European Health Risk Assessment Network on Electromagnetic Fields Exposure

EFHRAN

Work package 5

D3 - Report on the analysis of risks associated to exposure to EMF: *in vitro* and *in vivo* (animals) studies

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1 INTRODUCTION

The aim of work package 5 is to monitor and review systematically recent key research results from *in-vitro* and *in vivo* studies to identify any EMF health risks. The activities and tasks for this work package were essentially to collate and critically review the literature. Both cancer and non-cancer endpoints were considered. The task complements the risk analysis performed by EMF-NET in WP4. The four sub-tasks in WP5 are based on the frequency range: a) extremely-low frequency (ELF) b) intermediate frequency (IF), c) radiofrequency (RF) d) interaction mechanisms (whole frequency spectrum).

This report takes into account all results published after the 2009 SCENIHR report.[1] All publications were considered and none were excluded from the review on the basis of poor quality. It is also based on the conclusion of some recent expert group reports quoted below. Classification of the biological effects was done for exposure levels below the critical effect as defined by the International Commission on Non-Ionizing Radiation Protection (ICNIRP), e.g., 100 W/kg for local RF exposures of the head and neck.

In order to evaluate the strength of evidence for adverse effects arising as a consequence of exposure to EMF, EMF-NET had used a very simple, yet powerful, four point classification system that itself was based on the system used by the International Agency for Research on Cancer (IARC) to estimate the carcinogenic risk to humans from a wide range of chemicals and physical agents, including static and extremely low frequency electric and magnetic fields (IARC, 2002). EFHRAN decided to adopt the same classification system to evaluate the strength of evidence for any particular effect. The four classifications and criteria for inclusion into any particular category are shown in Table 1. Clearly, a classification of sufficient evidence requires there to have been much high quality research that produces a consistent outcome; independent replication is also considered a key element. Similarly, evidence suggesting a lack of effects indicates that several studies have reported the absence of field-related effects using a range of appropriate models and relevant exposure conditions.

Classification	Necessary inclusion criteria
Sufficient evidence	• when a positive relationship is observed between the exposure and the effect investigated • when the effect is replicated in several studies by independent investigators or under different protocols, and when there is a consistent exposure-response relationship • when confounding factors could be ruled out with reasonable confidence
Limited evidence	• when the evidence of the effect is restricted to a few studies, or when there are unsolved questions regarding the adequacy of the design, conduct or interpretation of the study • when confounding factors could not be ruled out in the studies with reasonable confidence
Inadequate evidence	• when the studies are of insufficient quality, consistency or statistical power to permit a conclusion
Evidence suggesting a lack of effects	• when no effects are reported in several studies by independent investigators under different protocols involving at least two species or two cell types and a sufficient range of field intensities

Table 1. The four point system used in this report to classify the strength of evidence for any particular effect; a similar

[1] SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks). Health Effects of Exposure to EMF. 19 January 2009

system was used by EMF-NET.

2 RADIOFREQUENCY FIELDS

2.1 Cancer studies

2.1.1 Discussion on cancer in the SCENIHR report

Seven recent studies with rodents have evaluated carcinogenicity of RF electromagnetic fields in vivo. Several different animal models were used including classical bioassays, studies using genetically predisposed animal models and co-carcinogenicity studies involving combined exposure to RF fields and known carcinogens. A few differences were reported for some endpoints, but no consistent dose-response pattern was observed, and the direction of the differences varied (increase or decrease in exposed animals), indicating that the few statistically significant differences are just statistical noise (false positive findings are unavoidable when many studies with multiple endpoints are conducted).

Overall, the results of the new studies are consistent with results from previous studies, and add to the evidence that the RF fields such as those emitted by mobile phones are not carcinogenic in laboratory rodents. Some of the new studies have also used exposure levels up to 4 W/kg, which is high, compared to most previous studies. Thus, these studies provide additional evidence that carcinogenic effects are not likely even at SAR levels that clearly exceed human exposure from mobile phones.

Different biological endpoints have been investigated in-vitro after RF field exposure using a variety of cell types and exposure conditions with diverse outcome. In the majority of studies no genotoxic effects were shown. A few studies suggest various biological effects (including genotoxic effects) from RF fields, alone or in combination with other factors, mostly at higher SAR values (above 2 W/kg). The biological relevance of these findings is however unclear. Inconsistent in-vitro findings and a lack of dose response relationships render any mechanistic understanding of potential non-thermal interactions between RF and living systems difficult. For RF fields below the recommended limits (2 W/kg) for energy absorption due to mobile phones, in-vitro studies have not identified reproducible effects by which carcinogenicity in living systems could be explained.

2.1.2 *In-vitro* cancer

2.1.2.1 Genotoxic effects

In two Italian studies from the same group the SXC-1800 exposure setup of the Swiss IT'IS foundation was used. The human trophoblast-derived HTR-8/SVneo cell line was used as *in-vitro* model of the female reproductive system. Indeed, trophoblasts play a crucial role in maintaining placental physiology. The alkaline comet assay was used to assess the level of DNA damage and three parameters were considered (tail length, % DNA in the tail, tail moment).

In the first paper (Valbonesi et al., 2008), it is reported that a 1-h exposure to amplitude-modulated GSM 1800 signals (2 W/kg) did not cause increased levels of DNA damage in HTR-8/SVneo cells, nor induced changes in the expression of HSP70 and HSC70 proteins and

hsp70A, B, C and hsc70 genes.

In the second paper (Franzellitti et al., 2009), cells were exposed for 4, 16 or 24 hours to 1800-MHz continuous-wave (CW) or amplitude-modulated GSM-217Hz and GSM-Talk. A 5-min ON, 10-min OFF intermittent exposure was applied. CW exposure was ineffective in inducing DNA damage. By contrast, the GSM 1800 basic signal induced a significant increase in all comet parameters after 16 and 24 hours of exposure while the GSM-Talk signal induced inconsistent increase in DNA damage in terms of comet parameters altered and exposure duration. Such a discrepancy in comet parameters outcome is unusual. Trophoblast cells, however, were shown to recover from DNA damage rapidly, within 2 hours after RF exposure, and their viability was not altered. These data suggest that CW RF fields did not induce DNA damage and that intermittent exposure to amplitude-modulated GSM signals may have induced, for exposure longer than 16 hours, reversible DNA damage without altering the viability of the cells.

In Germany, Schrader et al. (2008) evaluated the production of spindle disturbances in FC2 cells, a human-hamster hybrid (AL) cell line, after exposure to 835 MHz RF (90 V/m, 0.06 W/kg). In a previous paper they had shown that spindle disturbances were increased at low exposure levels (20 to 90 V/m) (Schmid and Schrader, 2007). Only two independent exposures were performed at room temperature in a transversal electromagnetic field (TEM) cell. RF exposure from 0.5 to 2 hours was shown to disturb spindle at the anaphase and telophase stages of cell division. However, spindle disturbances did not change the fraction of mitotic cells. This paper was criticized because of a lack of adequate controls at the different time points, especially since the cells were transferred from 37°C to 20-22°C for RF exposure and for up to 2 hours (Swicord, 2009).

The effect of GSM-1800 MHz RF (2 W/kg) on DNA repair was tested in HMy2.CIR human B-lymphoblastoid cells exposed to doxorubicin (DOX, 0.05-0.20 µg/ml) by Zhijian et al. in China (2009, 2010). Different types of combined exposure were assayed: Sham- (S) or RF-exposure (E) for 2 h before DOX treatment combined with sham- or RF- exposure for 2 h during DOX treatment (SS, ES, SE and EE) and sham- or RF-exposure after DOX treatment for 2, 6, 12, 16 and 24 h (SSS, SSE, SES, ESS, EEE). The exposure was intermittent, with a 5-min ON / 10-min OFF cycle and use of the SxC1800 setup (GSM 1800, IT'IS, Switzerland). The comet assay was used and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The authors showed that intermittent exposure to GSM-1800 did not induce DNA damage in HMy2.CIR cells. DOX-induced DNA damages were dose-dependent and unaffected by the double combination with GSM-1800 (ES, SE, EE). Among the different triple combinations of GSM-1800 exposure with DOX, the EEE scheme only (exposure to RF for 2 h, then simultaneous exposure to RF and DOX, and exposure to RF) could consistently impair DNA repair at 6 h and 12 h after exposure to DOX for DOX doses from 0.075 to 0.20 µg/ml. At 24 hr of RF exposure after DOX treatment, the level of DNA damage reached again the background level. The lack of effect of GSM-1800 intermittent exposure for up to 30 h seems contradictory to the data reported by Franzelletti et al. (2009), although the cell types were not the same in the two studies.

