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DEPARTMENT OF ANIMAL BREEDING AND HUSBANDRY
ΕΡΓΑΣΤΗΡΙΟ ΓΕΝΙΚΗΣ ΚΑΙ ΕΙΔΙΚΗΣ ΖΩΟΤΕΧΝΙΑΣ

Ph.D. Thesis
ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

BIOLOGICAL EFFECTS OF ELECTROMAGNETIC RADIATION

ΒΙΟΛΟΓΙΚΕΣ ΕΠΙΠΤΩΣΕΙΣ ΤΗΣ ΗΛΕΚΤΡΟΜΑΓΝΗΤΙΚΗΣ ΑΚΤΙΝΟΒΟΛΙΑΣ

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To

my Wife and Son

‘Without their unconditional love,
strength and guidance they got form above
this work would not have been done’

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Amr Gabr

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LIST OF ABBREVIATIONS

B	magnetic flux density	**MRI**	magnetic resonance imaging
C	celsius	**mT**	millitesla (10^{-3} T)
Ca	calcium	**mW**	milliwatt (10^{-3} W)
cm	centimeter	**MW**	micro wave
CNS	central nervous system	**n**	number of samples of a test series
D	electric flux density	**nW**	nanowatt (10^{-9} W)
DNA	deoxyribonucleic acid	**p**	level of significance
E	electric field strength	**RF**	radio frequency
ELF	extremely low frequency	**RNA**	ribonucleic acid
EMF	electromagnetic field(s)	**s**	second
F1,F2,F3	(1^{st}, 2^{nd}, 3^{rd}) filial generation	**SAR**	specific absorption rate
G	gauss (1 G = 100 μT)	**SW**	short wave
g	gram	**T**	tesla
GHz	Gigahertz (10^{9} Hz)	**THz**	Terahertz (10^{12} Hz)
GMS	Global System for Mobile Communication	**U**	voltage
H	magnetic field strength	**UHF**	ultra-high frequency
h	hour	**USW**	ultra-short wave
HF	high frequency (English: RF)	**UV**	ultraviolet light
Hz	hertz	**V**	volt
kg	kilogram	**V/m**	volt/meter
kHz	kilohertz (10^{3} Hz)	**W**	watt
m	meter	**W/kg**	watt/kg
mG	milligauss (10^{-3} G)	**μ**	micro (10^{-6})
MHz	megahertz (10^{6} Hz)	**μT**	microtesla

