (lasting 1 - 2 hours) was induced by injection of iron beads and 5-min exposure to the static magnetic field, but this was only seen when the middle part of the body was exposed and when there were short intervals between bead injections and static magnetic field exposure. Hypotension was prevented or reverted by a platelet activating factor antagonist.

Okano and Ohkubo (2001) examined the effects of static magnetic fields on blood pressure in conscious rabbits. Blood pressure was pharmacologically altered and a flux density of 1 mT was applied locally to the ear for 30 min. Blood pressure was decreased by nicardipine, a Ca²⁺ channel blocker, or increased by the nitric oxide synthase inhibitor N(omega)-nitro-L-arginine methyl ester (L-NAME). Static magnetic field exposure counteracted the effects of both drugs. However, the field was only locally applied to part of the ear. The exposed area is probably too small to alter blood pressure in the entire animal, especially in the presence of the pharmacological agents. The effect might possibly have been mediated by the autonomic nervous system.

The study by Okano and Ohkubo (2003a) is a continuation of the above-mentioned paper (Okano & Ohkubo, 2001). In this case, they investigate anti-pressor effects of whole-body exposure to a static magnetic field (5.5 mT for 30 min) on pharmacologically-induced hypertension in conscious rabbits. A suppression of both noradrenaline-induced and L-NAME-induced vasoconstriction and hypertension was observed, but there was no effect on microcirculation and blood pressure without pharmacological treatment. The mechanisms of the reduction of artificial hypertension are unknown.

Okano and Ohkubo (2003b) investigated the effects of static magnetic fields on the development of hypertension using young male, stroke resistant, spontaneously hypertensive rats (SHRs) beginning at 7 weeks of age. The animals were exposed to static magnetic fields of 3.0 - 10.0 mT or 8.0 - 25.0 mT for 12 weeks. Static magnetic fields suppressed and retarded the development of hypertension for several weeks in both exposed groups to a statistically significant extent when compared with an unexposed group. A recent paper by Okano et al. (2005a) indicated

that a static magnetic field at 5 mT, but not at 1 mT, suppressed and retarded hypertension, and also reduced plasma concentrations of NO metabolites, angiotensin II and aldosterone, in rats. The antipressor effects are probably related to the extent of reduction in plasma levels of angiotensin II and aldosterone in the SHRs. Okano et al. (2005b) also reported that static magnetic fields in the range of 7.5 - 25.0 mT applied for 2 - 12 wks reversed reserpine-induced hypotension and bradykinesia in rats.

Table 21. Blood pressure					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Meszaros, 1991)	Rat	Blood pressure	40 mT 5 min	No effect after saline+ SMF. Rapid decrease in arterial blood pressure, lasting 1 - 2 h after iron+SMF; only seen when middle part of body was exposed and with short interval between iron and SMF; hypotension prevented or reverted by PAF antagonist.	
(Okano & Ohkubo, 2001)	Rabbit	Blood pressure (BP) and cutaneous micro-circulation	1 mT 30 min	Reduced vasodilatation, enhanced vasomotion; antagonized reduction of BP; attenuation of vasoconstriction, suppression of elevation of BP.	
(Okano & Ohkubo, 2003a)	Rabbit	Pharmacologically induced hypertension	5.5 mT 30 min	No effects in normal animals. Hypertension suppressed by SMF.	
(Okano & Ohkubo, 2003b)	Rat	Blood pressure	3 - 10 or 8 - 25 mT 12 wk	SMF suppressed and retarded hypertension. Also reduction in angiotensin II and aldosterone. No dose effect.	

(Okano et al., 2005b)	Rat	Reserpine-induced hypotension	3.0 - 10.0, 7.5 - 25.0 mT 12 wk	SMF in the range of 7.5 –2 5.0 mT for 2 - 12 wk reversed reserpine-induced hypotension and bradykinesia compared with sham-exposed reserpine-treated rats.	
(Okano et al., 2005a)	Rat	Blood pressure	0.3 - 1 or 1 - 5 mT 12 wk	SMF at 1 - 5 mT suppressed and retarded hypertension. Also reduction in NO, angiotensin II and aldosterone.	

7.2.2.3.3 Blood flow

One possible site of the action of static magnetic fields is on blood flow. As a dynamic system of movable charges, this system is one that is at a susceptible point for static magnetic field action on the level of organism. Ohkubo and co-workers in Japan have performed a series of experiments in rodents. Most studies concerned acute effects of short exposures to low flux densities.

Ohkubo and Xu (1997) studied cutaneous microcirculation in conscious rabbits using an ear chamber, a device that allows direct observation of cutaneous capillaries. A flux density of 1, 5 or 10 mT was applied for 10 min. Static magnetic field exposure induced non-dose-dependent changes in vasomotion in a biphasic manner. With high amplitude of vasomotion, static magnetic fields induced vasoconstriction; with low amplitude of vasomotion static magnetic fields induced vasodilation. The physiology of this effect is still unclear. It could be the result of autonomic nervous system regulation mechanisms, or stimulation and suppression of normal metabolism in tissues that is related to changes in oxygen consumption. Immobilization stress cannot, however, be excluded.

In another study from this group, Okano et al. (1999) performed a more extensive investigation of the biphasic effect. They exposed conscious rabbits to 1 mT for 10 min while either increasing the vascular tone by noradrenaline administration, or decreasing it using acetylcholine. Static magnetic fields resulted in vasodilatation and increased vasomotion under high vascular tone and in vasoconstriction and decreased

vasomotion under low vascular tone. Thus, static magnetic fields counteract the effects of noradrenaline and acetylcholine.

Gmitrov et al. (2002) studies the effect of exposure to 0.2 and 0.35 T static magnetic fields on haemodynamics in anaesthetized rabbits. They found an increased blood flow during and after exposure, when compared with pre-exposure baseline and sham exposed controls. The authors postulated that long exposure to high level non-uniform static magnetic fields modifies microcirculatory homeostasis through modulation of the local release of endothelial neurohumoral and paracrine factors that act directly on the smooth muscle cells of the vascular wall, presumably by affecting ion channels or a second messenger system. However, data analysis in this paper is not completely clear. In the controls, anaesthesia resulted in a decrease in blood flow. A similar mechanism might be the cause of the observed trend for a decrease in blood flow after cessation of static magnetic field exposure.

In a study by Xu et al. (1998), subchronic effects of a locally applied static magnetic field on cutaneous microcirculation in rabbits were observed. The microcirculation was studied using ear chambers in conscious animals after exposure, by Sm-Co permanent magnets attached to the ear chamber, to 180 mT for 24 hours to 4 weeks. Exposures for 1 - 3 weeks significantly increased the amplitude of long-lasting vasodilatation and enhanced the vasomotion. The increased vasomotion in the exposed group decreased again to the initial values after exposure. It is peculiar that a short 1-mT exposure still has a measurable effect after prolonged 180 mT exposure. Xu et al. (2001) studied acute microhaemodynamic effects of whole-body exposure in anaesthetized mice to static magnetic fields. Exposure was to 0.3, 1 and 10 mT for 10 min. An increased peak blood velocity was observed for flux densities of 1 mT or higher. The effect remained for up to 35 hour after exposure.

The effects of short exposures to very high flux densities of 8 T on blood flow were investigated in three studies by Ichioka. Such a strong magnetic field could evoke induction of electrical potentials and magnetomechanical forces. Ichioka et al. (1998) exposed anaesthetized rats for 20 min and measured microcirculatory blood flow. Following exposure, blood flow initially increased for about 5 min, and gradually decreased and returned to control level thereafter. The effect is probably a rebound of the reactive hyperaemia and the action of magneto-hydrodynamic forces that induced a reduction of blood flow during exposure.

In a more elaborate study that followed this preliminary one, Ichioka et al. (2000) observed that a high-intensity static magnetic field (again 8 T applied for 20 min) can simultaneously modulate skin microcirculation and body temperature in anaesthetized rats. A decrease of the skin blood flow and temperature during exposure were observed,

both of which tended to recover after cessation of exposure. It is possible, however, that the anaesthesia reduced the temperature-controlling mechanisms. In a subsequent study, Ichioka et al. (2003) demonstrated that an 8-T field induced water vapour movement over the body of the animals, thereby decreasing the humidity in the air around the animals. This may well explain the decrease in skin temperature observed in the earlier experiments. In a study with both mice and rats exposed to a uniform 1.5 T field and to a 60 T m⁻¹ gradient field, Tenforde (1986b) found no effects on core body temperature of exposures up to 3 h in duration.

