

## 9 IMMUNE SYSTEM AND HAEMATOLOGY

Haematology is the branch of medicine that is concerned with blood, the blood-forming organs and blood diseases. Studies encompass the growth and development of the leukocyte (white blood cell) populations that form part of the immune system in addition to the erythrocyte (red cell) populations and the non-cellular serum constituents such as serum iron and serum alkaline phosphatase concentrations. Haemopoiesis, the formation of blood cells, occurs primarily in bone marrow, where there is progressive division and maturation from stem cells through to the formation of mature erythrocytes and leukocytes. Erythrocytes and leukocytes circulate in the bloodstream, from which cell populations and other haematological parameters may be readily sampled. However, there is a continual and active exchange of leukocytes with other body compartments such as the lymphoid system. In the adult human body, for example, only about 2% of the total lymphocyte pool is present in the blood and the lymphocyte subset composition can be varied by a number of different factors including disease. Few studies have examined ELF effects either on immune system function or on haematology. The tables below summarize the results of studies conducted on the immune system and haematology. Only the more significant ones are discussed in the text.

### 9.1 Immune system

The immune system identifies and responds to invading microorganisms such as viruses, bacteria, and various single-celled or multicellular organisms, and to “foreign” macromolecules including proteins and polysaccharides. Thus, it serves to protect individuals from infectious diseases and can also act against tumour cells, although these responses are fairly weak. Immunological responses are mediated through intercellular signalling pathways via chemical messengers such as cytokines and interleukins.

The first line of defence against pathogens is sustained by relatively nonspecific (natural or innate) parts of the immune system. These are natural killer (NK)-cells, mononuclear phagocytes and granulocytes. The protein “complement system” mediates many of the cytolytic and inflammatory effects of humoral (non-cell-mediated) immunity. These innate responses are followed by the adaptive (or acquired) antigen-specific responses of the immune system. The cells that mediate the antigen-specific (or acquired) responses are the B-lymphocytes, which secrete antibodies (humoral immunity) that circulate in body fluids, and the T-lymphocytes, that can function as cytotoxic cells (cell-mediated immunity) or as helper T-cells which assist in B- or T-cell activation. Activated cytotoxic T-lymphocytes specifically recognise and kill cells having foreign molecules on their surface and are implicated in anti-tumour responses. The acquired immune responses also involve the recruitment and amplification of the responses of the innate parts of the immune system.

### 9.1.1 Human studies

Selmaoui, Lambrozo & Touitou (1996) showed that a one-night (23.00 to 08.00) exposure to either continuous or intermittent (1 hour off and 1 hour with on/off switching every 15 s) 50-Hz, 10-T magnetic fields did not affect immunological parameters (CD3-, CD4-, CD8-lymphocytes, NK-cells and B-cell populations) in 16 healthy men aged 20–30 years as compared to 16 healthy sham-exposed men.

In 2000, Tuschl et al. (2000) published some results on immune parameters of ten workers exposed to the magnetic fields associated with induction heaters (50–600 Hz, up to 2 mT, or 2.8–21 kHz, 0.13–2 mT, for at least two years). Overall, there were no differences between exposed and control subjects in the levels of B- and T-cells, cytokines and immunoglobulins. However, the numbers of NK-cells and oxidative bursts of monocytes, implicated in cytotoxic responses were significantly increased in the exposed group while monocytes had significantly reduced phagocytic activity compared with those from unexposed personnel. The authors considered that overall the non-specific immunity of the exposed subjects was normal and that the most peculiar finding was the increase in NK-cell population.

Recently, the Mandeville group has reported effects of 60 Hz magnetic fields on 60 workers of power utilities (Ichinose et al., 2004). They monitored the activity of ornithine decarboxylase (ODC) in white blood cell, the activity of NK-cells, lymphocyte phenotypes, and differential cell counts. They monitored exposure over three consecutive days before collecting peripheral blood. There was no alteration of NK-cell activity nor of the number of circulating neutrophils, eosinophils, basophils, or T-lymphocytes. However, there was an association between exposure intensity and a decreased ODC activity and lower NK-cell counts.

The production of melatonin, which is known to stimulate the immune system, was quantified on the night preceding immune marker determinations. While no alteration in melatonin levels could be observed in the exposed subjects, the decrease in ODC activity, counts of NK- and B-cells, and monocytes were strongest for the workers with lowest melatonin production. According to the authors, the health consequences associated with these changes are not known.