Abstract

ΒΙΟΛΟΓΙΚΕΣ ΕΠΙΠΤΩΣΕΙΣ ΤΗΣ ΗΛΕΚΤΡΟΜΑΓΝΗΤΙΚΗΣ ΑΚΤΙΝΟΒΟΛΙΑΣ

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Περίληψη

Σκοπός της διδακτορικής διατριβής ήταν η μελέτη των βιολογικών επιδράσεων της έκθεσης εμβρύων (όρνιθας) σε ηλεκτρομαγνητική ακτινοβολία χαμηλής ισχύος 900 MHZ. Η συχνότητα αυτή αφορά την περιοχή λειτουργίας των κεραιών κινητής τηλεφωνίας (GSM-900). Το πρωτόκολλο έκθεσης περιλάμβανε τη συνεχή έκθεση των εμβρύων κατά τις 5 πρώτες ημέρες της εμβρυογένεσης. Στα πλαίσια του πειραματισμού κατασκευάστηκαν δύο όμοιες εκκολάπτικές συσκευές περιορίζοντας στο ελάχιστο την ύπαρξη μεταλλικών τμημάτων προς αποφυγή φαινομένων παρεμβολής (interference). Η μια από τις δύο συσκευές αποτέλεσε τη μονάδα μάρτυρα και η άλλη τη μονάδα έκθεσης με τοποθέτηση της κεραίας εκπομπής (τύπου Yagi) σε απόσταση 1,5 m από το κέντρο της. Ακολούθησαν μετρήσεις ηλεκτρικού πεδίου τριών σημείων (ανώτερο, κέντρο και κατώτερο) στη μονάδα έκθεσης. Οι τιμές αυτές τροφοδότησαν ειδικό λογισμικό το οποίο προσομειώνει τις δεδομένες συνθήκες έκθεσης επιτρέποντας τον υπολογισμό των επιπέδων της παρεχόμενης ακτινοβολίας σε επίπεδο αυγού (Ειδικός Ρυθμός Απορρόφησης, SAR: Specific Absorption Rate σε mW/kg) καθώς και της αναμενόμενης αύξησης της θερμοκρασίας ως αποτέλεσμα της έκθεσης. Η ανάλυση δοσιμετρίας έδειξε ότι ο μέσος όρος (±ΤΣ) του EPA ανήλθε σε χαμηλά επίπεδα της τάξεως του 0,13±0,02 mW/kg ενώ τα επίπεδα και η διάρκεια έκθεσξς δεν οδηγούν σε σημαντική αύξηση της θερμοκρασίας των αυγών (μέγιστη τιμή 0,03 ºC). Στα πλάισια της διδακτορικής διατριβής, ακολουθώντας το ίδιο πρωτόκολλο έκθεσης διενεργήθηκαν συνολικά 4 πειράματα. Στο πρώτο πείραμα διερευνήθηκαν τυχόν επιδράσεις της ακτινοβολίας στην εμβρυϊκή ανάπτυξη. Ακολούθησαν 2 πειράματα στα οποία εξετάστηκε εάν η έκθεση επηρεάζει συγκεκριμένες πτυχές της συμπεριφοράς νεαρών ορνίθων και τέλος στο 4^{o} πείραμα διερευνήθηκε εάν η ακτινοβολία τροποποιεί το ανοσοποιητικό σύστημα των νεαρών ορνίθων. Στο πρώτο πείραμα χρησιμοποιήθηκαν συνολικά 360 γονιμοποιημένα αυγά ενός κρεοπαραγωγικού υβριδίου (ISA Brown) σε τρείς πειραματικές σειρές (3x120 αυγά). Μετά από πενθήμερη έκθεση στην ηλεκτρομαγνητική ακτινοβολία, ακολούθησε στερεοσκοπική εξέταση της μορφολογίας όλων των εμβρύων. Συγκεκριμένα, καταγράφησαν μορφολογικές ανωμαλίες σχετιζόμενες με τη κεφαλή ή /και τα άκρα καθώς και δυσπλασίες της σπονδυλικής στήλης. Η εξέταση αυτή κατέδειξε μεγαλύτερο αριθμό εμβρύων με μορφολογικές ανωμαλίες (15 έναντι 4) καί έντονες δυσπλασίες στην σπονδυλική στήλη (27 έναντι 7) στην ομάδα των ακτινοβοληθέντων εμβρύων σε σύγκριση με τον μάρτυρα (10 έναντι 2). Επιπλέον, συγκριτικά με τους μάρτυρες, τα ακτινοβοληθέντα έμβρυα παρουσίασαν υψηλότερο βάρος (1001 έναντι 835 mg). Στο δεύτερο πείραμα χρησιμοποιήθηκαν συνολικά 360