Table 22. Blood flow					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Ohkubo & Xu, 1997)	Rabbit	Cutaneous micro-circulation in rabbit ear chamber	1, 5, 10 mT 10 min	Non-dose-dependent variation of vasomotion; 10 sec. latency	
(Okano et al., 1999)	Rabbit	Cutaneous micro-circulation in rabbit ear chamber	1 mT 10 min	Vasodilatation, vasomotion under noradrenaline-induced high vascular tone; vasoconstriction, reduced vasomotion under acetylcholine-induced low vascular tone.	
(Gmitrov et al., 2002)	Rabbit	Micro-circulation	0.25 T 80 min	Increased blood flow during and after exposure when compared with pre-exposure baseline and control experiments.	Data analysis not fully clear.
(Xu et al., 1998)	Rabbit	Cutaneous micro-circulation in rabbit ear chamber	180 mT 24 h - 4 wk	Vasodilation, enhanced vasomotion at 1 - 3 wk, disappears thereafter.	
(Xu et al., 2001)	Mouse	Muscle capillary micro-circulation	0.3, 1, 10 mT 10 min	Peak blood velocity increased at 1 and 10 mT.	

(Ichioka et al., 1998)	Rat	Cutaneous micro-circulation in dorsal skinfold chamber	8 T 20 min	After exposure, 5 min increase in blood flow, then return to control values.	
(Ichioka et al., 2000)	Rat	Cutaneous micro-circulation in dorsal skin pocket	8 T 20 min	Decreased skin blood flow and temperature during exposure; rectal temperature tended to decrease.	
(Ichioka et al., 2003)	Rat	Skin temperature changes	8 T 5 min	Skin temperature decrease related to SMF-induced decrease in humidity around the body.	
MRI or combined exposure studies					
(Tenforde, 1986b)	Rat, mouse	Temperature changes	1.5 T + 60 T m ⁻¹ up to 3 h	No effects on core temperature.	
Studies considered to be uninformative					
(Lud & Demeckiy, 1990) (Steyn et al., 2000)					

7.2.2.3.4 Blood brain barrier

The tight junctions between capillary endothelium in the brain and between the epithelial cells in choroid plexus effectively prevent proteins from entering the adult brain and slow the penetration of smaller molecules. The rate of passage of molecules is inversely proportionate to their size and directly proportionate to their lipid solubility. This barrier to the exchange of substances between the brain and the blood and cerebrospinal fluid is referred to as the blood-brain barrier.

Shivers et al. (1987) reported that exposure to a short clinical MRI procedure (0.15 T) elicited a temporary dysfunction of the blood-brain barrier in rats. Recovery of normal blood-brain barrier function was completed 15 - 30 min following cessation of the MRI exposure.

Prato et al. (1990) exposed adult rats to a clinical MRI procedure at 0.15 T. They measured the concentration of a radioactive tracer, ¹⁵³Gd-DTPA, in whole-brain homogenates and in blood samples. A significant increase in the blood-brain barrier permeability was observed in the MRI exposed group compared to a sham exposed group. Prato et al. (1994)

also exposed adult rats to various exposure scenarios. An MRI examination was simulated in two animal groups. A third group was exposed to a pure static field of 1.5 T and a fourth group to 1.89 T. A significant increase in the blood-brain barrier permeability was observed in one of the MRI exposed groups, whereas a decrease was found, compared to a sham exposed group, in the other MRI exposed group. Both groups exposed to static magnetic fields showed significant increased blood-brain barrier permeability only when compared to their respective sham controls. However, the extent of increase in permeability was small and within the range of diurnal variation. In addition and as the authors noted, interpretation is complicated by the fact that the increased tracer concentration in brain tissue could also have resulted from an increase in cerebral blood volume, without any change in the permeability of the blood-brain barrier.

Table 23. Blood Brain Barrier					
Authors	Animal	Endpoint	Exposure	Results	Comments
MRI or combined exposure studies					
(Shivers et al., 1987)	Rat	Blood brain barrier (BBB)	0.15 T (MRI) 23.2 min	Temporary opening of BBB, recovered 15 - 30 mins after exposure.	
(Prato et al., 1990)	Rat	Blood brain barrier permeability (BBBP)	0.15 T (MRI) 2 x 23.2 min	BBBP significantly increased.	
(Prato et al., 1994)	Adult male Sprague-Dawley rat	Blood brain barrier permeability (BBBP)	1.5 T (MRI); 1.5 T (MRI with reduced RF and increased gradient field); 1.5 T (SMF only); 1.89 T (SMF only) 2 x 22.5 min	BBBP increased after regular MRI, but decreased after modified MRI. Both SMF only levels increased BBBP.	

7.2.2.3.5 Blood cells

The results from several researchers are quite contradictory. This may be due to different testing systems, as well as to different magnetic field exposures (static magnetic fields alone, static magnetic fields in combination with RF exposure, etc.).

Atef et al. (1995) reported effects on haemoglobin structure and function of 10 min exposures to magnetic fields of 0.4 T. No changes in dimensions and shape of haemoglobin molecules were observed, but a decrease in auto-oxidation reaction rate was found. It was suggested that auto-oxidation might be reduced by static magnetic field exposure through a stabilizing effect on the tertiary conformation of haemoglobin. Bhatia (1999) detected an increased phagocytic activity at 37 °C and a decreased activity at 27 °C after exposing mice to 1.4 T for 60 min. However, the changes involved a maximum of 25% and the clinical relevance of this observation is questionable.

Feinendegen and Muhlenziepen (1987) reported an increase in thymidine kinase activity in mouse bone marrow cells after static magnetic field exposures from 0.2 to 1.4 T for 5 to 30 minutes. The mice were anaesthetised and body temperature was manipulated using a thermostatically controlled chamber. Thymidine kinase activity was increased at a body temperature of 27 °C and decreased at 37 °C. The effects were not lasting. The measured values returned to baseline within several minutes after completion of static magnetic field exposures. The differences in the size of the experimental groups (with a range from 9 to 45 mice) and the lack of information concerning the controls are weaknesses of the study. Stegemann et al. (1993) reported about the effects on the activity of acetylcholinesterase in mouse bone marrow cells, which was inhibited by about 20% after a static magnetic field exposure of 30 min to a 1.4 T field. However, they did not mention the number of the control animals nor the conditions that they were kept in. As the experiments were obviously not blinded, it is unclear whether or not there was bias in the reporting of static magnetic field-associated effects.

The viability of bone marrow cells *in vivo* can be tested by isolating these cells, injecting them in recipient animals, and counting the number of spleen colonies formed. In a follow-up experiment to the one cited above, Peterson et al. (1992) used this technique to study the effect of variations in field strength (0.5 - 1.4 T), exposure time (15 - 60 min) and body temperature (20 - 37 °C) on murine bone marrow cells. An increase in cell viability was observed after a 30 min exposure to 1.4 T only with a body temperature of 27 °C, but not with lower or higher temperatures. No effect was seen with lower field strengths. When the body temperature was kept constant at 27 °C and the field strength at 1.4 T, an effect of exposure duration was found: that is, longer exposure resulted in higher cell viability. The authors speculated that the specificity of the effect might be membrane related.

Several other studies reported on the effects of long-term continuous or intermittent static magnetic field exposure. Tenforde and Shifrine (1984) examined the effects of long continuous magnetic field

exposure (6 d at 1.5 T) on the immune system of mice being exposed to a static magnetic field. No effects were detected.

Gorczyńska and Wegrzynowicz (1983) and Gorczyńska (1987b) published several studies regarding long intermittent magnetic field exposure (maximum 0.3 T, 1 h d⁻¹, 7 weeks maximum). Gorczyńska described alterations in the coagulation system, in blood cell numbers, and in the haematopoietic organs of guinea pigs. In nearly every study published by this group, effects were described which were more or less linked to static magnetic field exposure. Unfortunately, only limited information is given about the experimental and environmental conditions. The data reported in the publications appear to be incomplete and the statistical analysis, if mentioned, seems to be questionable.

Table 24. Blood cells					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Atef et al., 1995)	Mouse	Haemoglobin structure and function	0.1 - 0.4 T 10 min	No changes in dimensions or shape of Hb molecule; decrease in auto-oxidation reaction rate.	
(Bhatia, 1999)	Mouse	Membrane and receptors of the reticulo-endothelial cells of bone marrow	1.4 T 60 min	Increased phagocytic activity at 37 °C and decreased activity at 27 °C	The increase or decrease of phagocyte activity is a max of ~ 25 %. Relevance is unclear.
(Feinendegen & Muhlensiepen, 1987)	Mouse	Thymidine kinase activity	0.2 - 1.4 T 5 - 30 min	Dose-dependent increase at body temperatures of 27 °C and 29 °C, decrease at 37 °C. Max effect in 30 min, return to baseline in 5 - 10 min. Membrane effect?	Effects were only measurable for minutes, not lasting. Relevance is unclear.
(Peterson et al., 1992)	Mouse spleen colonies (CFU-S7d)	Numbers of spleen colonies at different body temperatures	> 1.4 T > 15 min 20 - 37 °C	Increase in number of spleen colonies if SMF exposure was at least for 30 min at 1.4 T at a body temperature of 27 °C. Specificity of effect may be membrane effect.	
(Stegemann et al., 1993)	Mouse	Acetylcholinesterase activity	1.4 T 30 min	Max inhibition at 3.5 (27 °C) or 2 h (37 °C); incomplete recovery at 15 h.	Reduction of approximately 20%. Relevance is unclear.