Using a cross-section approach, Chinese scientists investigated the effects of ELF fields on the immune system. Zhu and coworkers (2002; 2001) systematically explored its effects on red blood cell, platelets and white blood cells of peripheral blood taken from people who were working with the electric railway system. They reported that the fields (50 Hz, 0.01–0.938 mT, or 0–12 kV m<sup>-1</sup>) decreased the number of white blood cells and the level of IgA and IgG (Immunoglobulins A and G) antibodies. They also found that the percentage of lymphocytes showing DNA damage was higher in the exposed group than in the control group. The authors concluded that ELF fields might induce DNA damage in lymphocytes, then cause apoptosis

of these cells, and further result in the decrease of cell number and immunoglobulin level in the blood.

Dasdag et al. (2002) compared blood cell counts, hematocrit and lymphocyte surface antigens of a group of 16 welders with that of a group of 14 healthy male control subjects. Although CD4 and CD8 levels were decreased in the welders and the hematocrit increased, the authors concluded that the differences were not clinically significant and that the results were not suggestive of an ELF effect on immunologic parameters.

Table 61 summarizes the studies on immune responses in humans exposed to ELF fields.

**Table 61. Immune system responses in humans**

Test	Exposure	Results	Comments	Authors
Numbers of CD3+, CD4+, CD8+ lymphocytes, of NK-cells and B-cells Healthy young men exposed: n=16 sham-exposed: n=16	50 Hz 10 µT Continuous or intermittent (1 h off, 1 h with on/off switching every 15 s) Exposure for one night (23:00 to 08:00).	No effect with either exposure protocol.	Well controlled study. Low power.	Selmaoui, Lambrozo & Touitou, 1996
Number of B- and T-cells, levels of cytokines and immunoglobulins Numbers of NK cells and oxidative bursts of monocytes Monocyte phagocytic activity Workers exposed to induction heaters (n=10)	50–600 Hz up to 2 mT or 2.8–21 kHz 0.13–2 mT Exposure for at least two years	No effect on B- and T-cells, cytokines and immunoglobulins. Increase in NK cells and in bursts of monocytes. Decreased phagocytic activity.		Tuschl et al., 2000
Activity of ornithine decarboxylase (ODC) in white blood cells Activity of NK cells Lymphocyte phenotypes Differential cell counts Power-utility workers (n=60)	60 Hz Personal magnetic field monitor for 3 consecutive working days	Decreased ODC activity. No alteration of NK activity. No change in number of circulating neutrophils, eosinophils, basophils, and T-lymphocytes, lower NK-cell counts		Ichinose et al., 2004

**Table 61. Continued**

Numbers of blood cells, levels of immunoglobulins, levels of DNA damage in lymphocytes (comet assay)	50 Hz 0-12 kV m <sup>-1</sup> , 0.01-0.92 mT 4.59±2.64 h / day, 9.72±3.09 year	Increase in red blood cells, platelets, and haemoglobin. Decrease in white blood cells and lymphocytes. Decrease in IgA and IgG. Increase in DNA damage of lymphocytes.		Zhu, Way & Zhu, 2001
Daily exposed workers: n=192 Unexposed control workers: n=106				
Numbers of blood cells, levels of immunoglobulins, levels of DNA damage in lymphocytes (comet assay)	50 Hz 1.69-3.25 kV m <sup>-1</sup> , 0.245-0.938 mT 4.59±2.64 h / day, 9.4±3.2 year	Increase in red blood cells and platelets. Decrease in white blood cells and lymphocytes. Decrease in IgA and IgG. Increase in DNA damage of lymphocytes.	Extension of Zhu et al., 2001	Zhu et al., 2002
Daily exposed workers: n=33 Unexposed control workers: n=106				
Red blood cells; hemoglobin; hematocrit; platelets; total white blood cells; neutrophils; lymphocytes; eosinophils; and CD3, CD4, CD8, and CD4/CD8	Welders exposed 3-4 hours per day per week and for at least 10 years	CD4, CD8 lower, hematocrit higher in welders. Differences "not clinically significant".		Dasdag et al., 2002
Male welders: n=16 Male controls: n=14				

### 9.1.2 Animal studies

Animal studies have been carried out using several approaches: some authors have examined the responsiveness of the whole immune system, while other used blood cell counts and standard in vitro tests on cells taken from the peripheral blood or spleen of exposed animals. This section discusses all experiments done with exposure of the animals even if the tests on their immune cells were done in vitro. Many of these studies have been previously reviewed by ICNIRP (2003) and the general conclusion was that "there is little consistent evidence on any inhibitory effect of power-frequency EMF exposure on various aspects of immune system function".

The Löscher group (Mevissen et al., 1996) had reported a decreased spleen T-lymphocyte proliferation in rats chronically exposed to 50 Hz magnetic fields. In a follow-up study, the same authors (Mevissen et al., 1998) found that this proliferation was initially increased, after 2 weeks, but then decreased, after 13 weeks, compared to sham-exposed animals.