γονιμοποιημένα αυγά σε τρείς πειραματικές σειρές ενός κρεοπαραγωγικού υβριδίου (RedBro). Ακολουθώντας το ίδιο πρωτόκολλο έκθεσης εκκολάφθηκαν συνολικά 236 ορνίθια από τα οποία τα 110 αποτέλεσαν το μάρτυρα. Από την 1^{η} μέρα της εκκόλαψης έως την 7^{η} ημέρα μετά την εκκόλαψη καταγράφηκαν τυπικές συμπεριφορές των ορνιθίων όπως: επισκέψεις στην ταϊστρα ή/και την ποτίστρα καθώς και η γενικότερη κινητικότητά τους. Η σύγκριση των δύο ομάδων (έκθεσης-μάρτυρα) κατέδειξε ίδια ποσοστά εκκόλαψης και παρόμοια πρότυπα ημερήσιας συμπεριφοράς. Ωστόσο, η ομάδα έκθεσης παρουσίασε υψηλότερα επίπεδα δραστηριότητας έναντι του μάρτυρα με μορφή επισκέψεων στην ταϊστρα, την ποτίστρα και υψηλότερη κινητικότητα εν γένει. Στο τρίτο πείραμα χρησιμοποιήθηκαν συνολικά 240 γονιμοποιημένα αυγά σε 2 πειραματικές σειρές ενός κρεοπαραγωγικού υβριδίου (RedBro). Χρησιμοποιήθηκαν συνολικά 40 ορνίθια ανά ομάδα (έκθεσης και μάρτυρα). Κατασκευάστηκαν 2 λαβύρινθοι σχήματος Υ και τα ορνίθια υποβλήθηκαν σε απλές δοκιμασίες εύρεσης της τροφής εντός των λαβυρίνθων στην ηλικία των 14-17 ημερών μετά την εκκόλαψη με σκοπό την αποτίμηση της ικανότητας χωρικής εκμάθησης (spatial learning) και μνήμης. Κατά το πείραμα αυτό διαπιστώθηκε ότι η ακτινοβολία προκαλεί έλλειμμα στη μνήμη μακράς διαρκείας χωρίς εμφανείς επιδράσεις στην ικανότητα χωρικής εκμάθησης. Διαφορές οι οποίες διαπιστώθηκαν σε μία μόνο παράμετρο κατά τη διαδικασία την χωρικής εκμάθησης αποδόθηκαν στο συναισθηματικό status των ζώων. Στο τέταρτο πείραμα χρησιμοποιήθηκαν συνολικά 240 γονιμοποιημένα αυγά σε 2 πειραματικές σειρές ενός κρεοπαραγωγικού υβριδίου (RedBro). Διενεργήθηκαν αιμοληψίες σε ορνίθια κατά την 1^{η}, 6^{η} και 10^{η} μέρα μετά την εκκόλαψη με σκοπό την μέτρηση παραμέτρων του ανοσοποιητικού συστήματος οι οποίες σχετίζονται με τη γενικότερη ικανότητα ανοσολογικής αντίδρασης. Συγκεκριμένα μετρήθηκαν ο ενεργοποιητής του πλασμινογόνου (u-PA) και τα επίπεδα του ανιόντος υπεροξειδίου (SO) στα ουδετερόφιλα και μακροφάγα καθώς και τα επίπεδα του μονοξειδίου του αζώτου (NO) στα μακροφάγα κύτταρα. Η σύγκριση των τιμών των παραμέτρων μεταξύ των 2 ομάδων έδειξε ότι η έκθεση σε ακτινοβολία συχνότητας 900 MHz προκαλεί ανοσοκαταστολή.

Λέξεις κλειδιά Ηλεκτρομαγνητική ακτινοβολία 900MHz, Ανωμαλίες, Συμπεριφορά, Χωρική εκμάθηση και μνήμη, Ανοσοποιητικό σύστημα.

BIOLOGICAL EFFECTS OF ELECTROMAGNETIC RADIATION

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Abstract:

The objective of the present study was to investigate whether exposure of chicken eggs to 900 MHz continuous wave (CW) and low intensity electromagnetic radiation during the first five days of embryonic development may cause; teratogenic effect, behaviour aberrations and/or poor immune response ability. To verify such hypothesis, four series of experiments were carried out. For all the experiments, the eggs were incubated at 37 ºC and a relative humidity of 55-60%. The average (±SD) specific absorption rate (SAR) in the exposed eggs was determined numerically and it was calculated as high as 0.13 ± 0.02 mW/kg. Temperature rise due to radiation was theoretically estimated to a worst-case maximum of 0.03 ºC. The first experiment was to investigate the teratogenic effect at 5 days-old chicken embryos. Gross, head and limb embryo morphological malformations as well as anatomy anomalies (neural tube curvature) were assessed by stereoscopic examination. The number of the dead embryos remained very low (1-2 cases per batch) and did not discriminate between the two groups. The exposed embryos displayed significantly higher morphological anomalies when contrasted to controls (15 *vs.* 4 respectively). There were also significantly increased anatomical anomalies as shown by the light (55 *vs.* 43) and strong (27 *vs.* 7) cases of the neural tube curvature in the exposed embryos. In total, more multiple abnormalities cases were observed in the EMF-exposed embryos (10 *vs.* 2). Moreover, exposed embryos exhibited significantly higher body weights (1001 *vs.* 832 mg) when contrasted to the controls. No dose dependent effect on abnormality rate was established. The second series of experiments was to investigate the effect of exposure on the typical daily behaviour. The general activity as well as the feeding and drinking behaviour of 236 chicks (110 radiated and 126 controls) was recorded using 4 video cameras during the first 7 days after hatching. A total number of 8285 observations were obtained. Birds' feeding, drinking and moving probabilities were modeled using a trivariate logistic regression with correlated random effects. Radiation resulted in higher probabilities of moving (0.04 *vs.* 0.01), feeding (0.31 *vs.* 0.17) and drinking (0.03 *vs.* 0.02). The highest feeding and drinking activities were observed during morning times. Positive significant correlations between the probabilities of the feeding, drinking and moving were calculated. No radiation effect on the body weight at hatching (43.7 *vs.* 43.3 g) or at the 7 days of age (136 *vs.* 131 g) was detected. The third experiment was to investigate the effects of exposure on chickens' later spatial learning and spatial memory. The spatial learning and memory performance were assessed by simple Y-maze tasks on 40 chicks per group aged 14-17 days. Three distinct positions were used during learning and a hidden position during the short term (directly after the last learning trial) and the long memory test (24h after the