(Tenforde & Shifrine, 1984)	Mouse	Humoral and cell-mediated immune responses	1.5 T 6 d	No effects.	
(Gorczyńska & Wegrzynowicz 1983)	Guinea pig	Platelet count; platelet aggregation; prothrombin and partial thromboplastin times; fibrinogen and fibrinolysis	5, 300 mT 1 h d ⁻¹ , 7 wk	Decreased platelet count; increased platelet aggregation; increased prothrombin and partial thromboplastin times; decreased fibrinogen, increased fibrinolysis.	No statistical test specified.
(Gorczyńska 1987b)	Guinea pig	Myelopoiesis	0.05, 0.3 T 1 h d ⁻¹ , 7 wk	Alterations in numbers of various blood cells, independent of exposure time.	
(Gorczyńska 1987a)	Guinea pig	Ceruloplasmin activity and iron content in serum; liver and spleen morphology	5, 300 mT 1 h d ⁻¹ , 7 wk	Drop in ceruloplasmin activity; unchanged serum iron content; morphological changes of spleen; functional disturbances of liver.	
Studies considered to be uninformative					
(Barnothy & Barnothy, 1970) (Battocletti et al., 1981) (Turieva-Dzodzikova et al., 1995) (Zhernovoi et al., 2001)					

7.2.2.3.6 Blood serum enzymes

Blood serum is a colloid system with proteins and an inorganic component. Some of the proteins have a transport function, some have an immune defence function and some are enzymes. The accent in this section is especially on static magnetic field effects on enzyme activity in blood plasma. Most of the studies reviewed below were judged to be scientifically weak.

Gorczyńska and Wegrzynowicz (1984; 1985; 1986a; 1986b; 1989) and Gorczyńska (1986; 1987a; 1988) have published a series of experiments on the effects of static magnetic field exposure on various outcomes. Guinea pigs were used in the majority of experiments, but use was also made of rabbits and rats. The exposures were homogenous static magnetic fields created by a 'resistive electromagnet', with exposure levels varying between 0.005 T and 0.3 T. Animals were exposed for one hour per day, 1 - 7 weeks. Control animals were placed for one hour per day in glass vessels similar to the ones used with the exposed animals. For almost all of the studied outcomes, an effect of the exposure was found that was independent of exposure level, but that generally increased with the duration of exposure. The findings included an indication of decreased concentration of serum protein, an increase in acid phosphatase activity, increased Na^+ concentration, indications of increased K^+ concentration, decreased chloride concentration, decreased glutamic pyruvic transaminase (GPT) activity, an increased level of fibrinogen degradation products (FDP) and a corresponding decrease in the concentration of fibrinogen in plasma. No morphological changes in the cardiac or skeletal muscles, kidneys, cerebellum, and lung tissue were observed. In one experiment using Wistar rats (Gorczyńska & Wegrzynowicz, 1989), effects were independent of both magnetic field level and duration of exposure, showing increased GPT activity, increased glutamic oxaloacetic transaminase (GOT) activity, increased lactic dehydrogenase activity, increased alkaline phosphatase activity, and reduced cholinesterase activity for all exposure levels regardless of exposure duration (1 - 7 weeks). However, experimental setup was only briefly described, and there was no description of animal pre-experimental conditions or of their conditions during the experiment. The statistical methods used were also not described.

Watanabe et al. (1997) studied lipid peroxidation in the liver, kidneys, heart, lungs, and brain of mice exposed to 3.0 T or 4.7 T for 1, 3, 6, 24, or 48 hours, or to a sham control. No effect was found at 3.0 T exposures, whereas lipid peroxidation in liver, but no other organ, was increased after 3 hours or longer of exposure to 4.7 T. Increased lipid peroxidation in liver and GOT and GPT activities, were found after administration of CCL_4 combined with exposure to 4.7 T, exceeding the effect of each exposure alone.

Osbakken et al. (1986) studied effects of exposure to a 1.89 T superconductive magnet on adult and offspring mice. The study design included cage-control groups, control groups housed in the magnet room 20 feet from the magnet, and exposed groups housed in the magnet room. Animals were exposed during a certain part of the day, for one to three months, for a total of 360 - 624 h. Animal populations and their living conditions were clearly described. No consistent differences were found in gross and microscopic morphology, haematocrit and white blood cell

counts, or in plasma creatine phosphokinase, lactic dehydrogenase, cholesterol, triglyceride, or protein concentrations.

Papatheofanis and Papatheofanis (1989a; 1989b) studied acid and alkaline phosphatase activity in bone and blood, and calcium and phosphate ion concentrations, in serum from exposed, sham-exposed, and cage controlled groups of mice. Animals were exposed for 30 minutes per day during 10 consecutive days to a 1 T homogenous static magnetic field generated by an electromagnet. No effects were found on ion concentrations or any of the other studied endpoints.

Table 25. Blood serum, enzymes					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Gorczyńska & Wegrzynowicz, 1984)	Guinea pig	Serum protein concentration	0.005 - 0.3 T 1h d ⁻¹ , 6 wk	Indication of decrease in serum protein concentration dependent on duration of exposure, but not on magnetic field level.	Weak methodology.
(Gorczyńska & Wegrzynowicz, 1985)	Guinea pig	Acid and alkali phosphatase	0.005, 0.3 T 1 h d ⁻¹ , 1 - 7 wk	Increase of acid, but not alkali phosphatase regardless of exposure level.	Weak methodology.
(Gorczyńska & Wegrzynowicz, 1986a)	Guinea pig	K ⁺ , Na ⁺ and chloride concentrations in serum	0.005 - 0.3 T 1 h d ⁻¹ , 6 wk	Increasing Na ⁺ , decreasing Cl ⁻ in serum; no statistically significant effect on K ⁺ . No differences were found 14 days after exposure cessation.	Weak methodology.

(Gorczyńska & Wegrzynowicz, 1986b)	Guinea pig	Glutamic pyruvic transaminase (GPT) activity and morphological changes in the cardiac or skeletal muscles, kidneys, cerebellum, and lung	0.005, 0.05, 0.1, 0.3 T 1 h d ⁻¹ , 7 wk	Changes in enzyme activity related to exposure duration; no morphological changes. Decrease of GPT in guinea pig, but increase in rat (see 1989 paper below).	Weak methodology.
(Gorczyńska & Wegrzynowicz, 1989)	Rat	Cytoplasmatic enzymes, cholinesterase activity, alkaline phosphatase activity	0.008, 0.15 T 1 h d ⁻¹ , 1 - 7 wk	Increased activity of cytoplasmatic enzymes (GPT, GOT, LDH), decreased cholinesterase activity, increased alkaline phosphatase activity; independent of exposure intensity or duration; reversible, return to normal 2 months after experiment.	Weak methodology.
(Gorczyńska, 1986)	Rabbit	Fibrinogen degradation products	0.005, 0.12, 0.3 T 1 h d ⁻¹ , 2 - 4 wk	Increased fibrinogen degradation product related to duration of exposure, not to dose.	Weak methodology.
(Gorczyńska, 1988)	Rabbit	Fibrinolysis	0.005, 0.1, 0.3 T 1 h d ⁻¹ , 4 wk	Duration-dependent increase in rate of fibrinolysis.	Weak methodology.

(Watanabe et al., 1997)	Mouse	Lipid peroxidation, GOT and GPT activity	3.0, 4.7 T 1, 3, 6, 24, or 48 h	Increase of lipid peroxidation in the liver after exposure to 4.7 T, but not 3.0 T. The increase did not vary with duration of exposure. Effect of exposure to 4.7 T on GOT or GPT, only after treatment with CCL ₄ . No effect on lipid peroxidation in kidney, heart, lung, or brain.	
(Osbakken et al., 1986)	Mouse	Gross and microscopic morphology; haematocrit and white blood cell counts; plasma creatine phosphokinase, LDH, cholesterol, triglyceride and protein concentrations	1.89 T 360 h over 1 month - 624 h over 3 months	No effects.	Weak methodology.
(Papatheofanis & Papatheofanis, 1989a)	Mouse	Bone acid and alkaline phosphatase activity	1 T 30 min d ⁻¹ , 10 d	No effects.	Weak methodology.
(Papatheofanis & Papatheofanis, 1989b)	Mouse	Ionic and enzymatic constituents of the circulatory system, e.g. blood alkaline phosphatase, acid phosphatase, calcium ion concentration and phosphate ion concentration	1 T 30 min d ⁻¹ , 10 d	No effects.	Weak methodology.

7.2.2.4 Endocrine system

The endocrine system, like the nervous system, adjusts and correlates the activities of the various bodily systems to the changing demands of the external and internal environment of the body.

The pineal and pituitary neuroendocrine glands, both situated in the brain and under neural control, release hormones into the blood stream that exert a profound influence on body metabolism and physiology, partly via their influence on the release of hormones from other endocrine glands situated elsewhere in the body. For example, hormones from both the pineal and pituitary glands play a central role in the control of reproduction.

7.2.2.4.1 Pineal gland

The pineal gland, or epiphysis, secretes melatonin, which functions as a timing device to keep internal physiological processes synchronized with the light-dark cycle in the environment. Melatonin is produced and released by the pineal gland only during the dark. Changes in day length can be taken as a marker of season, and melatonin has an important role in controlling the onset of reproduction in seasonally breeding animals.