Later, the same group (Häussler et al., 1999) reported on two independent experiments on the *ex vivo* production of interleukins (ILs) by mitogen-stimulated splenic lymphocytes from female Sprague-Dawley rats exposed to 100  $\mu$ T 50 Hz magnetic fields. In the first experiment, the rats were treated with DMBA and exposed or sham-exposed for 14 weeks. There was no difference between exposed and sham-exposed groups in the level of production of IL-1 by mitogen-activated splenic B-cells. In the second experiment, rats were exposed for 1 day, 1 week, or 2 weeks, followed by collection and activation of spleen lymphocytes. There was no difference in IL-1 or IL-2 production from stimulated B- or T-cells. According to the authors, these negative findings suggested that the reported changes in T-cell proliferation in response to magnetic field exposure (Mevisen et al., 1996; 1998) was not mediated via alterations in IL production.

In another experiment, Thun-Battersby, Westermann & Löscher (1999) exposed female Sprague-Dawley rats to a 50 Hz, 100  $\mu$ T field for periods of 3 or 14 days or 13 weeks. They performed analyses of T-lymphocyte subsets and other immune cells: NK- cells, B-lymphocytes, macrophages, and granulocytes in blood, spleen and mesenteric lymph nodes. They also detected proliferating and apoptotic cells in the compartments of spleen tissue. No effect was found on different types of leukocytes, including lymphocyte subsets for any of the exposure durations. The authors concluded that exposure did not affect lymphocyte homeostasis, but did not exclude that functional alterations in T-cell responses to mitogens and in NK-cell activity, as described in some studies of exposed rodents, may be one of the mechanisms involved in the carcinogenic effects of magnetic field exposure observed in some models of co-carcinogenesis, such as the DMBA model used by this group.

A number of tests of NK-cell activity have been carried out, mainly on exposed mice. House et al. (1996) reported that the NK-cell activity of young B6C3F(1) female mice was reduced in some experiments after exposure to continuous or intermittent 60 Hz magnetic fields (2–1000  $\mu$ T) but not in male mice nor in male or female rats. The authors later did the experiment with older female mice, and observed a similar decrease in NK-cell activity at 1000  $\mu$ T but not at the lower field intensities (House & McCormick, 2000). They concluded that the inhibition of NK-cell activity caused by exposure was consistent across their experiments but had little biological significance, as it was not associated with an increase in neoplasms in separate investigations with the same type of exposure.

Arafa et al. (2003) investigated the bioeffects of repeated exposure to 50 Hz high-strength (20 mT) magnetic fields on some immune parameters in mice. The animals were exposed daily for 30 minutes three times per week for 2 weeks. Immune endpoints included total body weight, spleen/body weight ratio, splenocytes viability, total and differential white blood cell (WBC) counts, as well as lymphocyte proliferation induced by phytohaemagglutinin, concanavalin-A and lipopolysaccharide. Magnetic field

exposure decreased splenocyte viability, WBC count, as well as mitogen-induced lymphocyte proliferation (by approximately 20%).

The authors also tested the effects of two distinct anti-radical compounds: L-carnitine and Q10. Both drugs were given 1 h prior to each ELF exposure. L-carnitine, but not Q10 attenuated the adverse effects of exposure on the vast majority of the immune parameters tested. It was speculated by the authors that the effect of L-carnitine was due to its anti-ROS properties.

Ushiyama & Ohkubo (2004) and Ushiyama et al. (2004) studied the acute and subchronic effects of whole-body exposure to 50 Hz magnetic field on leukocyte-endothelium interaction using a dorsal skinfold chamber technique in conscious BALB/c mice. They performed an acute exposure experiment by exposing for 30 min at 0, 3, 30 and 30 mT and a subchronic exposure experiment by continuous exposure for 17 days at 0, 0.3, 1 and 3 mT. The intra-microvascular leukocyte adherence to endothelial cells significantly increased at 30 mT in the acute exposure and at 3 mT in the subchronic exposure conditions. In a companion study Ushiyama et al. (2004), however, they failed to find changes in serum tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1  $\beta$  levels under exposure to subchronic exposure to 30 mT.

The effect of long-term exposure to ELF electric and magnetic fields on the thymocytes of rats was studied by Quaglino et al. (2004). The 2-month-old Sprague-Dawley rats were exposed or sham exposed for 8 months to 50 Hz fields ( $1 \text{ kV m}^{-1}$ ,  $5 \text{ }\mu\text{T}$  or  $5 \text{ kV m}^{-1}$ ,  $100 \text{ }\mu\text{T}$ ). Simultaneous exposure to continuous light and ELF fields did not change significantly the rate of mitoses compared to sham-exposed rats, but the amount of cell death was significantly increased. The conclusion of the authors was that, in vivo, stress, such as that caused by continuous exposure to light and ELF exposure can act in synergy to cause a more rapid involution of the thymus and suggested that this could be responsible for an increased susceptibility to the potentially hazardous effects of ELF-EMF.