In China, DNA damage and intracellular ROS were assessed by Yao et al. (2008) in cultured human lens epithelial cells after 2-h exposures to GSM-1800 (1, 2, 3, and 4 W/kg). RF radiation at 3 and 4 W/kg induced significant DNA damage, assessed using the alkaline comet assay

(single-strand breaks), while there was no effects on double strand breaks, evaluated using the γH2AX foci test. HLECs exhibited significant intracellular ROS increases in the 2, 3, and 4 W/kg groups. Surprisingly, when RF was superposed with a 2-μT magnetic noise, the increase in ROS and DNA damage did not occur.

In three recent review articles (Vljayalaxmi & Prihoda, 2008; Ruediger, 2009; Habash et al., 2009), the genotoxic effects of RF exposure were described and discussed and the conclusions of these publications were taken into account in the present report.

2.1.2.2 Non-genotoxic cancer related effects

Using the mouse BALB/3T3 cell transformation model, Hirose et al. (2008) evaluated the effect of a continuous 6-week 2140-MHz RF exposure (W-CDMA) in an anechoic chamber at 0.08 and 0.8 W/kg on spontaneous and induced neoplastic transformation. 3-methylcholanthrene (MCA) was used as an initiator and phorbol-12-myristate-13-acetate (PMA) as a tumour promoter. The data showed that no induction (RF alone), promotional (MCA+RF) or co-carcinogenic (MCA+PMA+RF) effect of RF exposure was found on cell neoplastic transformation.

Two studies by a French group (Billaudel et al., 2009a&b) aimed at a confirmation of the increase in ornithine decarboxylase (ODC) activity in L929 mouse fibroblasts after modulated RF exposure (DAMPS-835 MHz, 2.5 W/kg, 8 h), as previously reported by the Litovitz group, and at testing different RF signals (GSM-900 and -1800) and cell types (SH-SY5Y human neuroblastoma cells) for ODC activity. Exposure of L929 cells was performed in a TEM cell (DAMPS-835 MHz, 8 h, 2.5 and 6.0 W/kg), in a wire-patch antenna (217 Hz modulated GSM-900, 2 h, 0.5, 1, and 2 W/kg), in a DAMPS-835 MHz waveguide (8 h, 0.5, 1, and 2.5 W/kg) or in SxC-1800 waveguide (GSM-1800, 2, 8 and 24 h, 2.5 W/kg) under temperature control (37°C). SH-SY5Y cells were exposed at 1 and 2.5 W/kg for 8 or 24 h to DAMPS-835 MHz and GSM-1800 signals. ODC activities were assayed using $^{14}CO_2$ generation from ^{14}C-labeled L-ornithine either in live or lysed cells. Whatever the exposure condition and ODC activity testing method, the authors did not show any effect of RF exposure on ODC activity in either cell line tested.

HSP70 gene and protein expression was evaluated in the human trophoblast cell line HTR-8/SVneo after exposure to 1800-MHz continuous-wave (CW) and different GSM-1800 signals (217 Hz and Talk) at 2 W/kg for 4 to 24 h (Franzellitti et al., 2008). There was no alteration of the inducible HSP70 protein expression nor of the inducible HSP70A, HSP70B or the constitutive HSC70 transcripts under RF exposure with all condition tested. By contrast, the inducible HSP70C transcript was found significantly enhanced after 24 h of exposure to GSM-217 Hz signal and reduced after 4 and 16 h exposure to the GSM-Talk signal. However, the level of HSC70 protein was not assayed.

An Italian study was designed to assess whether UMTS exposure (1950 MHz, 0.5 and 2.0 W/kg) induced oxidative stress in human lymphoblastoid cells, with RF alone or in combination with ferrous ions, $FeSO_4$ (Brescia et al., 2009). The production of ROS was measured by flow cytometry. Short 5-60 min or long 24 h duration exposures were carried out in a waveguide system. The non-thermal RF exposures did not increase spontaneous ROS formation under any of the experimental conditions investigated. There was no change in cell viability in Jurkat cells

exposed to RF for 24 h. Combined exposures to RF and $FeSO_4$ did not increase ROS formation induced by the chemical treatment.

2.1.3 *In vivo* cancer

2.1.3.1 Genotoxic effects

In a follow-up of the Perform-A study (Tillmann et al., 2006), genotoxicity was assessed in the peripheral blood erythrocytes of surviving mice (males and females, mean number of 39 animals / group) exposed for 2 years (2 h/d, 5 d/w) to GSM-900 or -1800 at whole-body SARs of 0.4, 1.3, and 4 W/kg (Ziemann et al., 2009). Micronuclei were counted in polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) to evaluate the index of damage induced by RF exposure within 72 hours (acute) and 30 days (chronic) of sampling, respectively. Moreover, the analysis was performed blind in 2000 cells of each type and in two laboratories. In contrast to male and female mice treated with mytomycin C, which showed significant increased MN in PCE and/or NCE (females/males), no excess in MN in PCE and NCE was detected in mice exposed to RF. In line with the lack of carcinogenic effects of RF found in the Perform-A study, this state-of-the-art study showed a lack of clastogenic and aneugenic effects of long-term, whole-body RF exposure up to 4 W/kg.

2.1.3.2 Non genotoxic effects

The Drosophila model system was used by a South-Korean group (Lee et al., 2008) to assess the effects of exposure at 835 MHz on flies. At 1.6 W/kg, more than 90% of the flies were viable after the 30-h exposure. At 4.0 W/kg the viability started to drop after 12 h of exposure, which triggered a stress response and increased the production of ROS. There was also an activation of extracellular signal regulated kinase (ERK) and signalling of c-Jun N-terminal kinase (JNK), but not of p38 kinase. In addition, at 4.0 W/kg, the number of apoptotic cells increased in the fly brains. The findings thus depended on the SAR level. However, the dosimetry of the exposure system could not be evaluated on the basis of the description.

2.1.4 Conclusion on RF & cancer

The available in-vitro data suggest, depending on the cell type and exposure duration, that some effects of amplitude–modulated RF signals on DNA damage or DNA repair are reversible in time. Effects reported on mitotic spindles, suggest that low level RF exposure could be aneugenic. However, in view of the data obtained using integrative models (neoplasic transformation assay, in vivo study), these arguments seem weak and the related health consequences of such in-vitro effects remain questionable.

A review has been recently published based on the ICNIRP blue book that took into account papers published in 1994-2008 (Juutilainen et al., 2010) on RF investigations on animals. The conclusion of the review is that the results from the available literature are « rather consistent and indicate no carcinogenic effects at exposure levels up to 4W/kg, which is clearly higher than the worst-case local maximum values (up to 1.5 W/kg) associated with use of mobile phones». The conclusion of the present report goes along the same lines.

2.2 Nervous system effects

2.2.1 Discussion on the nervous system in the SCENIHR report

With the exception of a few findings in otherwise negative studies, there is no evidence that acute or long-term RF exposure at SAR levels relevant for mobile telephony can influence cognitive functions in humans or animals […] There is no evidence that acute exposures to RF fields at the levels relevant for mobile telephony have effects on hearing or vision. Furthermore, there is no evidence that this kind of exposure has direct neurotoxicological effects. Most studies show lack of effects on supporting structures like the blood-brain-barrier. The positive finding is lacking dose- response relationships and needs independent replication in studies with improved methodology. The findings of activated glial cells at relatively high SAR-values could indicate gliosis and thus subsequent neurodegeneration after exposure, although exposures at lower levels did not reveal any such effects.