A series of experiments from the University of Mainz, Germany, have focused on the effects of static magnetic field exposure on pineal gland melatonin synthesis and cell activity. An artificial magnetic field was generated by a pair of Helmholtz coils, of either 1.5 m or 3.0 m in diameter, creating an inversion of the horizontal component of the Earth's magnetic field. The response of pineal cells from guinea pigs and rats to a direct stimulation of a static magnetic field was investigated in two experiments (Semmler et al., 1980; Reuss et al., 1983). The majority of cells (80% of cells from guinea pigs and 67% from rats), did not respond at all, while the remaining cells elicited different types of responses, e.g. inhibition in the guinea pig (Semmler et al., 1980) or excitation of activity in the rat (Reuss et al., 1983). A decrease in pineal serotonin-N-acetyltransferase (NAT) activity and melatonin content (measured per gland) in rats was found after exposure during 15 minutes or 2 hours before midnight in an initial experiment (Welker et al., 1983).

An additional series of experiments were performed to elucidate the mechanism(s) through which magnetic fields affect NAT activity and melatonin content. NAT activity and melatonin content in acutely blinded rats were not affected by 30 minutes exposure to a magnetic field, in contrast to the decrease that was found in intact animals (Olcese et al., 1985). In another experiment, the effect was found to be species dependent, but independent of ocular pigmentation. That is, a reduction of NAT activity and melatonin content was found both in albino Sprague Dawley rats and in Long-Evans hooded rats, but no effect was found in

golden hamsters (Olcese & Reuss, 1986). However, a later study showed that pigmentation was important; no effect was seen in pigmented gerbils, whereas NAT activity was decreased by exposure in male albino gerbils (Stehle et al., 1988). Another study demonstrated that the effect was found only in the presence of dim red light (Reuss & Olcese, 1986). The number of animals in most of the above-described experiments was small, it is unclear whether animals were randomized into experimental groups, the ages of animals were not clearly reported, and levels of NAT activity varied considerably between control groups of the same species in different experiments.

A Swiss research group exposed rats to a one hour inversion of the horizontal component of the Earth's magnetic field, and compared pineal cyclic adenosine monophosphate (cAMP) content to that in unexposed siblings (Rudolph et al., 1988). A 38% reduction of cAMP was found in exposed rats. In another rat experiment, no effect on NAT activity or melatonin content was found after exposure to an artificial magnetic field that compensated for the Earth's natural magnetic field (Khoory, 1987).

Kroeker et al. (1996) exposed rats to a static magnetic field that could vary between 5×10^{-5} T and 0.08 T. One daytime and one nighttime control group was used. The magnetic field was created by placing a disk magnet on the bottom of each animal cage. No effects of the magnetic field exposure on melatonin content were found in any of the experimental groups. The only group with lower melatonin content was the daytime control group.

Jankovic and collaborators studied aspects of the immune system and melatonin in rats. In one study (Jankovic et al., 1991), they implanted small magnets, or same size iron beads, a number of days before or after immunization or challenge with various biochemical agents. Several immune response markers showed significantly elevated levels with the various static magnetic field treatments, while they were not visible in sham exposed or sham operated subjects. It is difficult to determine any localization information from this study (while the magnets are small, so are rat brains). Following suggestions from other research in magnetosensitivity, the authors studied the influence of magnetic fields in combination with pinealectomy, with appropriate controls (Jankovic et al., 1993; 1994). Magnetic fields again increased the immune response, and the absence of the pineal reduced the same markers, but the papers failed to make a strong case for linking the two.

Lerchl et al. (1990; 1991) reported an influence of intermittent exposure to a 40 μ T static magnetic field on serotonin and melatonin metabolism in laboratory animals. The results were supported by Yaga et al. (1993), who reported changes in diurnal melatonin rhythm from pulsed static magnetic fields (40 μ T) that resulted in a net reduction of melatonin

secretion. In contrast, Levine et al. (1995) exposed mice to a 2 T pulsed magnetic field without an effect on melatonin secretion. In all these studies, the exposure contained time varying components due to rapid field switching or pulsing. Thus, firm conclusions on static magnetic field effects cannot be drawn from the results.

Table 26. Pineal gland					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Semm et al., 1980)	Guinea pig	Electrical activity of pineal cells	Artificial geomagnetic field strength	Depression of pineal activity by an induced SMF; restoration by the inverted SMF.	
(Reuss et al., 1983)	Rat	Electrical activity of pineal cells	Artificial geomagnetic field strength 4 - 5 min	Activation of pineal cells continued after switching off the magnetic stimuli.	Variable responses.
(Welker et al., 1983)	Rat	Pineal serotonin-N-acetyltransferase (NAT) activity; melatonin content	Artificial geomagnetic field strength 2 h	Reduction of nocturnal pineal NAT activity and melatonin content by changing the inclination of the ambient SMF, 63 - 58°, 68° or 78°.	Seem to report selected results. Weak methodology.
(Olcese et al., 1985)	Rat	Pineal serotonin-N-acetyltransferase (NAT) activity; melatonin content	Artificial geomagnetic field strength 30 min	Reduction of nocturnal pineal NAT activity and melatonin content in intact rats; no effects in blinded rats.	Incomplete description of methodology.
(Olcese & Reuss, 1986)	Rat, hamster	Pineal serotonin-N-acetyltransferase (NAT) activity and melatonin content	Artificial geomagnetic field strength 30 min	Reduction of nocturnal pineal NAT activity and melatonin content by 50° rotation of the horizontal component of the ambient SMF in rats; no effect in hamsters.	Incomplete description of methodology. NAT activity in unexposed rats was considerably lower than in their previous study (1985).

(Stehle et al., 1988)	Mongolian gerbil, rat	Pineal serotonin-N-acetyltransferase (NAT) activity and melatonin content	Artificial geomagnetic field strength 30 min	Decreases in nocturnal pineal NAT activity and melatonin content in albino gerbils and rats regardless of sex; no effect in pigmented gerbils.	Results not fully reported. Incomplete description of methodology.
(Reuss & Olcese, 1986)	Rat	Pineal serotonin-N-acetyltransferase (NAT) activity and hydroxyl-indole-O-methyltransferase (HIOMT) activities	Artificial geomagnetic field strength 15 min	Magnetic field reduces nocturnal NAT and activity, and HIOMT activity, only under dim red light. No effect of the field alone.	Methodological uncertainties; variable control data.
(Rudolph et al., 1988)	Rat	Pineal cAMP	Inverting of horizontal component of natural SMF 1 h	Decrease in pineal cAMP.	Methodology inadequately described.
(Khoory, 1987)	Rat	Pineal N-acetyltransferase (NAT) activity, melatonin content	Artificial geomagnetic field strength 30 min	No effect on NAT activity or melatonin content.	Unclear if randomized. Experiment not blinded.
(Kroeker et al., 1996)	Rat	Neurochemistry	80 mT or 7 T 12 h and 8 d	No effect on melatonin, catecholamines, serotonin, or their metabolites.	Unclear if randomized. Experiment not blinded.

(Jankovic et al., 1991)	Rat	<p>Weight change: spleen, thymus</p> <p>Immune response: plaque-forming cell (PFC) response, haemagglutinin production, local hypersensitivity skin reactions, experimental allergic encephalomyelitis (EAE), antibody production, peripheral blood CD4+ and CD8+ cells</p>	60 mT 14, 24, 34 and 36 d	The highest immune response was observed with exposure of the occipital brain region for 24 d in the SMF group.	
(Jankovic et al., 1993)	Rat	<p>Immune response: plaque-forming cell (PFC) response and hemagglutination reaction</p>	60 mT 21 d	<p>Increased immune response in the presence of implanted magnets.</p> <p>Reduced immune response in the absence of the pineal gland.</p>	

(Jankovic et al., 1994)	Rat	Weight change: spleen, thymus Immune response: plaque-forming cell (PFC) response, haemagglutinin production, local hypersensitivity skin reactions,	60 mT 29, 39 d	Various immune responses were increased by SMF. SMF recovered decrease of immune reactions caused by pinealectomy.	Results are more supportive of interactions with some other structure than the pineal gland. No description of methods for statistical analysis.
MRI or combined exposure studies					
(Lerchl et al., 1990)	Mouse, rat	Pineal serotonin metabolism	Inversion of horizontal component of Earth's magnetic field 5 min, 5 min off, 1 h; rapid on/off switching of field	Pineal serotonin increased; in rats: increase of pineal 5-hydroxyindole acetic acid, decrease of serotonin-N-acetyltransferase activity; pineal and serum melatonin levels not altered.	Age of animals not reported. Unclear if randomized. Experiment not blinded.
(Lerchl et al., 1991)	Rat	Serotonin-N-acetyltransferase, melatonin, serotonin, 5-hydroxyindole acetic acid	Inversion of horizontal component of Earth's magnetic field Rapid on/off switching of field	Reduced serotonin-N-acetyltransferase activity, lower melatonin; increased serotonin, 5-hydroxyindole acetic acid; effects not due to SF, but to on/off switching	Age of animals not reported (but little variation in body weight). Unclear if randomized. Experiment not blinded.
(Yaga et al., 1993)	Rat pineal glands	Pineal serotonin-N-acetyltransferase (NAT) activity, melatonin content	40 μ T, pulsed 45 min	Suppression of pineal NAT activity, and melatonin content by exposure during mid- or late dark phase; no changes after exposure early in the dark phase or during the day.	Age and sex of animals not reported, however, little variation in weight. Unclear if randomized.