Table 62 summarizes the studies on immune system responses found in experimental animals.

### **9.1.3 Cellular studies**

Jandova et al. (1999; 2001) found that the adherence of leukocytes taken from cancer patients to solid surfaces (such as glass surfaces or plastic materials) was increased after 1 hour of exposure to a 50 Hz sinusoidal magnetic field (1 mT and 10 mT), while it was decreased in T-lymphocytes taken from healthy donors. The leukocyte surface properties manifest cell-mediated immunity, since, in the presence of antigens, leukocytes taken from cancer patients exhibit less adherence than leucocytes from healthy humans. The authors concluded that the response of cell-mediated immunity was altered by external magnetic field exposure and hypothesized about different biophysical mechanisms, among which were the free radical reactions.

**Table 62. Immune system responses in animals**

<b>Biological endpoint</b>	<b>Exposure conditions</b>	<b>Results</b>	<b>Comments</b>	<b>Authors</b>
<b>T-cell proliferation</b>				
Spleen lymphocyte proliferation Swiss-Webster mice	60 Hz 100 kV m <sup>-1</sup> 90–150 days	No effect.		Morris & Phillips, 1982
Spleen T-lymphocyte proliferation Sprague-Dawley rats	50 Hz 50 µT 13 weeks	Decreased T-cell proliferation		Mevisen et al., 1996
Spleen T-lymphocyte proliferation Sprague-Dawley rats	50 Hz 100 T 13 weeks	Increase in T-cell proliferation after 2 weeks; decrease after 13 weeks; no effect on B-cells		Mevisen et al., 1998
Peripheral blood lymphocyte proliferation Baboons	Pilot study: 60 Hz 9 kV m <sup>-1</sup> , 20 µT 5 weeks Main study: 60 Hz 30 kV m <sup>-1</sup> , 50 µT 5 weeks	Reduced B-lymphocyte response in pilot study. No effect in main study.	Considerable heterogeneity in results of sham exposed animals.	Murthy, Rogers & Smith, 1995
<b>T-cell function</b>				
Ex vivo production of interleukins (ILs) by mitogen-stimulated splenic lymphocytes Female Sprague-Dawley rats treated with DMBA	50 Hz 100 µT 14 weeks 1 day, 1 week, 2 weeks	No effect on production of IL-1. No difference in IL-1 or IL-2-pro- duction by stimulated B- or T- cells.		Häussler et al., 1999

**Table 62. Continued**

T-lymphocyte subsets; NK-cells, B-lym- 50 Hz phocytes, macrophages and granulocytes in blood, spleen and mesenteric lymph nodes; proliferating and apoptotic cells in the compartments of spleen tissue Sprague-Dawley rats	No effects.	Thun-Battersby, Westermann & Löscher, 1999
Delayed-type hypersensitivity to oxazolone B6C3F1 mice	60 Hz 2, 200, 1000 $\mu$ T continuous, 1000 $\mu$ T intermittent (1 h on/off) 13 weeks	Generally well described study. House et al., 1996
Resistance to <i>Listeria monocytogenes</i> infection Mice (BALB/C)	60 Hz 2, 200, 1000 $\mu$ T continuous, 1000 $\mu$ T intermittent (1 h on/off) 4 or 13 weeks	Experimental and control data not shown. House et al., 1996
Long-term effects on IL-1 and IL-2 activity Sheep	60 Hz transmission lines 1.07, 3.5 $\mu$ T 12–27 mo	Hefeneider et al., 2001
<b>NK-cell activity</b>		
Spleen and blood NK cells SENCAR mice treated with DMBA and TPA	60 Hz 2 mT 6 h / day, 5 days / week, 21 weeks	McLean et al., 1991