2.2.2 Animal studies

2.2.2.1 Blood-brain barrier (BBB)

A Japanese group has reviewed its studies on the microcirculatory parameters assessed through a cranial window (Hirota et al., 2009). Vascular diameter, plasma velocity, leukocyte behavior and BBB function were evaluated. After acute (10 min, brain-averaged SAR of 0.6, 2.4, and 4.8 W/kg) and chronic exposure (60 min, 5 days/week, 4 weeks, 2.4 W/kg) with a monopole antenna at 1439 MHz, no changes were observed, irrespective of the exposure conditions.

The Salford group had shown that GSM-900 exposure alters the permeability of the blood–brain barrier (BBB), resulting in albumin extravasation 14 days after 2 h of exposure and increase of dark neurons 28 days but not 14 days after exposure. These effects did not show a dose-response relationship.

In a recent study, the same group (Nittby et al., 2009) examined the effects of the same exposure conditions (SARs of 0, 0.12, 1.2, 12, and 120 mW/kg) in rats sacrificed 7 days after exposure. Albumin extravasation was greatest in animals exposed at 12 mW/kg and no effects on the incidence dark neurons were described.

In 2009, three groups published the results of studies which attempted to corroborate some of the work of Salford and colleagues using the same rat strain, but avoiding some of the technical weaknesses in the original studies such as the use of rats of widely different ages, sexes and trying to improve fixation and staining methods.

In the USA, McQuade et al (2009) used a similar transverse electromagnetic transmission line (TEM) exposure cell and similar exposure parameters to those used by Salford and colleagues. Extravasation of albumin in rat brain tissue was examined at the end of exposure. Brains from exposed or sham-exposed rats for 30 min to CW 915 MHz or 915 MHz pulse-modulated at 16 or 270 Hz at whole-body SARs ranging between 1.8 mW/kg and 20 W/kg showed little or no extracellular extravasation of albumin in contrast to the effects seen in the positive control groups.

In Japan, Masuda et al. (2009) examined the effects of a single 2-h exposure or sham exposure to GSM-915 MHz radiation also in a similar TEM cell at whole-body SARs between 20 mW/kg and 2.0 W/kg. The effects on the extravasation of albumin and on the appearance of dark

neurons were evaluated histologically 14 or 50 days after exposure. The authors reported that they were unable to find any evidence of increased albumin extravasation or dark neurons in the brain tissue of exposed animals. Clear increases of both parameters were seen in the positive control groups

In France, Poulletier de Gannes et al. (2009) also used improved staining techniques, in order to identify albumin extravasation and the presence of dark neurons in rat brains 14 or 50 days after the head-only exposure or sham exposure of rats for 2 h to a GSM-900 signal at brain averaged SARs of 140 mW/kg and 2.0 W/kg. In addition, a more specific marker for neuronal degeneration was used and the presence of apoptotic neurons was looked. The authors reported that they were unable to find any evidence of increased albumin extravasation, neuronal degeneration, dark neurons or apoptosis in 12 different regions of rat brain tissues of exposed animals, although clear increases in both were seen in the positive control group.

Thus, the observations of Salford and colleagues have not been successfully confirmed by these three groups, which indicates that the original observations are loosing credibility.

2.2.2.2 Stress response

The Australian group of Finnie (Finnie et al., 2009) examined the effects of GSM-900 signals throughout gestation on HSP expression in foetal mouse brains. Pregnant mice were exposed or sham-exposed (10 per group) to GSM-900 signals at a whole-body SAR of 4 W/kg for 1 h per day every day from day 1 to day 19 of gestation. Following exposure, pregnant animals were sacrificed and one foetal brain was selected from each litter for heat shock response. HSP25, HSP32 and HSP70 expressions were assessed in three coronal brain sections. There was no evidence of induction of HSP32 or HSP70 in the mouse brains, and HSP25 expression was limited to two brainstem regions in both exposed and sham-exposed animals.

The same group studied the effects of a whole body exposure to GSM-900 (4 W/kg) on microglial cell activation (Finnie et al., 2010). After acute and long-term (2 years) exposures, no differences were observed between sham and exposed-groups. The positive controls obtained after a focal stab wound validated the method.

The effects of 900 MHz exposures were investigated in Turkey on rat brain apoptosis and oxidative stress (Dasdag et al., 2009). After 10 months of exposure (2 h/day, 7 days/week), brains were immuno-histochemically stained for apoptosis markers (active caspase-3 and p53). Catalase, total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index were measured. The apoptosis score was significantly lower in the exposed group compared with the sham and the cage control groups, but p53 was not significantly affected by exposure. The TAC and catalase were increased in the exposed group while TOS and oxidative stress index were not statistically different between exposed and sham groups.

The de Seze group in France (Ammari et al., 2008) assessed the effect of exposure to GSM-900 signals on rat brain metabolic activity by measuring cytochrome oxidase levels. The brains of the rats were exposed or sham- exposed at a SAR of 1.5 W/kg for 15 min per day or at 6 W/kg for 45 min / day for 7 days. Animals were sacrificed 7 days after the end of exposure. Compared to the sham-exposed group, significant decreases were found in cytochrome oxidase

activity of animals exposed at 6 W/kg in areas close to the RF loop antenna (the prefrontal and frontal cortex) and in deeper structures (the posterior cortex, the hippocampus and septum) but not in those exposed at 1.5 W/kg, raising the possibility that the effects were thermal in nature.

2.2.2.3 Gene expression

In a collaborative project between Italy and Australia, Paparini et al. (2008) carried out microarray analyses of 22,600 genes in the whole brain tissue of a total of 30 mice (15 per group) exposed or sham-exposed to GSM-1800 MHz signals at a brain SAR of ~ 0.2 W/kg for 1 hour. In contrast to the study by Nittby et al (2008a) described below, gene expression in the brain tissue of exposed mice was not significantly different from that of the sham-exposed. Applying a weakly stringent constraint revealed that 75 genes modulated their expression between 0.67 to 2.8 fold, including several gene ontology functions such as transcription regulation and transporter activity. However, real-time RT-PCR analysis did not confirm the expression changes observed.

In Sweden, Nittby et al (2008a) carried out microarray analyses of 31,099 genes from hippocampal and cortical tissue of the brains of a total of 8 rats (4 per group) following exposure or sham-exposure to GSM-1800 signals at an average whole-body SAR of 13 mW/kg (brain SAR of 30 mW/kg) for 6 h. The authors reported significantly altered expression in some categories of gene in both cortex and hippocampus of the exposed rats compared to those from sham-exposed controls using gene ontology analysis to examine the expression of various functional categories of genes. Four of the 10 most significantly altered categories were associated with membrane receptor function. The authors reported that these results might have a relation to their previous observation of albumin transport through brain capillaries. However, the number of animals per group and the exposure SAR levels were very low.

A recent review by McNamee and Chahan (2009) on gene and protein expression in vivo and in-vitro under RF exposure, came to the conclusion that “Numerous studies have investigated the potential ability of RF radiation to modify gene transcription and protein levels in a variety of cellular and animal models. A selected few of these investigations have reported RF-radiation-induced effects, but under conditions where the possibility of thermal confounding cannot be excluded. Other studies report RF-radiation-induced alterations in gene/protein expression under non-thermal RF-radiation exposure conditions, but typically in unique (unreplicated) conditions/ models or under experimental conditions with methodological shortcomings. Furthermore, there are no clear trends in the list of responsive genes/proteins across studies of various experimental designs, thereby diminishing the biological plausibility that responses observed in individual studies are genuine. When taken collectively, the weight of evidence does not support the notion of specific, non-thermal responses to RF radiation at the gene or protein level. Nevertheless, a few well-conducted studies have observed sufficient evidence of possible RF-radiation-induced gene/protein interaction to warrant further investigation.”