(Levine et al., 1995)	Mouse	Serum melatonin levels	2 T 100 min	No effect on melatonin levels.	
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7.2.2.4.2 Other endocrine gland effects

Other endocrine gland functions under static magnetic field action were reported by Gorczynska and Wegrzynowicz (1991a). They measured glucose homeostasis in rats after exposure to 1 mT and 10 mT fields for 10 days. They observed relatively small, but significant and consistent, changes, such as increased glucose levels, decreased insulin release. They interpreted the observed immune system changes as stress responses. Sutter et al. (1987) also studied effects on body weight and insulin release in rats after long, intermittent exposures to 0.4 T or 0.8 T fields. They saw no changes in organ or body weights. Although the two field levels affected glucose oxidation, the changes for the two field levels had different signs.

Teskey et al. (1987) exposed rats to MRI fields for 20 minutes per day for 5 or 21 days and evaluated survivability, hormone levels and weight parameters 13-22 months after the exposure. They found no differences from controls at this distant observation point.

Table 27. Other endocrine glands					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Gorczyńska & Wegrzynowicz 1991a)	Rat	Glucose homeostasis	1, 10 mT 1 h d ⁻¹ , 10 d	Slight increase in glucose, insulin release decreased, glucagons content increased as compared to controls.	Subtle but relatively solid effects.
(Sutter et al., 1987)	Rat	Pancreatic insulin content and <i>in vitro</i> insulin release of Langerhans islets; glucose and insulin plasma levels; body and pancreas weight	400, 800 mT 2h d ⁻¹ , 5d wk ⁻¹	In adipocytes, 400 mT increased insulin-stimulated 1- ¹⁴ C-glucose oxidation and 800 mT diminished it; no effect on body weight.	Opposite signs of the effects on glucose oxidation.
MRI of combined exposure studies					
(Teskey et al., 1987)	Rat	Survival, stress reactions	0.15 T 22.5 min d ⁻¹ , 5 d; 23.3 min d ⁻¹ , 21 d	No effect on hormone levels and weight parameters at 13 - 22 months after the exposure. No change in survivability.	.

7.2.2.5 Reproduction and development

Few studies have examined the effect of static magnetic fields on fertility. Most studies concern possible effects on the developing embryo and fetus (teratogenic effects). Key factors in the investigation of the potential teratogenic effects of any agent include an awareness of the potential sensitivity of the different developmental stages and the underlying developmental processes. Periods of cell proliferation and migration are particularly vulnerable to many teratogens. With regard to statistical analyses, those based on the numbers of affected fetuses (as used by some authors) will tend to overestimate the significance of any effect seen. This is because the assumption that individual fetuses within a litter are independent leads to an underestimate of the true variance.

7.2.2.5.1 Male fertility

Withers et al. (1985) did not detect any effect on spermatogenesis after exposing mice to a magnetic field of 0.3 T for 66 hours. Narra et al. (1996) reported slight changes in spermatogenesis and embryogenesis in mice exposed at 1.5 T for 30 min, but no abnormalities in sperm head shape (although the data were rather variable). Tablado et al. (1996; 1998; 2000) reported that maturation of sperm movement in mice, as well as postnatal testicular and epididymus development, was largely unaffected by either single, short-term exposure, or intermittent (1 h per day) or continuous, long-term exposure at 500 - 700 mT. In 1996, no effects on sperm motility, maturation and production were reported after exposing mice to a maximum of 0.7 T, 24 h d⁻¹ for a maximum of 35 days. Tablado et al. (1998) used the same experimental set up two years later and reported sperm head abnormalities. However, developmental changes in the testes were not detected by the same authors in a subsequent experiment when dams were exposed, starting at the 7th day after gestation until birth (Tablado et al., 2000).

Table 28. Male fertility					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Withers et al., 1985)	Mouse	Spermatogenesis	0.3 T 66 h	No effect on spermatogenesis.	
(Narra et al., 1996)	Mouse	Spermatogenesis, embryogenesis	1.5 T 30 min	Reduction in testicular sperm; no increase in sperm head shape abnormalities; decreased survival of pre-implantation embryos.	
(Tablado et al., 1996)	Mouse	Sperm development	0.7 T 1 or 24 h d ⁻¹ , 10 or 35 d	No effect on sperm motility, maturation, production	
(Tablado et al., 1998)	Mouse	Sperm development	0.7 T 1 or 24 h d ⁻¹ , 35 d	More sperm head abnormalities with continuous exposure; no effect on tail.	
(Tablado et al., 2000)	Mouse	Testis development	0.5 - 0.7 T d 7 of gestation to day of birth	No effect up to 35 days of age.	

7.2.2.5.3 Mammalian development – static field exposure

Studies of possible teratogenic effects on mammalian species are more relevant to humans than are those on non-mammalian species. An

early, rather comprehensive study by Sikov et al. (1979) did not find any effect of exposure to a static field of 1 T, either before implantation, during organogenesis or during fetal development, on the pre-natal and post-natal development of mice. A later study by Konermann and Monig (1986), which focused particularly on cortical development in mice, also found no developmental effect of exposure to fields of 1 T. Similarly, Zimmermann and Hentschel (1987) also reported a lack of effect on development in mice following exposure to a static magnetic field of 3.5 T over the whole period of gestation. More recent studies of mice exposed to fields of 4.7 T (Okazaki et al., 2001) and 6.3 T (Murakami et al., 1992) confirmed the lack of effect of exposure during organogenesis on *in utero* mouse development.

More variable results have been seen in two studies looking at possible developmental effects in rats. Mevissen et al. (1994) reported a significant decrease in the number of live fetuses per litter in rats exposed for the entire period of gestation to a 30 mT static field. The authors suggested that such exposure might be embryotoxic. A significant increase in the total number of resorptions and number of fetuses with common skeletal variants was also reported, although, as indicated above, the significance of findings based on individual fetuses may well be overestimated.

Table 29. Mammalian development - static field exposure					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Sikov et al., 1979)	Mouse	Prenatal and postnatal development	1 T for varying periods for up to the whole of gestation	No consistent effects seen on prenatal or post-natal development.	Small litter numbers and large litter variability preclude firm conclusions
(Konermann & Monig, 1986)	Mouse	Prenatal development	1 T 1 h, 7, 10, 13 d post conception	No effect.	
(Zimmermann & Hentschel, 1987)	Mouse	Reproduction, development, haematology	3.5 T during mating and whole period of pregnancy (18 d)	With mating during 7-d period in field, reduced number of pregnancies (less mating); no teratology and pathology.	

(Okazaki et al., 2001)	Mouse	Fetal development	4.7 T 2 d	No effect on prenatal death and malformations but enchondral ossification enhanced and vascular endothelial growth factor reactivity altered.	
(Murakami et al., 1992)	Mouse	Fetal development	6.3 T 1 h d ⁻¹ , d 7- d 14 of gestation	No significant effects on litter size, fetal weight, intrauterine mortality rate, or skeletal abnormalities.	
(Mevisse et al., 1994)	Rat	Reproduction, fetal development	30 mT d 1-d 20 of pregnancy, or whole period of pregnancy	Decreased fetal survival; no malformations; increased skeletal ossification; postnatal growth enhanced after exposure during whole pregnancy.	
Studies considered to be uninformative					
(High et al., 2000)					

7.2.2.5.4 Mammalian development – MRI exposure

The exposures of pregnant mouse dams to all three magnetic field used in MRI (static, gradient and RF), have been examined by three research groups. While such exposures are more realistic (regarding MRI), any observed effects cannot be reliably attributed to any single field component. Significant heating, which can result from excessive RF magnetic field absorption, is a known teratogen (see Edwards et al., 2003). In addition, high levels of acoustic noise, resulting from rapid gradient field switching, may induce stress-related effects. A lack of effect, however, indicates that if the experimental model used was appropriate and the experimental design of sufficient power, none of the above conditions would have significantly affected the outcome.

Tyndall (1993) examined the effects of exposure for 36 min to a 1.5 T field (plus unspecified gradient and RF fields) on day 7 of gestation, during development of the anterior neural plate. An increased percentage of fetuses per litter with reduced craniofacial perimeter and crown-rump length was reported in the exposed groups compared to sham exposed animals. The author discussed RF-induced heating as a possible mechanism, although no rise in body temperature was recorded. Earlier

studies (Tyndall, 1990; Tyndall & Sulik, 1991) reported that a similar exposure increased the incidence of eye abnormalities in the same strain of mouse (which is prone to this condition), but did not enhance the effect of x-ray-induced increases in this endpoint.