**Table 62. Continued**

Spleen natural killer cells BALB/C mice	0.8 Hz (pulsed) 10–120 mT 10 h / day, 5 days	Enhanced activity at 30 mT and above.	de Seze et al., 1993
NK-cell activity Young mice and rats	60 Hz 2–1000 $\mu$ T, continuous or intermittent	Reduced NK-cell activity in some experiments in female mice but not in male mice nor in male or female rats.	House et al., 1996
NK-cell activity Older mice	Repeat of above study	Reduced NK-cell activity.	House & McCormick, 2000
Spleen NK-cells F344 rats	60 Hz 2, 200, 1000 $\mu$ T continuous, 1000 $\mu$ T intermittent (1 h on/off) 6 or 13 weeks	No consistent effect in males or females.	House et al., 1996
Spleen NK-cells F344 rats	60 Hz 20 $\mu$ T–2 mT 20 h / day, 6 weeks	Trend for enhanced activity with exposure.	Fully described study; but significant effects with control rather than sham comparison. Tremblay et al., 1996
<b>Macrophage activity</b>			
Peritoneal macrophages F344 rats	60 Hz 20 $\mu$ T–2 mT 20 h / day, 6 weeks	Trend for enhanced hydrogen peroxide release with exposure.	Fully described study; but significant effects with control rather than sham comparison. Tremblay et al., 1996
<b>Antibody cell activity</b>			
Circulating antibody levels to keyhole limpet haemocyanin Immunised Swiss Webster mice	60 Hz 100 kV m <sup>-1</sup> 30 or 60 days	No effect.	Morris & Phillips, 1982

**Table 62. Continued**

Antibody-forming spleen cells Immunised BALB/C mice	60 Hz 500 $\mu$ T 5 h on three alternate days	No effect.	Putinas & Michaelson, 1990
Antibody-forming spleen cells Immunised BALB/C mice	0.8 Hz (pulsed) 10–120 mT 10 h / day, 5 days	No effect.	de Seze et al., 1993
Antibody-forming spleen cells Immunised B6C3F1 mice	60 Hz 2, 200, 1000 $\mu$ T continuous 1000 $\mu$ T intermittent (1 h on/ off) 3 or 13 weeks	No effect.	Generally well described House et al., 1996 study; positive controls.
Body weight, spleen/body weight ratio, splenocytes viability, total and differen- tial WBC counts, lymphocyte prolifera- tion induced by PHA, Con-A and LPS Effect of anti-radical compounds L-car- nitine and Q10 Mice	50 Hz 20 mT 30 min / day, 3 days /week, 2 weeks	Decreased splenocyte viability, WBCs count, and mitogen- induced lymphocyte prolifera- tion. Only L-carnitine attenuated the effects of exposure.	Arafa et al., 2003
Leukocyte-endothelial interaction BALB/c mice	50 Hz 0, 3, 10 and 30 mT 30 min	Increased leukocyte adherence at 30 mT.	Ushiyama & Ohkubo, 2004
Leukocyte-endothelial interaction; serum TNF-alpha and IL-1 beta BALB/c mice	50 Hz 0, 0.3, 1 and 3 mT continuous for 17 days	Increased leukocyte adherence at 3 mT, no change in serum TNF-alpha and IL-1 beta levels.	Ushiyama et al., 2004
Rate of mitosis in thymocytes 2-month-old Sprague-Dawley rats	50 Hz 1 kV m <sup>-1</sup> , 5 $\mu$ T 5 kV m <sup>-1</sup> , 100 $\mu$ T 8 months	No change in rate of mitoses; cell death significantly increased.	Quaglino et al., 2004

Ikeda et al. (2003) studied the immunological functions of human peripheral blood mononuclear cells (PBMCs) from healthy male volunteers. They assessed the activities of NK and lymphokine activated killer (LAK) cells and the production of interferon- $\gamma$  (IFN- $\gamma$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-2 (IL-2), and interleukin-10 (IL-10). The PBMCs were exposed for 24 hours to linearly (vertical), or circularly, or elliptically polarised fields, at 50 and 60 Hz (2–500  $\mu$ T for the vertical field and 500  $\mu$ T for the rotating fields). They found no effect of exposure on the cytotoxic activities and the cytokines production of human PBMCs.

The Simko-group in Germany has been very active in recent years studying the effects of 50 Hz, 1 mT magnetic fields on various immune cells. The effects on the production of free radicals was studied by Lupke, Rollwitz & Simko. (2004) in monocytes from the blood of human umbilical cord and in human Mono Mac6 cells. In monocytes a significant increase of superoxide radical anion production was observed (up to 40%) and an increase in ROS release (up to 20%) upon 45-min exposure of monocytes. The increases were even larger in Mono Mac6 cells.

Rollwitz, Lupke & Simko (2004) gave some evidence of the cell-activating capacity of ELF magnetic fields by reporting a significant increase in free radical production after exposure of mouse bone marrow-derived (MBM) promonocytes and macrophages. The superoxide anion radicals were produced in both types of cells. The authors suggested that the NADH-oxidase pathway was stimulated by exposure, but not the NADPH pathway.