short memory trial), respectively. Survival analysis was used to compare time-to-an event i.e. latency of the various tasks among controls and exposed animals. Radiation resulted in prolonged latencies (40.09 *vs.* 18.3 sec) during the first (acquisition) trial at the first learning position and the long term-memory test (13.42 *vs.* 8.32 sec). Radiation had no statistically significant effect(s) on the starting time, at the accomplishment times in the remaining two learning as well as the short memory trials. Finally, the fourth series of experiments aimed at investigating whether exposure of chicken eggs affects immune response ability of young chicken. The blood samples were collected at 1, 6 and 10 days-old from 66 chicks that randomly selected after hatching. Blood macrophages and heterophils were isolated and the following parameters were determined: total membrane-bound urokinase plasminogen activator (u-PA) and superoxide anion production on heterophils and microphages as well as nitric oxide production by macrophages. Data analysis showed an overall statistically significant effect of radiation on all parameters studied with lower values for the exposed eggs**.** Age and batch had no statistically significant effect on the parameters studied. In conclusion, the present study suggests that 900 MHz electromagnetic radiation exposure during the first five days of the embryonic development resulted in increased morphological as well as anatomical abnormality rates and embryo weight. Moreover, the exposure resulted in modifications of the daily behvioural pattern as well as impairment of the long-term memory function. Additionally, the reduced levels of important macrophages and heterophils parameters studied here may suggest a serious adverse effect of the EMF exposure on the immune system.

Keywords: 900MHz electromagnetic radiation, embryos abnormalities, behaviour, spatial learning and memory, immune system.

Introduction

1. Introduction

All populations are now being exposed to varying degrees of man-made sources of electromagnetic fields (EMFs) and the exposure levels will likely continue to increase as technological inventions advance. EMFs are thus becoming a major health concern and generate intense debate within the scientific community and in the fellow citizens. Man-made EMFs are currently created mostly by mobile phone communication systems and broadcasting transmitters. Of particular interest is the 900 MHz as it represents the frequency band of GSM900, the first widely deployed and most used cellular telephony system.

Little is known about populations' exposure from combined EMF sources and even less about the relative importance of each source. Moreover, there is no available method to infer the individual measurements of each EMF source at the same time. Therefore, it is difficult to evaluate the biological effects explicitly for 900 MHz EMF exposure in an open-field study. A feasible alternative is to carry out experimental studies in which experimental conditions are controlled as most as possible. Here, exposure can be highly and accurately defined providing detailed knowledge of exposure parameters and their variation over time and space. In addition, measures to prevent or at least minimize any non-EMF related interference can be more easily employed (Krawczyk et al., 2008). The advantages of experimental studies become more pronounced when the aim of the study is to investigate effect(s) of exposure during critical periods such as embryogenesis.

Embryogenesis is highly sensitive to external factors and this period is critically important for normal embryo development. Thus, any teratogenic effect, as a result of EMF exposure, could be clearly detected during or at later stages of development. Moreover, during this period the central nervous system (CNS) is more prone to external environmental stimuli such as the EMFs (D'Andrea et al., 2003). Thus, any defects caused by EMFs during this period may reasonably be manifested at postnatal stages, in form of, for instance, behavioral aberrations and/or deficits. This holds also for the immune system makeup since a large number of cell types are formed during early development stages.

While human embryos are unethical to be used in EMFs research, numerous studies use rodents as the study subjects because a direct extrapolation of the results could be accepted for humans. However, among mammalian and non-mammalian species, the chicken (*Gallus domesticus*) displays major advantages to be used as an animal model. It is self-sufficient, there is considerable knowledge on its embryogenesis and large numbers of observations over a short time of period i.e. incubation period, can be attained. The major advantage, however, relates to results interpretation. Since no maternal protective mechanism is involved, such as in mammals, results may be directly attributed to only EMFs action (Ubeda et al., 1994).

There are some studies reporting effects of 900 MHz exposure on the reproduction and development, the nervous, endocrine, cardiovascular, immune and hematological systems in animals and/or human (for a detailed review, see ICNIRP, 2009). Most of these studies have considerable differences in experimental design (e.g. flux density, field parameters, duration and time of exposure) leading to controversial conclusions. Thus, there is still great interest on investigating possible adverse biological effects of the 900 MHz system on living organisms.