Heinrichs et al. (1988) carried out a comprehensive study of the effects of exposure of mice for 16 h around the same period (~ day 9 gestation) to MRI fields where the static magnetic field was 0.35 T. Pulsed gradient and RF magnetic fields were also present. There were no effects on the incidence of prenatal deaths, nor that of skeletal defects, but crown-rump length was significantly reduced in the MRI-exposed group. This effect may have been overestimated; however, since the analysis was based on the number of affected fetuses and appeared to neglect litter effects (see above). The authors noted that the noise generated within the magnet (by the switched gradient fields) may have been stressful. They further commented on the 10% reduction in body weight seen in both exposed and sham exposed groups due to dehydration over the 16 h treatment period.

An earlier study (Carnes & Magin, 1996) had reported significantly reduced fetal weight, which is strongly influenced by litter size, in mice exposed for 8 h on day 9 gestation to a 4.7 T static magnetic field, a switched gradient field and a 200 MHz RF field where the whole-body power absorption (specific energy absorption rate, or SAR) was estimated as 0.015 mW kg^{-1} . Sound levels were not provided. No effect was seen after exposure on day 12 of gestation, nor after exposure on day 9 and day 12 combined. In addition, no effect was seen on the number of fetal deaths in any MRI exposure group. Sperm production, which was not significantly affected in the study described above (Magin et al., 2000), was significantly reduced in mice exposed on day 12 gestation, but not on day 9, nor on day 9 and 12 combined. Overall, even ignoring the differences in experimental protocol, it is difficult to conclude that the effects described in the two studies are reproducible, either within or between the studies. A more likely explanation is of spurious differences introduced by small numbers, incomplete analysis, and variable data.

Later studies by Magin, Carnes and colleagues (Carnes & Magin, 1996; Magin et al., 2000) examined the effects of exposure of mice on days 9 and/or 12 gestation, i.e. during organogenesis, to static magnetic fields of around 4-5 T, combined with switched gradient and RF fields. In the study by Magin et al. (2000), mice were exposed to a static field of 4 T, a switched gradient field and a 170 MHz RF field, for which the average whole-body SAR was estimated to be 0.2 W kg^{-1} . Litter size was unaffected, but significantly increased numbers of resorptions and fetal deaths occurred in the group exposed on day 12 of gestation, but not on day 9, nor on days 9 and 12 combined. In addition, a significant increase in the rate of acquisition of motor skills was seen in the mice exposed on day 9 of gestation, whereas this was decreased in the group exposed on

day 12. However, the numbers of pregnant dams per treatment group were rather small, the analyses often based on total numbers rather than numbers affected per litter, and the data were rather variable. Furthermore, only the exposed groups experienced the loud (90 - 100 dB) acoustic noise generated by the switched gradient fields.

Table 30. Mammalian development - MRI exposure					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Tyndall, 1993)	Mouse	Crown-rump length and craniofacial perimeter	1.5 T 36 min	Decreased crown-rump length and craniofacial perimeter.	
(Tyndall, 1990)	Mouse	Eye developmental abnormalities induced by x-radiation at up to 30 cGy	1.5 T less than 1 h on d 7 of gestation	No effect on x-ray-induced eye abnormalities.	
(Tyndall & Sulik, 1991)	Mouse	Eye development	1.5 T 36 min, d 7 of gestation	Increased number of malformations.	
(Heinrichs et al., 1988)	Mouse	Placental resorptions, stillbirths, fetal weight at birth and crown-rump length	0.35 T 16 h, beginning on d 8 of gestation	Reduction of crown-rump length and fetal weight.	
(Carnes & Magin, 1996)	Mouse	Fetal growth, postnatal development, testicular development	4.7 T 8 h, d 9, d 12 of gestation	Reduction of sperm production in adults.	Small number, incomplete analysis, variable data.
(Magin et al., 2000)	Mouse	Fetal growth, postnatal development	4 T 1 or 2 x 9 h	No effect.	Small number, incomplete analysis, variable data.

7.2.2.5.2 Development in non-mammalian vertebrate embryos

In contrast to mammals, where development of the embryo and fetus occur *in utero*, amphibian and avian embryos develop in eggs, which are in some ways easier to experimentally manipulate. However, effects on such development may be less directly relevant to humans than studies on mammals.

An early, preliminary study by Brewer (1979) reported an inhibitory effect of static magnetic field exposure of 0.05 T on the reproduction of fish. However, the data were not analyzed. In contrast, Asahishima et al. (1991) reported that magnetic shielding (5 nT) induced early developmental abnormalities in the newt. The control data were, however, rather variable.

The possibility that strong magnetic field gradients may affect embryonic development in amphibia has been raised by IARC (2002). Early studies (Neurath, 1968; Ueno et al., 1984) had described abnormal growth and increased malformations in such embryos exposed to a static field of 1 T with field gradients of 10 - 1000 T m⁻¹. However, Mild et al. (1981) reported a lack of effect on the development of amphibian embryos of exposure to a spatially homogeneous static magnetic field of 0.25 T for up to 7 days. In addition, later studies by Ueno et al. (1990; 1994) briefly reported a lack of developmental effects following exposure during the early stages of development to 6.34 and 8 T fields, but it was not clear whether such exposure included strong field gradients.

More recently, Denegre et al. (1998) investigated the effect of exposure to static magnetic field of up to ~ 17 T on the first three cleavages of the fertilized egg of the African clawed toad *Xenopus laevis* used previously by Ueno et al. (1984; 1994). The authors found that the second and third cleavage oriented parallel to the plane of the magnetic field. The proportion of cleavages parallel to the field increased with field strength above around 2 T, to a maximum effect at around 17 T. The largest effects occurred in the homogeneous field rather than the gradient field, and the authors suggested that the effect resulted from the interaction with diamagnetically anisotropic molecules in the mitotic apparatus, possibly the microtubules of the spindle formation. These effects on the mitotic apparatus of cells exposed to high magnetic fields (up to 22 T) were confirmed in a further study by Valles et al. (2002). However, it is not clear whether the effects reported by Denegre et al. (1998) altered the proportion of eggs that developed into normal tadpoles.

Espinar et al. (1997) reported effects on cell migration and on differentiation of the cerebellar cortex in chickens after a long continuous exposure to 20 mT. The changes included cell degeneration and delay in the process of neuronal differentiation. Jové et al. (1999) found slight changes in the rate of development of chick embryos, including the pineal gland, in chicks exposed to static magnetic fields of 18 or 36 mT for up to 15 days incubation.

Behr et al. (1991) found no effects on embryonic development from exposure to a 4 T static magnetic field before and during incubation of chick embryos. MRI-specific exposure combinations with ELF and RF fields were also investigated in the study, but they are not relevant in this context.

Prasad et al. (1982; 1990) evaluated the effects of MRI exposure in an amphibian (frog) system. In one study (Prasad et al., 1982) frog spermatozoa, eggs and embryos were exposed to 0.7 T in combination with RF fields for 20 minutes. In another experiment fertilized frog eggs were exposed to a maximum of 4.5 T, again in combination with RF fields, for 60 minutes (Prasad et al., 1990). No deleterious effects were detected. Yip et al (1994a; 1994b; 1995) found no effect on the development of the central nervous system of exposed chickens in a total of three studies highlighting the effects of long continuous MRI field exposure (6 hours at 1.5 T in combination with RF and gradient fields). Kay et al. (1988) evaluated the exposure effects of MRI on frog embryogenesis. They found no abnormal morphology, function, or developmental delays of frog embryogenesis.

Table 31. Development in non-mammalian vertebrate embryos					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Brewer, 1979)	Fish	Reproduction	0.05 T 3 generations	1st generation: reduced gestation time; 2nd: reduction spawn rate; 3rd: reproduction inhibited. Increase in size with exposure. Effects reversed after removal from field.	No statistics.
(Asashima et al., 1991)	Newt	Development	5 nT 5 d	Increased number of abnormalities.	Variable data
(Ueno et al., 1984)	Frog	Embryonic development	1.0 T; different gradients 8 - 12 h	No effect of 1 T field. Minor malformations with gradient field exposure.	
(Mild et al., 1981)	Frog	Embryonic development	0.25 T up to 7 d	No effect.	
(Ueno et al., 1990)	Frog	Embryonic development	4.5, 6.34 T up to 20 h	No effect of 6.34 T up to 7 h or 4.5 T up to 20 h on rapid cleavage and differentiation.	
(Ueno et al., 1994)	Frog	Embryonic development	40 nT, 8 T up to 20 h	No difference in development between control (Earth magnetic field), magnetic shielded (40 nT) and 8 T.	