2.2.2.4 Neurodegenerative *diseases and neuronal loss*

The paper by Arendash et al. (2010) is the first describing cognitive benefits after RF exposure (918 MHz; ca. 0.25 W/kg) in both normal and transgenic mice developing Alzheimer's disease (AD). The cognitive interference task used in that study was designed to mimic the corresponding human task. The improvement in cognitive functions in transgenic mice was linked to reduced brain amyloid-β (Aβ) deposition through Aβ anti-aggregation actions as demonstrated *in-vitro*. Several hypothesis on the possible mechanisms of RF action were proposed by the authors including increased Aβ clearance from the brains of AD mice, increased neuronal activity, and increased cerebral blood flow and glucose utilization. Nevertheless, the authors noted an increase in both body and brain temperature during exposure that could be a key feature of the mechanism. If confirmed, RF exposure may represent a new therapeutic approach against AD. However, the poor description of the dosimetry warrants a replication study.

In South Korea, the influence of RF exposure (CDMA 835 MHz) on calcium homeostasis was followed by immuno-histochemistry through changes in the expression of calcium binding proteins (CaBP) such as calbindin D28-k (CB) and calretinin (CR) in the hippocampus of mice (Maskey et al., 2010). Various exposure durations and SARs were tested: 1 h/day for 5 days at 1.6 or 4 W/kg, 5 h/day for 1 day at 1.6 or 4 W/kg, and daily exposure for 1 month at 1.6 W/kg. The main effect was observed after a one-month exposure yielding a complete loss of pyramidal cells in the CA1 area stained by CR. Image analysis of CaBP and CR labelling also revealed significant differences in all of the brain hippocampal areas studied and for the majority of exposure conditions. The exposure system was designed for free-running animals but the experimental and numerical dosimetry was not well described and thermal effects cannot be ruled out.

2.2.2.5 Neurogenesis

The possible effects of whole-body pulsed 2.45 GHz exposures (2.8 mW/cm^2) was studied in the Slovak Republic on rat postnatal neurogenesis using the BrdU proliferating cell marker (Orendácová et al., 2009). Two animal ages were taken into account: newborn (P7) and old (24 months) rats. Two exposure durations were compared: 2 days (4 h/day) and 3 days (8 h/day), considered by the authors as short and long term exposures, respectively! The recovery period was acute (24 h) or chronic (1-4 weeks). The most significant changes were only observed in the newborn rats after either short or long exposures, in terms of altered proliferation of cells in the rostral migratory stream. The RF source was an ill-defined microwave oven and no SAR determination was done.

2.2.2.6 Behaviour

In South Africa, Daniels et al. (2009) exposed rat pups to a 840 MHz signal from post-natal day 2 to 14 for 3 h per day and behaviour tests were performed at P58 using the Morris water maze to assess spatial memory and the ability to learn a specific task, and the Open Field test to assess anxiety-like behaviour and mood disturbances. At P62, the presence of dark neurons

and corticosterone levels were evaluated. No significant differences were observed in the spatial memory test and dark neurons in the hippocampus. However, in exposed males, increased grooming and decreased locomotor activity were noticed, with no significant effect on basal corticosterone levels. The decrease in locomotor activity together with the increase in grooming suggested that RF exposure might lead to behavioural abnormalities, at least in males. These results should be taken with caution as the dosimetry was not carried out and the reported incident power in the far field was only 60 $\mu W/m^2$!

2.2.3 *In-vitro* studies

A Japanese group has examined the consequences of a 2-h exposure at 1950 MHz (0.2, 0.8, and 2.0 W/kg) on microglial cells, which constitute the immune system of the brain (Hirose et al., 2010). Classical markers of microglial activation were assayed in primary microglial cell cultures, 24 and 72 h after exposure, such as histocompatibility complex class II. No statistically significant differences were observed between sham and exposed groups. The production of Tumour Necrosis Factor-α, interleukin-1β, and interleukin-6 were also measured and did not reveal any effect. These findings suggest that exposure to 1950 MHz did not activate microglial cells *in-vitro*.

In a collaborative work of research groups in Bologna, Italy, the viability, proliferation, and vulnerability of two models of neuronal cells (SN56 cholinergic cell line and rat primary cortical neurons), were assessed after exposure to GSM-900 and in the presence of neurotoxic molecules, (glutamate, 25-35AA beta-amyloid, and hydrogen peroxide) (Del Vecchio et al., 2009). Exposure to RF did not change viability/proliferation of the SN56 cholinergic cells or viability of cortical neurons. The neurotoxic effect of hydrogen peroxide was increased by RF exposure in SN56 but not in primary cortical neurons. These results suggest that combined exposure to RF and some neurotoxic agents might alter oxidative stress in cells.

The purpose the Chinese study by Xu et al. (2010) was to determine whether RF exposure caused oxidative damage to mitochondrial DNA mtDNA, known to be susceptible to oxidative stress. Primary cultures of cortical neurons were exposed for 24 hours to GSM-1800 signals (2 W/kg, ITIS' sXc-1800 system, Switzerland). Exposure induced a significant increase in the levels of a common biomarker of DNA oxidative damage: 8-hydroxyguanine in the mitochondria of neurons. The number of copies of mtDNA and the levels of RNA transcripts were also affected after RF exposure. All these damages diminished in the presence of melatonin, a well-known antioxidant.

2.2.4 Conclusion on RF & the nervous system

The most surprising finding is the beneficial effect of RFR on cognitive function in Alzheimer disease mice.

The very recent animal studies relevant to mobile telephony show a lack of effect on the BBB, microglial cell activation, and stress response.

There is some evidence that RF exposure influences gene expression, behaviour, and the number of neurons but these studies were done with small number of animals or absence of

dosimetry. Some in-vitro studies seem to show an effect of mobile telephony signal on oxidative stress that must be confirmed.

2.3 RF Development and reproduction

2.3.1 Discussion on development and reproduction in the SCENIHR report

The recent studies that addressed RF field effects on prenatal development in animals and the association of maternal mobile phone use with behavioural effects in children have not provided new information that would change the conclusions of the previous opinion that there are no adverse effects at nonthermal exposure levels. Studies on male fertility are inadequate due to low statistical power and/or methodological problems.

2.3.2 Development, teratology

In Japan, Ogawa et al. (2009) examined the effect of head-only exposure to a 1950 MHz W-CDMA RF signal on embryogenesis in rats. The authors exposed or sham-exposed 60 pregnant rats for 90 min/day from day 7 to 17 of gestation at average brain SARs of 0.67 or 2 W/kg (whole-body SARs of 0.4 W/kg). The pregnant rats were sacrificed on gestational day 20. All organs of dams were macroscopically observed and foetuses examined for a number of conventional teratological parameters. There were no statistically significant differences in any parameters either for health or pregnancy of the dams or for embryo or foetal development.

The consequences of exposure to RF emitted by a “real” mobile telephone were tested on the retina of developing chicken embryos (Zareen et al., 2009) in Pakistan. At different ages of development, the retinal thickness and epithelial pigmentation grades were measured. Statistical differences between sham and exposed embryos were noticed, depending on the duration of exposure. According to the authors, EMF modified the normal developmental processes of retina in chick embryos. However, the use of a phone as RF source precludes any validation of the dosimetry making the results of this study very difficult to interpret.

A Turkish group (Tomruk et al., 2010) has evaluated the possible biological effects of whole-body 1800 MHz exposure of rabbits (15 min/day for a week) on liver lipid peroxidation and oxidative DNA damage. Nonpregnant and pregnant rabbits and their newborns in both control and exposed conditions were followed. No significant differences were found in DNA damage using the 8-OHdG marker.[2] Lipid peroxidation using malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased in the exposed non-pregnant group. In newborns, no significant differences were found in 8-OHdG and MDA levels while liver FOX levels were found significantly decreased in exposed newborns. The authors did not discuss the absence of effect in pregnant rabbits and the decrease in FOX level observed both in exposed pregnant rabbits and their newborns. The dosimetry of the exposure system (horn antenna in the near field) was not done and no SAR was given, making the data difficult to interpret.