The same research group (Simko & Mattsson, 2004) has concluded that some of the effects of ELF magnetic field exposure might be caused by increasing levels of free radicals. They considered four different types of processes: (i) direct activation of macrophages (or other immune cells) by short-term exposure leading to phagocytosis (or other cell specific responses) and consequently, free radical production, (ii) exposure-induced macrophage activation including direct stimulation of free radical production, (iii) increase in the lifetime of free radicals under exposure leading to long-term elevation of free radical concentrations, (iv) long-term exposure leading to a durable increase in the level of free radicals, subsequently causing an inhibition of the effects of the pineal gland hormone melatonin. However, there are no well-established data showing that free radical production is affected by ELF magnetic field exposure.

Table 63 summarizes the results of ELF in vitro studies on immune system responses.

**Table 63. Immune system in vitro studies**

<b>Biological endpoint</b>	<b>Exposure conditions</b>	<b>Results</b>	<b>Authors</b>
Adherence assay Leukocytes taken from venous blood of normal donors and cancer patients	50 Hz 1 and 10 mT (measure- ments gave 1.02 and 9.52 mT, respectively) 1 h Test tubes placed in the center of a coil. Expo- sure performed at 37°C. Sham exposure not mentioned.	Decreased adherence in normal leukocytes which normally are adherent. Increased adherence in cancer leukocytes that are usually not adherent to solid surfaces. Similar effect for longer exposure duration (2, 3 and 4 h tested but no data shown).	Jandova et al., 1999; 2001
Several CD markers and transcription and expression of CD4.	50 Hz 24, 48, 72 h	Slight effect on CD4, CD14 and CD16 recep- tor expression, other CD receptors not affected.	Conti et al., 1999
Peripheral blood mono- nuclear cells CD4 expression	50 Hz, pulsed (2 msec. impulse duration) gen- erated by a BIOSTIM apparatus 1.5 mT 24, 48 and 72 hours	DNA CD4+ expression increased mRNA CD4+ expression increased in resting cells exposed for 24 h, but not 48 or 72 h Increase in percentage cell cycle progression in S phase	Felaco et al., 1999
Activity of NK and LAK cells; production of IFN-gamma, TNF- alpha, IL-2, and IL-10 PBMCs from healthy male volunteers	50 and 60 Hz linearly (vertical), circu- larly, or elliptically polarised magnetic fields 2–500 µT (vertical field) 500 µT (rotating fields) 24 h	No effects.	Ikeda et al., 2003
Monocytes from blood of human umbilical cord and human Mono Mac6 cells Production of free radi- cals	50 Hz 1 mT 45 min	Increase in superoxide radical anion production in monocytes; increase in ROS release upon 45- min exposure of mono- cytes (larger in Mono Mac6 cells).	Lupke, Roll- witz & Simko, 2004
Mouse bone marrow- derived (MBM) promonocytes and macrophages Production of free radi- cals	50 Hz 1 mT 45 min to 24 hours	Increase of free radical production: superoxide anion radicals were pro- duced in both types of cells.	Rollwitz, Lupke & Simko, 2004

## **9.2 Haematological system**

Haematological parameters include: leukocyte and erythrocyte counts, haemoglobin concentration, reticulocyte and thrombocyte counts, bone marrow cellularity and prothrombin times, serum iron and serum alkaline phosphatase concentrations and serum triglyceride values. Most studies have included assessments of the differential white blood cell count, that is, the overall concentration of white cells (leukocytes) and their various sub-groups. However, the importance of small alterations of the levels of circulating leukocytes is not clear as there is a continual and active exchange with other body compartments such as the lymphoid system which can be affected by a number of different factors including disease.

### **9.2.1 Human studies**

Very few studies have been performed on volunteers and none in recent years.

Selmaoui et al. (1996) exposed or sham exposed 32 male volunteers to 10  $\mu\text{T}$ , 50 Hz horizontally polarised magnetic fields between 23.00 and 08.00 on two separate days. Blood samples were taken from each subject at 3-hourly intervals from 11.00 to 20.00 and hourly from 22.00 to 08.00. One month later, the exposed group was subjected to an intermittent 10  $\mu\text{T}$ , 50 Hz magnetic field between 23.00 and 08.00. In the intermittent regimen, the magnetic field was turned on for one hour and off for the next hour; during the on-period, the field was cycled on and off every 15 s. Counts of all cell types showed a strong circadian rhythm with the possible exception of neutrophils and NK-cells; However, values in the group exposed continuously and in those exposed intermittently were always very similar to values in the sham exposed groups. Moreover, inter- and intra-individual variations were so high that small effects due to exposure were unlikely to be detected.

Bonhomme-Faivre et al. (1998) monitored a few subjects exposed for 8 hours per day for more than 1 year in their hospital laboratory to 50 Hz, 0.2–6.6  $\mu\text{T}$  magnetic fields. CD3 and CD4 lymphocyte counts were significantly lower than those measured in six control workers, but NK-cell counts were increased. Since exposure levels were measured at ankle level, the whole-body exposure of the individuals was unknown and no health consequences could be attributed to field exposure.