(Denegre et al., 1998)	Frog	Development	1.74 - 16.7 T duration?	Alteration of cleavage planes (dose response). 50% abnormal embryos with parallel SMF (no dose effect), no abnormal embryos with perpendicular SMF.	
(Valles, Jr. et al., 2002)	Frog	Embryonic development	17 - 22 T duration?	Exposure during first two cell cycles induced third cycle mitotic apparatus and the third cleavage plane to align without changing cell shape.	
(Espinar et al., 1997)	Chicken	Brain development	20 mT 24 h at d 6, or from d 0- d 13	Exposure-dependent irreversible effects on cell migration and differentiation of cerebellar cortex.	
(Behr et al., 1991)	Chicken	Embryonic development	1, 4 T 18.8, 37.6, 56 or 75.1 min	No effect.	Includes SMF only group, but no statistics.
(Jové et al., 1999)	Chicken	Embryo pineal gland development	18, 36 mT from d 0 - d 5,10,15	Unequal promotion of embryo pineal gland development. Effect depended on exposure intensities and duration.	

MRI or combined exposure studies					
(Prasad et al., 1982)	Frog	Development after exposure of spermatozoa, eggs and embryos	0.7 T + RF 20 min	No effect.	
(Prasad et al., 1990)	Frog	Embryonic development	0.15, 4.5 T + RF + gradient 1 h	No effect.	.
(Yip et al., 1995)	Chicken	Axonal outgrowth	1.5 T + RF + gradient 6 h	No effect.	Controls not exposed to noise and vibration as in MRI.
(Yip et al., 1994a)	Chicken	Brain	1.5 T + RF + gradient 6 h	No effect.	
(Yip et al., 1994b)	Chicken	Embryonic development	1.5 T + RF + gradient 6 h	No immediate effects, but after 6 d higher abnormality and mortality rates.	More detailed studies should be performed, as the effect of noise and vibration cannot be ruled out.
(Kay et al., 1988)	Frog	Embryogenesis	1.5 T + RF + gradient $2 \times 1 \text{ h d}^{-1}$, prolonged exposure	No effect.	.

7.2.2.5.5 Developmental effects in non-vertebrate embryos

A few studies have been carried out with non-vertebrate species. These are phylogenetically distant from mammalian species, but may nevertheless (in principle) provide useful information. Levin and Ernst (1997) detected an effect of long continuous low field exposure (10 mT - 100 mT) in a sea urchin model. Ho et al. (1992) reported that static magnetic field exposure up to 9 mT during early embryogenesis of the fruit fly caused a dose-dependent increase in the number of abnormalities. Ramirez et al. (1983) evaluated the exposure effect of weak static magnetic field (4.5 mT) exposure on oviposition and development of the fruit fly. They found no effects on oviposition, but an increase in mortality of eggs and larvae.

These studies were regarded as relatively uninformative for health risk assessment.

Table 32. Other developmental effects					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Levin & Ernst, 1997)	Sea urchin	Embryo development	10 mT - 0.1 T 26 h	Onset of mitosis delayed, species-dependent; exogastrulation by 30 mT, not 15 mT in one species, none in other.	Methodology weak.
(Ho et al., 1992)	Fruitfly larvae	Embryo-genesis	up to 7 mT 30 min 9 mT 24 h	Dose-dependent increase in abnormalities.	
(Ramirez et al., 1983)	Fruitfly	Oviposition, development	4.5 mT 14 d (oviposition) 48 h (development)	No difference in oviposition between control and SMF on d 1 - 7, avoidance of SMF on d 8 - 14. Mortality of eggs, larvae increased; adult viability decreased.	Variable data; inappropriate analysis.

7.2.2.6 Genotoxicity and cancer

Animal studies are often used in the evaluation of suspected human carcinogens, either screening for an increased incidence of spontaneous tumours or for the incidence of tumours induced by known carcinogens.

7.2.2.6.1 Genotoxicity and mutagenesis

Genotoxic effects of exposure to static magnetic fields have been mostly examined in cell cultures (see section 7.1.7). Few *in vivo* studies of genotoxicity or possible effects on other carcinogenic processes have been carried out.

Kale and Baum (1979) were unable to detect an enhanced mutation rate in the fruit fly *Drosophila melanogaster* exposed up to ten

days to 1.3 T, and up to seven days to 3.7 T. In a subsequent study, Kale and Baum (1980) examined the effect of a long, continuous magnetic field exposure (166 h at 3.7 T) on chromosomal mutations in *D. melanogaster*, but were not able to detect any measurable magnetic field-induced changes. Koana et al. (1995; 1997) exposed *D. melanogaster* and their larvae to a 0.6 T magnetic field for 24 hours and observed a decrease of the surviving mutant genotype adults. The same group (Koana et al., 1997) also studied 5 T static magnetic fields for 24 hours and observed an enhancement of somatic recombination that was suppressed by vitamin E supplement. However, the genotoxicity equalled the effect of half to a quarter of the daily sunlight in Japan, thus making its clinical relevance questionable.

More recently, Suzuki et al. (2001) used a standard micronucleus assay and reported a significant, time-dependent and dose-dependent increase in micronucleus frequency in mice exposed to static magnetic fields of 2, 3 or 4.7 T for 24, 48 or 72 h. Micronucleus frequency was significantly increased following exposure to 4.7 T for all three time periods, and to 3 T after exposure for 48 or 72 h, whereas exposure to 2 T had no significant effect. The authors suggested that exposure to higher fields may have induced a stress reaction, or directly affected chromosome structure or separation during cell division.

With regard to combined exposures, Prasad and his co-workers (1984) examined the possible effects of exposure to RF and static magnetic fields on DNA in a study using mice. They did not find any chromosomal damage after exposing mice to 30 MHz in a magnetic field of 0.75 T for 60 minutes.

In 1995 Rofsky et al. reported the effects of MRI exposure (1.5 T static magnetic field with gradient and RF magnetic fields) with and without administration of the MRI contrast agent gadopentetate dimeglumine in a rat model (Rofsky et al., 1995). Neither magnetic fields alone nor the combination with contrast agent resulted in measurable chromosomal damage.

Table 33. Genotoxicity and mutation					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Kale & Baum, 1979)	Fruitfly, egg, larva, pupa and adult	Lethal mutations	1.3 - 3.7 T 24 h, 10 d low field, 7 d high field	No effect.	
(Kale & Baum, 1980)	Fruitfly	Lethal mutations	3.7 T 166 h	No effect on production of induced sex-linked, recessive, lethal mutations.	
(Koana et al., 1995)	Fruitfly, 1st and 2nd instar larvae	Genotoxicity	0.6 T 24h	Decrease of surviving mutant genotype adults.	
(Koana et al., 1997)	Fruitfly, 3rd instar larvae	Genotoxicity and mutagenesis	5 T 24 h	Enhancement by SMF of somatic recombination, no effect on non-disjunction, terminal deletions, gene mutations. Effect suppressed by vitamin E supplement.	
(Suzuki et al., 2001)	Mouse	Micronucleus frequency	2, 3, 4.7 T 24, 48, 72 h	Increased number of micronuclei at higher exposure intensities and longer durations.	
MRI or combined exposure studies					
(Prasad et al., 1984)	Mouse	Chromosomes in bone marrow	0.75 T 1 h	No effects, no chromosomal damage.	Exposure to SMF and RF.
(Rofsky et al., 1995)	Rat	Unstable chromosomal damage in regenerating liver cells	1.5 T + RF + gradient T1 (5.45 min) and T2 (10.45 min) imaging sequences	No effect of MRI alone or in combination with gadopentetate dimeglumine.	

7.2.2.6.2 Cancer

Few studies investigating the potential carcinogenicity of static magnetic fields have been carried out. With regard to possible effects on induced tumours, Bellossi (1984) reported a lack of effect on survival time in mice with chemically-induced epidermal tumours that were exposed to up to 800 mT for up to 1 h per day for 5 days per week until death was reported. In a later study, Mevissen et al. (1993) reported that exposure of rats to a magnetic field of 15 mT for 13 weeks did not significantly affect the incidence of chemically-induced mammary tumours, nor did it affect the number of tumours per animal compared with controls, although the weight per tumour was significantly increased. A complication is that the tumour multiplicity was reduced in rats treated with a static magnetic field as compared to the reference control, although the difference was not statistically significant. However, it is hard to draw any conclusions from this study because of the large range of tumour weights and the relatively small group size.

The growth of transplanted tumours were reported to be unaffected by exposure of mice to static fields of at least 1 T (Bellossi & Toujas, 1982; Bellossi, 1986c). Bellossi and colleagues studied the effect of static field exposure on the growth of tumours in mice injected with Lewis Lung tumour cells. Exposure to uniform static fields of up to ~ 1 T for up to 8 h per day for 5 days per week until death had no effect on the survival time (Bellossi & Toujas, 1982). Neither did exposure to non-uniform static magnetic fields of up to ~ 1 T, with gradients of up to 3 T m^{-1} (Bellossi, 1986c). The same group also studied the influence of static magnetic field exposure on spontaneous development of lymphoblastic leukaemia in mice (Bellossi, 1986b). Neither uniform nor non-uniform static magnetic fields had an effect. A somewhat longer lifespan was observed after intermittent exposure to 600 - 800 mT, but the number of animals in this group was quite small. The experimental procedures and analysis of the data in all these studies were rather briefly described, reducing the confidence that can be placed in them.