There seems to be a scientific consent that low density 900 MHz exposure does not generally cause adverse effects. In addition, almost nothing is known as to whether exposure during embryogenesis is of biological importance. The present thesis was designed to provide more knowledge to this crucial issue. The general idea of the thesis was to subject chicken embryos to continuous radiation during early embryogenesis. To serve this purpose, a specialized exposure system was devised that allowed for continuous wave exposure of the subjects during the first 5 days of development. Direct measures before and during experimentation as well as dosimetry analysis allowed for detailed knowledge of the exposure parameters such as the electric field strength, the spatial average background value and given separately for the albumen and the yolk the total electromagnetic power absorbed and the mean specific absorption rate SAR, as well as the possible temperature rise due to exposure. To possibly eliminate any interference phenomena, two incubators were constructed with deliberately minimum use of metallic parts. Following the above exposure protocol, three series of experiments were carried out. In the first series, it was investigated whether 900 MHz radiation may cause teratogenic effects. The assessment of teratogenic action was based on the number of abnormalities as well as the degree of malformations in important organs or body parts such as the spinal cord, head etc. The main idea behind the second series of experiments was that exposure may have a detrimental effect on the CNS of developing embryos and this effect could be detected at postnatal stages in form of behavioral aberrations. To verify such a scenario, two experiments were carried out. In the first experiment, exposed hatchlings were video recorded and then evaluated according to their typical daily behaviour (e.g. eating, drinking, and moving). In the second experiment, animals were subjected to spatial learning and memory tasks to assess any deficits in their spatial learning and memory ability. Finally, the third series of experiments aimed at investigating whether 900 MHz exposure causes immunosuppression by measuring blood parameters that are related to general immune ability.

STRUCTURE OF THE THESIS

The present thesis is divided into three parts;

- **Part A, Literature Review:** provides a thorough literature review with regard to electromagnetic fields, the interaction mechanisms and the biological effects of EMFs.
- **Part B, Experiments:** presents the four specific experiments of the thesis. Each experiment has the typical form of a scientific paper, with introduction, material and methods, results and discussion.
- **Part C, General Discussion:** gives a conclusive summary of the present findings, general conclusions and provides recommendations for future research.

Part A
Literature Review

Part A: Literature Review

This chapter provides an overview of the operating principles of electromagnetic field, and discusses the mechanism(s) underlying the effect. Additionally, we review EMF interaction mechanisms with biological systems, deals with the different effects of EMFs.

2.1. Electromagnetic Radiation

In this section we give some information about electromagnetic radiation definition and mechanism of biological effects, see (e.g. Krawczyk et al., 2008; Harper and Buress, 2008; ICNIRP, 2009) for additional details.

The non-ionizing radiation could be divided in terms of frequency to extremely low-frequency (ELF), radio frequency (RF) and microwaves (MW) frequencies (Figure 2.1). ELF radiation has very long wave lengths (on the order of a million meters or more) and frequencies in the range of 100 Hertz or cycles per second or less. While, radio frequencies have wave lengths of between 1 and 100 meters and frequencies in the range of 3 kHz to 300 MHz. However, the MWs have wavelengths ranging from as long as one meter to as short as one millimeter, or equivalently, with frequencies between 300 MHz and 300 GHz (Pozar, 1993). Microwave is used in broadcasting and telecommunication transmissions because, due to their short wavelength, highly directive antennas are smaller and therefore more practical than they would be at longer wavelengths (lower frequencies)(Bittner, 1991). Moreover, MW are good for transmitting information from one place to another, because microwave energy can penetrate haze, light rain and snow, clouds, and smoke. The MW is electromagnetic field (EMF), can be viewed as the combination of an electric field and a magnetic field. The electrical part is produced by a voltage gradient and is measured in volts/meter. The magnetic part is generated by any flow of current and is measured in Tesla. Electromagnetic power is the rate at which energy is consumed or produced and it is the product of voltage and current (ICNIRP, 2009). However, power density, also called the power flux density, is a distribution of power over certain area. Power density is expressed in units of power per area, typically in units of milliwatts per square centimeter (mW/cm^2). The quantity used to measure how much EMF radiation is actually absorbed by the body is called the Specific Absorption Rate (SAR). The SAR is usually expressed in units of watts per kilogram (W/kg) or milliwatts per gram (mW/g).