These studies are summarized in Table 64.

### **9.2.2 Animal studies**

Boorman et al. (1997) exposed Fischer 344/N rats and B6C3F1 mice to 60 Hz magnetic fields (2200 and 1000  $\mu\text{T}$ ) for 8 weeks (18.5 h per day, 7 days per week). An additional group of rats and mice was exposed intermittently (1 h on and 1 h off) to 1000  $\mu\text{T}$  magnetic fields. There were no haematological alterations that could be attributed to magnetic field exposure.

**Table 64. Human haematological studies**

Biological endpoint	Exposure conditions	Results	Comments	Authors
Counts of all blood cell types	50 Hz 10 $\mu$ T 23.00 to 08.00 on two separate days	No effect but strong inter and intra-individual variations.	Well controlled study. Low power.	Selmaoui et al., 1996
CD3 and CD4 lymphocytes and NK counts	50 Hz 0.2–6.6 $\mu$ T at ankle level 8 h / day, 1 year	Decrease in CD3 and CD4 and increase in NK cells.	Dosimetry not provided. Low number of subjects (6 exposed, 6 controls).	Bonhomme-Faivre et al., 1998

Zecca et al. (1998) assessed haematological variables before exposure and at 12-week intervals during exposure up to 32 weeks. Male Sprague-Dawley rats (64 animals per group) were exposed for 8 h per day, 5 days per week for 32 weeks at 50 Hz (5  $\mu$ T and 1 kV m<sup>-1</sup>, and 100  $\mu$ T and 5 kV m<sup>-1</sup>). Blood samples were collected at 0, 12, 24, and 32 weeks. No pathological changes were observed under any exposure conditions in animal growth rate, in morphology and histology of the tissues collected from the liver, heart, mesenteric lymph nodes, testes and bone marrow or in serum chemistry.

Three studies were performed by Korneva et al. (1999) in male CBA mice exposed to 50 Hz, 22  $\mu$ T magnetic fields for 1 h, at the same time of day, for 5 successive days. In the first study, spleen colony formation was examined and the number of colony-forming units was not higher than in sham-exposed animals. Significant changes were seen in the thymus weight and thymus index of exposed animals when compared to sham-exposed animals. In a second study, mice were given a sublethal dose of X-rays (6 Gy) followed 2 h later with the same magnetic field exposure as above. The number of colonies per spleen showed a consistent, significant increase with exposure and the number of colony forming units per femur was decreased. In the third study, bone marrow was taken from mice that had been exposed in still the same way, and injected into mice that had been exposed to a lethal dose of X-rays (9 Gy). The number of colony forming units per femur in the recipient mice was significantly reduced at days 1 and 4 after injection.

A summary of these studies is presented in Table 65.

**Table 65. Animal haematological studies**

Biological end-point	Exposure conditions	Results	Comments	Authors
Differential white blood cell count Swiss-Webster mice and Sprague-Dawley rats	60 Hz 100 kV m <sup>-1</sup> 15 (rats only), 30, 60 or 120 days	No consistent effects seen in replicate studies.	Replicate studies; some results variable.	Ragan et al., 1983
Differential white blood cell and bone marrow progenitor cell count CBA/H mice	50 Hz 20 mT 7 days	No effect.		Lorimore et al., 1990
Splenic lymphocyte subgroup analysis B6C3F1 mice	60 Hz 2, 200, 1000 µT continuous 1000 µT intermittent (1 h on/off) 4 or 13 weeks	No effect.	Generally well described study.	House et al., 1996
Differential white blood cell count F344 rats	60 Hz 20 µT–2 mT 20 h / day, 6 weeks	Trend for reduced T-cell count with exposure; reduced total, cytotoxic and helper T-cells.	Fully described study; significant effects with control rather than sham comparison.	Tremblay et al., 1996
Differential white blood cell count Sprague-Dawley rats	50 Hz 100 µT 3 days, 14 days or 13 weeks	No effect.	Extensive lymphocyte sub-set analysis.	Thun-Battersby, Westermann & Löscher, 1999
Differential white blood cell count Baboons	Pilot study: 60 Hz 9 kV m <sup>-1</sup> , 20 µT 5 weeks Main study: 60 Hz 30 kV m <sup>-1</sup> , 50 µT	Reduced helper T-lymphocyte count in pilot study; no effect in main study.	Considerable heterogeneity in sham exposed results.	Murthy, Rogers & Smith, 1995
Haematology Fischer rats and B6C3F1 mice	60 Hz 1000 or 2200 µT continuous 1000 µT Intermittent (1 h on, 1 h off) 18.5 h / day, 7 days / week, 8 weeks	No effect.		Boorman et al., 1997