Table 34. Cancer					
Authors	Animal	Endpoint	Exposure	Results	Comments
Static magnetic field effects					
(Bellossi, 1984)	Mouse	Methylcholanthren carcinogenesis	25 - 600 mT 2 h d ⁻¹ , 5d wk ⁻¹ 400 mT 5, 10, 15, 30 or 60 min d ⁻¹ , 5 d wk ⁻¹ 300, 600 or 800 mT 30 min d ⁻¹ for unspecified time	No effect on splenic index or survival.	Methods incomplete.
(Mevisse et al., 1994)	Rat	Development of mammary tumors induced by DMBA	15 mT 91 d	Increased tumour weight.	Small sample size and large range of tumour weights; source of increased tumour weight not stated. Histopathology showed no obvious peculiarities.
(Bellossi & Toujas, 1982)	Mouse	Tumour growth	13 - 900 mT 0.5, 1, 2, 3, or 4 h d ⁻¹ , 5 d wk ⁻¹ , from 2 months of age until death	No effect on survival after grafted Lewis Lung tumour.	Small group size. Also missing statistics, but there are no obvious differences.
(Bellossi, 1986c)	Mouse	Tumour growth	0.4 mT uniform SMF; non-uniform SMF: average gradient 3 T m ⁻¹	No effect on life span, splenic weights or thymic weights.	
(Bellossi, 1986a)	Mouse	Spontaneous carcinogenesis, survival	400 - 800 mT 2 h d ⁻¹ , 5 d wk ⁻¹ 4.6 mT from age 9 wk up to death	Neither uniform nor non-uniform SMF had effects on development of viral lymphoblastic leukaemia. Longer lifespan after 600 - 800 mT (intermittent).	Small group size for 600 - 800 mT intermittent.

Studies considered to be uninformative
(Imajo et al., 1989) (Gray et al., 2000) (Tofani et al., 2003)

7.2.2.7 Other biological endpoints

This summary covers the category of studies that did not fit neatly into the other animal sub-categories. These studies are generally of a poor quality and were not considered informative for human health assessment.

Table 35. Other biological endpoints					
Authors	Animal	Endpoint	Exposure	Results	Comments
Studies regarded as uninformative					
(Duda et al., 1991)	Rat	Liver and kidney concentration of copper, manganese, cobalt and iron	490 mT 0.5 - 4 h d ⁻¹ , total 8 - 64 h	SMF had no effect; 50 Hz MF changed metal concentration in kidneys of non-fertilized female rats.	Some differences observed, but these may well be due to multiple comparisons.
(Sato et al., 1996)	Mouse	Metallothionein (MT) synthesis in liver, kidney, brain	3.0, 4.7 T 1, 3, 6, 24, or 48 h	MT synthesis induced in liver; CCl ₄ -induced hepatic MT synthesis enhanced. No effect in kidney or brain.	Quite long exposure needed to see the reported effects.
(Danielyan & Ayrapetyan, 1999)	Rat	Hydration of tissues and cell volume (number of ouabain binding receptors)	200 mT 0 - 5 h	Decrease of hydration in brain and liver tissue for 3.5 - 5 h; decrease of cell volume in brain, liver and spleen; increase of cell volume in kidney.	

(Barnothy & Sumegi, 1969)	Mouse	Histopathology of various organs	0.9 T, 2 T m ⁻¹ gradient 13 d	Disorganization in 40% of adrenal cortex slices. Increased mitotic index in liver cells.	Changes in bone marrow megakaryocytes decreased, the opposite of normal stress response. No description of methods for statistical analysis.
(Bellossi et al., 1984)	Rat, mouse	Body weight	400, 600, 800 mT 2 h d ⁻¹ , 5 d w ⁻¹ (4.6 mT in some mice), rats exposed for 4 wk and mice for ≥ 250 d	No effect on growth observed 4 weeks after exposure.	Varying physical parameters without any apparent rationale. Generally shorter exposures than the preceding studies. No description of methods for statistical analysis
(Bellossi et al., 1981)	Mouse	Water structure in brain: brain relaxation times (spin-lattice T1 and spin-spin T2), measured 1 - 5 d after exposure	600 mT 2 h	No effect on brain relaxation times T1/T2 five days after exposure.	Using a magnetic method to analyse these effects does not seem like a very good idea. Waiting 5 days is very long for structured water. Statistical analysis deficient.

(Bellossi, 1983)	Mouse	Trypanosomiasis	400 mT 10, 30, 60, 120 min, 5 d 200 - 600 mT 90 min	No effect.	No statistical analysis. Very consistent survival after injection suggestive of a very robust disease progression. This animal model may not be adequate to detect SMF effect.
(Gorczyńska et al., 1986)	Rat	Cell respiration (liver mitochondria)	0.008, 0.15 T 1 h d ⁻¹ , 7 wk	Respiration through NADH dehydrogenase, succinic dehydrogenase and cytochrome oxidase influenced by duration, intensity (more at 0.008 T); reversible after 3 months.	
(Gorczyńska & Wegrzynowicz, 1991b)	Rat	Structural changes in hepatocytes mitochondria, endoplasmic reticulum and ribosomes; activity of mitochondrial respiratory enzymes; glycogen in hepatocytes; serum cortisol	1, 10 mT 1 h d ⁻¹ , 10 d	Structural changes in hepatocytes mitochondria, endoplasmic reticulum and ribosomes; increased activity of NADH dehydrogenase, succinic dehydrogenase, cytochrome oxidase; increase in glycogen in hepatocytes; high serum cortisol.	The reported changes may be due to temporary alterations in liver cell organelles.
(Parafiniuk et al., 1992)	Guinea pig	Structural changes in hepatocytes mitochondria	5, 300 mT 1 h d ⁻¹ , 3-7 wk	Structural changes in hepatocytes mitochondria.	

7.2.2.8 Conclusions

Few studies have been carried out on the effects of static electric fields. The evidence indicates that the surface electric charge can be perceived, and there is weak evidence to suggest that if the static field is sufficiently intense ($> 40 \text{ kV m}^{-1}$ in rats), then this may induce aversive behaviour.

There is good evidence that the movement of laboratory rodents in static magnetic fields equal to or greater than 4 T may be unpleasant, inducing aversive responses and conditioned avoidance. Such effects are thought to be consistent with magnetohydrodynamic effects on the endolymph of the vestibular apparatus. Otherwise, the data on behaviour are variable.

There is some evidence that several vertebrate and invertebrate species are able to use static magnetic fields, at levels as low as geomagnetic field strengths, for orientation. However, these responses are not thought to be relevant to human health.

There is some evidence of a stimulating effect on bone formation of static magnetic fields in the millitesla range. Stronger proof is present for such effects of tesla-strength static magnetic fields in a model system, but this treatment needs to be confirmed *in vivo*.

There is good evidence that exposure to fields greater than about 1 T (0.1 T in larger animals) will induce flow potentials around the heart and major blood vessels, but the physiological consequences of this remain unclear. Several hours of exposure to very high flux densities of up to 8 T in the heart region did not result in any cardiovascular effects in pigs. In rabbits, short and long exposure to fields ranging from geomagnetic levels to the millitesla range were reported to affect the cardiovascular system, although the evidence is not strong.

The results from one group suggested that the static magnetic fields of millitesla intensities may suppress early blood pressure elevation via hormonal regulatory system. The same group has reported that low-intensity SMF of up to 0.2 T may induce local effects on blood flow that may lead to improvement of microcirculation. In addition, another group reported that high static magnetic field flux densities of up to 10 T may lead to reduced skin blood flow and temperature. In all these cases, however, the end points are rather labile, a situation that may have been complicated by pharmacological manipulation, including anaesthesia in some cases, and immobilisation. In general, it is difficult to reach any firm conclusion without some independent replication.

There are several studies describing possible effects of magnetic field exposure on blood cells and the haemopoietic system. However, the results are equivocal, limiting the conclusions that can be drawn. The available evidence regarding effects of static magnetic field exposure on enzymatic and ionic constituents in serum comes primarily from one laboratory. These findings need to be confirmed by independent laboratories before conclusions can be drawn.

With regard to effects on the endocrine system, several studies from one laboratory suggest that static magnetic field exposure can affect pineal synthesis and melatonin content. However, some studies performed at other laboratories have been unable to demonstrate an effect. It is possible that differences in the study designs, e.g. exposure circumstances, species, outcome measures, or timing of exposure, can explain the conflicting results. The finding of a suppressive effect of static magnetic field exposure on melatonin production needs to be confirmed in further research before firm conclusions can be drawn. On the whole, few studies have investigated static magnetic field effects on endocrine systems other than the pineal and no consistent effects have emerged.

Reproduction and development is a very important issue in MRI exposure, for both patients and clinical staff. On this subject, only a few good studies of static magnetic field effects are available at field values above 1 T. MRI studies *per se* are uninformative in this respect, because it is difficult to distinguish the effects of the static fields from those of the other MRI fields. Further examination of the possible effects of static field exposure is urgently needed for the assessment of health risk.

With regard to genotoxicity and cancer, so few animal studies have been carried out that it is not possible to draw any firm conclusions.