Lee et al. (2009a) in South Korea exposed pregnant mice (whole-body at 2.0 W/kg) to a CDMA signal or simultaneously to CDMA and WCDMA signals throughout the entire gestation period

[2] 8-hydroxy-2-deoxyguanosine

(days 1-17). Mice received two 45-min RF-field exposures, separated by a 15-min interval. On the 18th day of gestation, foetuses were examined for teratological evaluation. Simultaneous experimental exposure to CDMA and WCDMA signals did not cause any observable adverse effects on mouse foetuses.

2.3.3 Reproduction

2.3.3.1 In vivo

Pregnant rats were exposed by a Turkish research group to a “real” mobile phone in a standby position for 11 h 45 min and turned on to speech position for 15 min during the whole 21-day pregnancy (Gul et al., 2009). The battery was charged continuously. The number and the weight of pups were followed after delivery. The ovaries of the female pups were removed 21 days after delivery and the volumes and number of follicles were measured and counted respectively. The statistical analysis revealed that the number of pups and follicles were decreased in the exposed group, but the use of a phone as an RF source precludes any validation of the dosimetry making the results of this study very difficult to interpret.

Rabbit testicular function and structure were compared among 800 MHz mobile-phone exposed animals (8 h daily for 12 weeks) placed in a specially designed cage, sham-group (stress control rabbit placed in the same kind of cages) and cage control group (Salama et al., 2008). The mobile phone was on the stand-by mode. A weekly analysis of sperm concentration revealed a significant drop after 8 weeks of exposure and in sperm motility at 10 weeks. Histological testicular sections showed also a significant decrease in the diameter of seminiferous tubules in the exposed group while testosterone concentration measurement did not show any differences. However, there was no dosimetry and moreover it s well known that a mobile telephone in the stand-by emits very rarely and therefore the total exposure of the animals was extremely small.

To find an explanation for the impact of RF exposure on sperm motility previously described, the same group studied the levels of both fructose and citrate known as important components in semen that facilitate sperm motility (Salama et al., 2009a). The exposure system and the tested groups were the same as used previously but the frequency was 900 MHz. The number of motile sperms and the fructose levels were significantly decreased after 10th week of exposure. The sham-exposed animals showed a similar tendency looking at the motility. No significant changes were observed for the other studied parameters (citrate level and histologic sections of prostatic complex, ampulla, and vesicular gland).

Rabbit male sexual behaviour was analyzed after exposure under the same conditions described above (Salama et al., 2009b). The copulatory behaviour (duration, frequency and type of mounts) was significantly modified in the exposed group. These modifications were not linked to hormonal disruption (testosterone, dopamine and cortisol).

The interpretation of these three papers is hindered by the lack of dosimetry and the likely absence of RF exposure!

Male mice were exposed to RFR coming from GSM base stations placed around an office block complex or around a residential quarter in Lagos, Nigeria (Otitoloju et al., 2010). Both exposure conditions caused an increase in sperm head abnormalities compared to the control group

located away from GSM base stations. The observed sperm head abnormalities were also found to be dose dependent. However the quality of the exposure assessment and living conditions of the mice seem to be so poor that the results must be taken with extreme caution.

2.3.3.2 In-vitro

In Australia, human spermatozoa from 22 donors were exposed in a home-made cylindrical waveguide to a GSM-1800 signals (de Iuliis et al., 2009). Different SARs from 0.4 to 27.5 W/kg were tested. Both spermatozoa motility and vitality were reduced in a dose-dependent manner above 1 W/kg. The authors also observed an increase in reactive oxygen species (ROS) generation after 1 W/kg and above which seemed to be of mitochondrial origin and not related to thermal effect. The ROS production seemed to be related with the observed increase in oxidative DNA damage and fragmentation. The dosimetry was done using crude methods and the validation of this exposure system is uncertain in terms of SAR determination and heating of the sample at high SAR.

2.3.4 Conclusion on Development and reproduction

Most of the studies described above have shown effects of RF on teratology and reproduction. Nevertheless, in all these papers, the exposure systems are not appropriate and dosimetry data are absent or not correct.
The conclusions of the SCENIHR report are thus still valid.

2.4 RF Miscellaneous

2.4.1 *In vivo* studies

2.4.1.1 Auditory

The cochlear functions of female infant and adult rabbits was investigated in Turkey by measuring the Distortion Product Otoacoustic Emission (DPOAE) response amplitudes under exposure to GSM-1800 signals, 15 min daily for 7 days (Budak et al., 2009a). DPOAE alterations were found under GSM exposure, more in adult than infant rabbits. According to the authors, prolonged exposure and hyperthermia, increasing the temperature in the ear canal, may have decreased the DPOAE amplitudes. However no dosimetry was performed for the near-field exposure system (horn antenna), which makes the data very difficult to interpret.
Within the framework of the European EMF nEAR Project, an Italian research group (Galloni et al., 2009) investigated the effects of UMTS exposure on the functionality of cochlear outer hair cells (OHCs) in rats, locally exposed on the right ear or sham-exposed at 1946 MHz, 10 W/kg, 2 h/d, 5 d/w, for 4 weeks. DPOAEs were performed before, during and 1 week after exposure. There were no statistically significant differences among the groups. These findings are very much in line with the consensus of an absence of effects of RF exposure on the auditory system of animals.

2.4.1.2 Immunology

An Italian research group (Prisco et al., 2008) examined the effects of exposure to a GSM-900 signal on the ability of bone marrow cells to differentiate, colonize lymphatic organs, and rescue lethally X-irradiated mice from death. X-irradiated mice were injected with medium containing bone marrow cells from either RF-exposed (2 W/kg, 2 h/day, 5 days/ week, 4 weeks) or sham-exposed donor mice. All mice that received bone marrow cells survived. Three and 6 weeks after bone marrow cell transplantation, no differences in thymus cellularity or in the frequency of differentiating cell sub-populations (identified by CD4/CD8 expression) were observed among the three transplanted groups. Mitogen-induced thymocyte proliferation yielded comparable levels in all transplanted groups. There were no effects of exposure on spleen cell number, percentages of B and T cells, proliferation, and IFN-γ production in transplanted mice.

2.4.2 *In-vitro* studies

2.4.2.1 Immunology

In Croatia, Pavicic and Trosic (2008) studied the influence of CW 935 MHz exposure on the structure and growth of V79 cells. A Gigahertz Transversal Electromagnetic Mode cell (GTEM-cell) was used to expose the cell cultures and the SAR was estimated at 0.12 W/kg. Cell samples were exposed for 1, 2, and 3 h. Cell growth was determined during 5 days. Microtubule structure was altered after 3 h of exposure and cell growth was decreased three days after exposure. The authors concluded that the 935 MHz exposure altered microtubule proteins, which consequently may have affected cell growth.

A similar study was performed by the same group (Trosic and Pavicic, 2009), who monitored the proliferation, cytoskeleton structure, and mitotic index of V79 cells after 1, 2, and 3 h of exposure (935 MHz, calculated SAR of 0.12 W/kg). The proliferation of exposed cells declined for those exposed for 3 hours, 72 h after irradiation. Microtubule structure was altered after 3 hours of exposure. The mitotic index was not affected. The authors suggested that exposure may have slowed down cell division kinetics as a consequence of microtubule impairment caused by exposure.

In both investigations by this Croatian group the GTEM exposure system was not well adapted to this type of studies and there was no accurate dosimetry.

2.5 Conclusion on RF effects

In the last 15 years most of the research on RF and health has been devoted to the search for nonthermal biological effects of exposure. This search has been unsuccessful so far in spite of the report of many uncorrelated findings. The collection of recent papers does not change the overall picture and, on the contrary, it appears that the quality of the work in particular in terms of exposure systems and dosimetry has not been satisfactory, despite the availability of such devices and methods. Results from the high-quality studies are mostly negative. A few major studies are ongoing that will not be completed before the coming health risk assessments. This

is true in particular for the bioassay investigation under progress in Chicago (McCormick et al., DMccormick@iitri.org).