**Table 65. Continued**

Blood cells count before exposure, at 12, 24 and 32 weeks of exposure	50 Hz 5 $\mu$ T, 1 kV m <sup>-1</sup> 100 $\mu$ T, 5 kV m <sup>-1</sup> 8 h / day, 5 days / week, 32 weeks	No effects.	Zecca et al., 1998
Morphology and histology of different organs (liver, heart, mesenteric lymph nodes, testes, bone marrow)			
Groups of 64 rats sham-exposed			
Spleen colony formation	50 Hz 22 $\mu$ T	No effect of EMF alone.	Korneva et al., 1999
Bone marrow injected to mice exposed to 9 Gy X-rays	1 h / day, same time of day, 5 successive days 6 Gy X-rays followed after 2 h by same exposure as above	Increase in number of colonies per spleen; decrease in colony forming units per femur. Number of colony forming units per femur significantly reduced in the recipient mice.	
Male CBA mice			
Total and differential white blood cell counts	50 Hz 20 mT 30 min / day, 3 days / week, 2 weeks	Decreased white blood cells count.	Arafa et al., 2003
Mice			

### 9.2.3 Cellular studies

Only one paper has been published recently on the effects on cells of the haematopoietic system: Van Den Heuvel et al. (2001) studied the effects of 50 Hz, 80  $\mu$ T magnetic fields on the proliferation of different types of stem cells, including haemopoietic cells. The cytotoxic effects of exposure were investigated on the proliferation of undifferentiated murine 3T3 cells using the neutral red test. Magnetic fields had no cytotoxic effect on this cell line.

When exposed to the same fields, a reduction in the proliferation and differentiation of the granulocyte-macrophage progenitor (CFU-GM) grown from the bone marrow of male and female mice was shown compared to non-exposed cells. Stromal stem cell proliferation (CFU-f) from female mice showed a reduction while CFU-f from male mice did not decrease. The authors concluded that these effects on CFU-f are equivocal.

Table 66 summarizes the results of ELF in vitro studies.

**Table 66. Cell proliferation studies**

Biological endpoint	Exposure conditions	Results	Authors
Cell numbers and colony following efficiency Mouse haemopoietic progenitor cells FDCP mix A4	Nullified fields, 50 Hz vertical fields, Ca <sup>2+</sup> ion cyclotron resonance conditions at 50 Hz 0.006, 1 and 2 mT 2 hours immediately after seeding 1, 4 or 7 days, one hour after seeding	No effects.	Reipert et al., 1997
Cell number K562 myeloid leukaemia cells	50 Hz 0.2–200 $\mu$ T up to 24 h	No effects.	Fiorani et al., 1992
<sup>3</sup> H-thymidine uptake CCRF-CEM human lymphoblastoid cells	72 Hz pulsed 3.5 mT 0.5–24 h	No effects.	Phillips & McChesney, 1991
Proliferation Stem cells Undifferentiated murine 3T3 cells	50 Hz 80 $\mu$ T 4 days	No effects.	Van Den Heuvel et al., 2001
Proliferation and differentiation of the granulocyte-macrophage progenitor	50 Hz 80 $\mu$ T 7 days	Reduction in proliferation and differentiation.	Van Den Heuvel et al., 2001
Stromal stem cell proliferation	50 Hz 80 $\mu$ T 10 days	Decrease in female mice and no change in male mice.	Van Den Heuvel et al., 2001

### 9.3 Conclusions

Evidence for the effects of ELF electric or magnetic fields on components of the immune system is generally inconsistent. Many of the cell populations and functional markers were unaffected by exposure. However, in some human studies with fields from 10  $\mu$ T to 2 mT, changes were observed in natural killer cells, which showed both increased and decreased cell numbers, and in white blood cell counts, which showed no change or decreased numbers. In animal studies reduced natural killer cell activity was seen in female, but not male mice or in rats of either sex. White blood cell counts also showed inconsistency, with decreases or no change reported in different studies. The Animal exposures had an even broader range of 2  $\mu$ T to 30 mT. The difficulty in interpreting the potential health impact of these data is due to the large variations in exposure and environmental conditions, the relatively small numbers of subjects tested and the broad range of endpoints.

There have been few studies carried out on the effects of ELF magnetic fields on the haematological system. In experiments evaluating differential white blood cell counts, exposures range from 2  $\mu$ T to 2 mT. No consistent effects of acute exposure to magnetic fields or to combined electric and magnetic fields have been found in either human or animal studies.

Overall therefore, the evidence for effects of ELF electric or magnetic fields on the immune system and haematological system is considered inadequate.