Overall, in agreement with the conclusions of recent international reports, one can conclude that there are no well-established positive effects of low-level RF exposure (SAR < 2 W/kg). The open questions that can be addressed in animal and cell investigations are mainly related to the possible greater sensitivity of children.

2.6 Strength of evidence for selected biological effects from exposure to RF fields.

Outcome	Strength of evidence
Cancer studies	
- Genotoxic effects	
In vitro	Limited evidence
In vivo	Lack of effect
- Non genotoxic effects	
In vitro	Inadequate evidence
In vivo	Inadequate evidence
Nervous system	
- BBB	Lack of effect
- Stress response	Limited evidence
- Gene expression	Inadequate evidence
- Neurodegenerative disease	Inadequate evidence
- Neurogenesis	Inadequate evidence
- Behaviour	Inadequate evidence
- *In vitro*	Limited evidence
Development and reproduction	
- Development, teratology	Inadequate evidence
- Reproduction	Inadequate evidence
- *In vitro*	Inadequate evidence
Miscellaneous	
- Auditory	Lack of effect
- Immunology	
In vivo	Inadequate evidence
In vitro	Inadequate evidence

3 EXTREMELY LOW FREQUENCY (ELF) FIELDS

3.1 Discussion on ELF effects in the 2009 SCENIHR report

The previous opinion stated that ELF magnetic fields are a possible carcinogen. This conclusion was chiefly based on childhood leukaemia results.

It was also concluded that a consistent relationship between ELF fields and self-reported symptoms has not been demonstrated.

Regarding breast cancer and cardiovascular disease, an association was considered unlikely. For neurodegenerative diseases and brain tumours, the link to ELF fields remained uncertain.

The new information available is not sufficient to change the conclusions of the 2007 opinion.

The few new epidemiological and animal studies that have addressed ELF exposure and cancer do not change the previous assessment that ELF magnetic fields are a possible carcinogen and might contribute to an increase in childhood leukaemia. At present, in-vitro studies did not provide a mechanistic explanation of this epidemiological finding.

No new studies support a causal relationship between ELF fields and self-reported symptoms.

New epidemiological studies indicate a possible increase in Alzheimer's disease arising from exposure to ELF. Further epidemiological and laboratory investigations of this observation are needed.

Recent animal studies provided an indication for effects on the nervous system at flux densities from 0.10-1.0 mT. However, there are still inconsistencies in the data, and no definite conclusions can be drawn concerning human health effects.

Very few recent in-vitro studies have investigated effects from ELF fields on diseases other than cancer and those available have very little relevance. There is a need for hypothesis-based in-vitro studies to examine specific diseases.

It is notable that in vivo and in-vitro studies show effects at exposure levels (from 0.10 mT and above) to ELF fields that are considerably higher than the levels encountered in the epidemiological studies (μT-levels) which showed an association between exposure and diseases such as childhood leukaemia and Alzheimer's disease. This warrants further investigation.

3.2 Cancer studies

3.2.1 Discussion on cancer in the 2009 SCENIHR report

The previous assessments are unchanged. The fact that the epidemiology findings of childhood leukaemia have little support from known mechanisms or experimental studies is intriguing and it is of high priority to reconcile these data. A recent study on rats has provided additional evidence of co-carcinogenic effects from exposure to ELF magnetic fields at 100 μT. However, the findings still need independent confirmation.

Although many earlier in-vitro studies did not show any effects, some studies indicated that ELF magnetic fields alone and in combination with carcinogens induce both genotoxic and other biological effects in-vitro at flux densities of 100 μT and higher. Recent studies support this effect. Direct field-inducing damage to DNA is unlikely; therefore alternative mechanisms must

be hypothesised. As already pointed out in the last opinion there is still a need for independent replication of certain studies suggesting genotoxic effects and for better understanding of combined effects of ELF magnetic fields with other agents and their effects on free radical homeostasis.

3.2.2 *In vivo* studies

In a Japanese study, cancer was initiated in new-born CD-1 mice with a single subcutaneous injection of 60 µg 7,12-dimethylbenz(a)anthracene (DMBA) within 24 h after birth (Negishi et al. 2008). At 4 weeks of age, groups of 50 males and 50 females mice either served as cage controls or were exposed to 0 (sham-exposed), 7, 70, or 350 µT circularly-polarized 50 Hz magnetic fields for 22 h/d, 7 d/w for 30 weeks in Merritt-type coils having two orthogonal sets of four square coils. Animals were observed daily and the development of malignant lymphoma/lymphatic leukaemia was examined histopathologically. The experiment was conducted twice for testing reproducibility. All examinations and histopathological evaluations were conducted blind.

The data showed that the incidence of malignant lymphoma/ lymphatic leukaemia and the cumulative proportions of mice with malignant lymphoma/lymphatic leukaemia in the MF-exposed groups were not significantly higher than those in the sham-exposed group of each sex in individual experiments and in males and females combined in each experiment, and in all the animals (experiments combined). Immunohistochemical analysis revealed that the lineage of the origin of all malignant lymphoma/lymphatic leukaemia was T-cell. Therefore, while showing no promotional effect of the magnetic fields, this state-of-the-art study used a model of adult leukaemia. Such study using an animal model of childhood leukaemia would be highly valuable.

The French study of Bernard et al. (2008) was the first using a chemically-induced rat model of acute pro-B lymphoblastic leukaemia. The possible co-initiating or co-promoting effects of sinusoidal 50 Hz MF (100 µT, with or without harmonics) were assessed on the development of leukaemia. Body weight, survival time, percentage of bone marrow blast cells, cumulative incidence of leukaemia and type of leukaemia were found to be similar between exposed and unexposed groups. By contrast, γ-irradiation induced significant changes in the leukaemia type.

3.2.3 *In-vitro* studies

A few previous studies had suggested that 50/60 Hz magnetic fields reduced the antiproliferative effect of melatonin on MCF-7 human breast cancer cells (Liburdy et al., 1993). In the German study by Girgert et al. (2009), the effect of a 48-h exposure to a 1.2 µT, 50 Hz MF was studied in oestradiol- and/or melatonin-stimulated MCF-7 cells and MCF-7 mel1a cells (i.e. MCF-7 cells transfected with the MT1 melatonin receptor gene). The authors showed that MF inhibited the melatonin-induced decrease in oestradiol-stimulated MCF-7 mel1a cell proliferation. Based on a single blot picture, the authors claimed that estradiol-increased binding of CREB to the promoter of BRCA-1 was diminished by treatment with melatonin, and that exposure to MF almost completely abolished binding of CREB. Expression of BRCA-1, p53, p21WAF, and c-myc (oestrogen-responsive genes) was increased by estradiol in both cell lines, except for p53 expression in MCF-7 mel1a cells, but the amplitude was always less than a factor 2. Melatonin mostly diminished the effect of estradiol, except for c-myc. In estradiol-treated cells, magnetic

fields had either no effect (c-myc in both cell lines, BRCA1 and p53 in MCF-7 Mel1a cells) or an effect similar to melatonin. The only exception was the combination of estradiol/melatonin and magnetic fields, which exhibited a significant 2.4 increase in c-myc expression in MCF-7 mel1a cells. Based on this study, the picture of the effects of 50 Hz MF is still unclear as the effects described here mostly mimic those of melatonin with some exceptions (proliferation and c-myc expression).

3.2.4 Conclusion on cancer and ELF

Of importance is the bioassay using a model of childhood leukaemia (proB lymphoblastic leukaemia), that showed no influence of 50 Hz MF. A new bioassay using polarised magnetic fields (as present in the environment) instead of linear fields also showed no influence on chemically-induced lymphoma/ lymphatic leukaemia.
New data on the influence of low-level 50 Hz electromagnetic fields on melatonin effects provide no clear evidence of melatonin being a target of MF.
The need is still of more bioassays using childhood leukaemia models.

3.3 Other health effects

3.3.1 *In vivo* studies

3.3.1.1 Auditory

A Turkish group had studied the effects of RF exposure on the auditory system of rabbits (see above). In the present study the same group (Budak et al., 2009b) assessed the effects of 50 Hz electric fields (5.1 and 10.2 kV/m, 3 h/day for 14 days). Electric field exposure altered hearing functions and high-field exposure caused an increase in cochlear activity.

3.3.1.2 Behaviour

The long-term consequences of 50 Hz magnetic field exposure on the development of chronic stress and stress-induced psychopathology in rats were investigated in Hungary by Szemerszky et al. (2010). Adult male Sprague-Dawley rats were exposed to 50 Hz, 0.5 mT fields for 5 days, 8 h daily or for 4-6 weeks, 24 h daily. Anxiety was assessed in elevated plus maze test, and depression-like behaviour of the long-exposure group in the forced swim test. After behavioural tests, organ weights, blood hormone levels as well as proopiomelanocortin mRNA level from the anterior lobe of the pituitary gland were measured. Both exposure protocols were ineffective on somatic parameters. An enhanced blood glucose level was found after prolonged exposure. The hormonal stress reaction was similar in control and short-term exposed rats, but significant proopiomelanocortin elevation and depressive-like behaviour were found following long-term ELF exposure. The authors concluded that long and continuous exposure to 0.5 mT magnetic fields may be a mild stress situation and could contribute to depressive state or metabolic disturbances.
In a Turkish study (Gulturk et al., 2010), the effects of long-term magnetic field exposure (5 mT, 165 min/d, 30 days) or insulin, and their combination on blood-brain barrier (BBB) permeability in a diabetic rat model (diabetes mellitus, induced with streptozotocin) were investigated. Both

diabetes mellitus and ELF exposure increased BBB permeability and even more in combination. Insulin decreased their effect on the BBB.

3.3.1.3 Memory

Memory impairment in newly hatched chicks was investigated in China (Sun et al., 2010) following *in-ovo* exposure on embryonic days 12-18 to a 50 Hz magnetic field at 2 mT (60 min/day). The effect of stress during training, and memory retention were tested at 10, 30, and 120 min, following exposure to a bitter-tasting bead. While sham chicks had good memory retention levels at 30 and 120 min, those exposed to MF did not. This suggests a potential disruption of memory formation caused by *in-ovo* exposure. This effect only occurred in the more stressed isolated chicks.

3.3.1.4 Haematology

In still another study from Turkey, Cakir et al. (2009) investigated the effects of ELF exposure (0.97 mT, 50 and 100 days, 3 h/day) on whole-blood haematological parameters in rats. Eosinophil, hemoglobin and MPV levels decreased significantly in rats exposed for 50 days. There were no alterations in total leukocyte, neutrophil, lymphocyte, monocyte, eosinophil and basophil counts, or in erythrocytes, between exposed and sham groups. Exposure had no effect on body weight. The authors concluded that exposure induced only slight but statistically significant alterations in some haematological parameters, but within the physiological range.

3.3.2 *In-vitro* studies

3.3.2.1 Calcium ion

A study was conducted by Piacentini et al. (2008) in Italy to determine whether ELF magnetic fields influenced the neuronal differentiation of neural stem/progenitor cells (NSCs) of mice by modulating the Cav1-channel function. In cultures exposed to 1 mT 50 Hz fields, the proportion of cells with immunoreactivity for neuronal markers and for Cav1.2 and Cav1.3 channels was increased. There was also a significant increase in spontaneous firing, percentage of cells exhibiting Ca^{2+} transients in response to KCl stimulation, amplitude of these transients and Ca^{2+} currents generated by the activation of Cav1 channels. According to the authors, these data suggest that field exposure promotes neuronal differentiation of NSCs by upregulating Cav1-channel expression and function.

3.3.2.2 Reactive Oxygen Species (ROS)

In Rostock, Germany, the Mattsson group investigated the effects of exposure to 50 Hz, 1 mT magnetic fields on the production of ROS in macrophages of mice (Frahm et al., 2010). The ROS level was increased by exposure and there was a modulation of the expression level of important proteins acting in redox regulatory processes: there was slight and transient decreases after short-term exposures (2 h or less) of clathrin, adaptin, PI3-kinase, protein kinase B (PKB) and PP2A, whereas longer exposures had no effect. The levels of the NAD(P)H oxidase subunit gp91phox oscillated between increased and normal levels. The stress proteins Hsp70 and Hsp110 exhibited increased levels at certain time points. The effects of MF on

protein levels were different from the effects exerted by TPA or LPS, although all factors caused increases in ROS release. As suggested by these authors, ELF magnetic fields initially and immediately influenced redox-mediated pathways by modulating NAD(P)H oxidase as shown in the altered gp91phox level. The effect is not on the transcriptional level, but either on recruitment of pre-existing mRNA or on stability of the enzyme.

Henrykowska et al., (2009) in Poland assessed the influence of the shape of an ELF magnetic field on catalase and superoxide dismutase activity, malondialdehyde concentration and free radicals generation in human blood platelets. The suspension of platelets was exposed for 15 min to 50 Hz magnetic fields of different shapes, at 10 mT. Free radicals, malondialdehyde and catalase levels were increased following exposure, regardless of the shape of the magnetic field applied, but superoxide dismutase activity was lowered.

An Italian study was carried out to investigate the influence of ELF magnetic fields on protein oxidation and on the 20S proteasome functionality, the complex responsible for the degradation of oxidized proteins (Eleuteri, 2009). Caco 2 cells were exposed, for 24-72 hours, to 1 mT 50 Hz magnetic fields. The treatment induced a time-dependent increase in both cell growth and protein oxidation, while there was no change in cell viability. EGCG, a natural antioxidant compound, counteracted the field-related pro-oxidant effects. According to the authors, this suggests that the increased proteasome activity was due to an enhancement in free radicals.

3.3.2.3 Genotoxicity

Exposure to 50 Hz 1 mT for 24 h had been reported to induce DNA damage in human fibroblast cells using the Comet assay (Ivancsits et al. 2002). These findings have been criticised because of a lack of reproducibility, technical and statistical concerns, and the absence of a plausible mechanism.

However, in a confirmation study by Focke et al. (2010), intermittent (but not continuous) exposure of human primary fibroblasts to 1-mT magnetic fields (15 h) was shown to induce a slight but significant increase in DNA fragmentation, and it is suggested that the magnetic rather than the electric field plays a role. According to the authors, the effect depended on cell proliferation, with a concomitant reduction of actively replicating cells and an increase in apoptotic cells, but no contribution of oxidative DNA base damage. However, these latter demonstrations appeared weak, mostly based on duplicated experiments. Globally, the statistical analysis is not adequate for these small samples. Indeed, for the comet assay, the sample unit is the culture (or the animal in *in-vivo* experiments), not the cell. The authors suggest that the processes involved in DNA replication rather than DNA itself may be affected by MF exposure.

3.3.3 Conclusion on ELF effects

Many new findings have been published in the last years. They are mostly uncorrelated and performed under high-level exposure, i.e., in the mT range. Moreover, most of them are not addressing the main issue, which is the association between exposure and childhood leukaemia. In the context of decreasing funding, this situation is not likely to change in the coming years.

3.4 Strength of evidence for selected biological effects from exposure to ELF magnetic fields.

Outcome	Strength of evidence
Cancer studies	
- *In vivo*	Lack of effect
- *In vitro*	Inadequate evidence
Other health effects	
In vivo	
- Behaviour	Limited evidence
- Memory	Limited evidence
- Haematology	Inadequate evidence
In vitro	
- Calcium ion	Limited evidence
- ROS	Limited evidence
- Genotoxicity	Limited evidence

4 INTERMEDIATE FREQUENCIES

4.1 Discussion on IF effects in the 2009 SCENIHR report

The previous opinion expressed its concern that very little useful epidemiologic data on intermediate fields and health risks are available. Furthermore, it was noted that in vivo and in-vitro data are very sparse. Well-established acute effects occur and these are explained by extrapolation from ELF and RF field mechanisms. Thus it was concluded that there was no basis for an appropriate assessment of long term effects.

Occupational exposure to IF fields in certain areas is considerably higher than exposure to the general public. However, very little research on IF and health risks in occupational settings or for the general public have been presented since the previous opinion, and no epidemiological studies have appeared. Consequently, the data are still too limited for an appropriate risk assessment.

In view of the increasing occupational exposure to IF among workers in e.g. security, shops, and certain industries it is important that research in this area is given priority.

4.2 Development

A previous study of the Lee group in Korea had shown no effect of a 20 kHz exposure at 6.26 μT on foetal development of pregnant mice. The same authors (Lee et al., 2009) performed a complementary teratological evaluation by increasing the peak intensity to 30 μT, which is the occupational exposure limit at 20 kHz in Korea. The animals were killed on the 18th day of gestation and foetuses were examined for mortality, growth retardation, changes in head size and other morphological abnormalities. Exposure to IF fields did not cause any observable adverse effects on mouse foetuses.

White fertile eggs were either exposed in a blind fashion to a 20 kHz, 1.1 mT sinusoidal magnetic

field or sham-exposed during the first 2, 7, or 11 days of embryogenesis (Nishimura et al., 2009). Data from the present study indicate that exposure did not induce any significant embryotoxicity. No dose–response relationship was also found, in a range from 0.01 to 1.1 mT, in the 2-day embryos. Additional eggs treated with all-trans-retinoic acid, a known teratogen, before sham or IF exposures, did not show any potentiation of the embryotoxic action of retinoic acid.

4.3 Genotoxicity

In Japan, the mutagenicity, co-mutagenicity, and gene conversion after 48 h of IF magnetic field exposure (2, 20 and 60 kHz) was investigated using bacterial mutation and yeast genotoxicity tests (Nakasono et al., 2008). Cells were exposed in a Helmholtz type exposure system that generated vertical and sinusoidal IF magnetic field: 0.91 mT at 2 kHz, 1.1 mT at 20 kHz and 0.11 mT at 60 kHz. Mutagenicity was tested in four strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537), and two strains of Escherichia coli (WP2 uvrA and WP2 uvrA/pKM101), co-mutagenicity was assayed in TA98, TA100, WP2 uvrA and WP2 uvrA/pKM101 with five chemical mutagens, and gene conversion assays, as well as DNA repair assay, were performed in Saccharomyces cerevisiae XD83. No statistically significant effects were observed between exposed and control groups in any of the genotoxicity tests. Moreover, the IF magnetic field tested did not induce gene conversion nor affect the repair process of DNA damage induced by UV irradiation.

In another Japanese cellular study (Sakurai et al., 2009), the effects of 23 kHz IF fields (6.05 mT, 2 h) were evaluated in CHO-K1 cells on cellular genotoxicity as determined by cell growth, comet assay, micronucleus formation and hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation, and on stress responses measured through the expression and activation of heat shock protein (Hsp) 27, 70, 105 and phosphorylated Hsp27. Irrespective of the parameter studied, no effect of the IF exposure could be detected.

4.4 Conclusion on IF effects

Very few new findings have been published on effects of IF exposure. Most of them originated from South Korea and they were negative.

In the context of the continuous development of the related technologies, further research is warranted but the current conclusion based on few investigations is that no health hazards are foreseen.

4.5 Strength of evidence for selected biological effects from exposure to IF fields

Outcome	Strength of evidence
Development	Lack of effect
Genotoxicity	Lack of effect

5 MECHANISMS

5.1 Radiofrequency

The search for mechanisms of RF bioeffects other than heating has continued without success. The main difficulty is that there are no well-established bioeffects for which mechanisms must be elucidated! A recent review by Sheppard et al., (2008) evaluated the various hypotheses (cooperativity, signal averaging, coherent detection, or by nonlinear dynamical systems, radical pair mechanism, role of magnetite). None of them have been validated experimentally.

The only new publications were related to the attempt of the groups of Balzano in the USA and Exell in the UK to detect demodulation by living cells (Balzano et al., 2008): a doubly resonant cylindrical microwave cavity to allow a search for nonlinear RF energy conversion in biological cells. Cells with a diode-like nonlinearity could demodulate a modulated RF carrier wave and generate low frequency signals in an exposed biological sample. The cavity operates in the TE(111) mode at 890 MHz and in the TE(113) mode at 1780 MHz. In none of the tested biological samples exposed at 890 MHz has a signal at double the frequency been observed. The demodulation process thus does not seem to occur at this frequency range and is likely to be confined below around 10 MHz.

The consensus of the heating mechanism as the only mechanism occurring in the GHz range is still valid.

5.2 ELF

The search for mechanisms of ELF bioeffects is also active and there a few observations of bioeffects that are under replication. There are still the two open possibilities for the magnetic field itself or for the time-varying magnetic-field induced currents to elicit bioeffects.

One of the mechanisms that is still being actively investigated is the one behind animal navigation. Many species, including birds, mammals, reptiles, amphibians, fish, crustaceans and insects, are known to orient and navigate in the geomagnetic field. The biophysical mechanisms that underlie the avian magnetic compass are poorly understood. One mechanism, based on magnetically sensitive free radical reactions, is gaining support. Maeda et al. (2009) have used spectroscopic observation of a carotenoid-porphyrin-fullerene model system to demonstrate that the lifetime of a photochemically formed radical pair is changed by application of 50 μT magnetic fields, and to measure the anisotropic chemical response that is essential for its operation as a chemical compass sensor. These experiments established the feasibility of chemical magnetoreception and gave insight into the features required for detection of the direction of the geomagnetic field.

6 REPORTS

Scientific reports written by groups of experts have recently been published. They have been taken into account in this report in terms of health risk assessment.

- The ICNIRP[3] has just published a "blue book"[4] on the biological effects and health consequences of exposure to RF fields in the range 100 kHz-300 GHz (ICNIRP, 2009). Its conclusions, based on publications collected up to the beginning of 2009, are in line with those of the present report.

- The annual report of the SSM[5] was published at the beginning of 2010 and spans over 2008 and 2009. This issue was devoted to RF bioeffects and the summary on animal and cell work was the following: "A large number of cell studies are done on both genotoxic and non-genotoxic outcomes, such as apoptosis and gene expression. There are no new positive findings from cellular studies that have been well established in terms of experimental quality and replication. Potential heating of the samples is still seen as a major source of artefacts. Moreover, these few positive results are not related to each other and/or are not relevant for health risk assessment.
  There are animal studies on brain structure and brain function as well as on genotoxicity and cancer. Also reproductive effects are looked at. However, animal studies have not identified any clear effects on any of a number of different biological endpoints following exposure to RF radiation typical of mobile phone use, generally at levels too low to induce significant heating."

- The French Agency for Environmental and Occupational Health Safety (Afsset)[6] is a public body reporting to the French Ministers for ecology, for health and for employment.
  It recently published a major report on RF and health: More than 1,000 studies were reviewed by AFSSET, focussing on mobile phones, Wi-Fi emitters, microwave ovens, cordless home phones and other devices that use frequencies of between 9 kilohertz (kHz) and 300 gigahertz (GHz). Most of the studies did not show any negative impacts. Some research, however, did point to possible health problems, including cell damage, reduced male fertility and a lower blood flow to the brain. All of these studies had been performed at levels far above ambient and close to the thermal threshold.

[3] International Commission on Non Ionizing Radiation Protection
[4] www.icnirp.de/documents/RFReview.pdf
[5] Swedish Radiation Safety Authority www.stralsakerhetsmyndigheten.se
[6] www.afsset.fr

7 CONCLUSION

For the three frequency ranges examined, the conclusions of the 2009 SCENIHR report are still valid in spite of the publication of several positive findings.

Many of the new publications originate from laboratories and countries that are new to bioelectromagnetics research. This translates sometimes into unsatisfactory dosimetry or statistical analysis. Health risk assessment to be performed in the coming years (e.g., WHO EMF project) will need to be carried out with strict quality criteria.

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