Radiation can affect the body in many ways, and the health effects may not become apparent for many years. These effects range from mild symptoms, such as skin reddening, to serious effects such as cancer and death. These effects are dependent upon the amount of radiation absorbed by the body (the dose), the type of radiation, whether or not the exposure was internal or external, and the length of exposed time (Haddow et al., 2008). The body attempts to repair the radiation damage, but sometimes the damage is too severe or widespread. Lapses may also occur in the body's natural repair process in its attempt to compensate for the damage caused by radiation. Biological effects of radiation are typically divided into two categories (NCRP, 2007). The first category

consists of exposure to high doses of radiation over short periods of time producing acute or short term effects. The second category represents exposure to low doses of radiation over an extended period of time producing chronic or long-term effects.

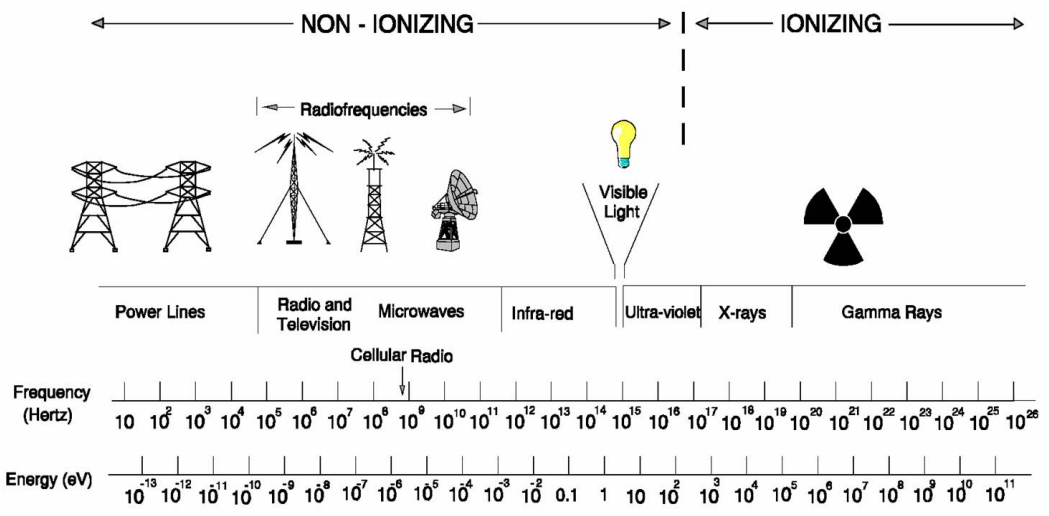


Figure 2.1 Types of non-ionizing radiation

EMFs are found to produce both thermally and non-thermally a large number of biological effects, in many cellular and animal studies (Banik et al., 2003). The thermal mechanisms that may convey detection of MWs by mammals is the heating of tissues by MW exposure that can be detected by thermal receptors in the skin and elsewhere in the body and central nervous system (CNS)(Barnes and Greenebaum, 2007). The identification of a family of transient receptor potential (TRP) ion channels that are gated by specific temperatures has been an important advance in the elucidation of the molecular mechanisms of thermosensitivity. Research has revealed a family of TRP proteins that sense heat and cold at the cellular level (Patapoutian et al., 2003). The international 900 MHz exposure guidelines limit level reported as maximum SAR of 2 (ICNIRP, 1998) or 1.6 W/kg (IEEE, 1992)]. According to ICNIRP (1998) the threshold for biological effects is seen at SAR values above 4 W/kg. A SAR value of 4 W/kg is associated with temperature increase of more than 1 ºC, and although the sensitivity of various types of tissue to thermal damage varies widely, irreversible effects occur above this value. However, EMF may affect a biological system, causing a biological effect, without necessarily causing an adverse change in health (Barnes and Greenebaum, 2007).

In the present review we emphasize on the studies that indicate different possible biological effects of the EMF exposure on living organisms. Additionally, because of the large number of studies relating MW radiations in general, we concentrate in this review on those that regard to radiations with high frequencies and intensities around and close to the frequency used in the present study (900 MHz).

2.2. Biological effects of EMF radiation:

2.2.1. Embryo Development and Teratologies

The following studies have the intention to present an up to-date general survey of published studies on teratogenic effects of high frequency electromagnetic fields. Studies are examining the high frequency fields depending on the applied frequency ranges. Additionally, they were divided according to used test animals or test models (e.g. chicken, mammalian embryos). Neutra et al. (2002) and Thalau (2002) reviewed studies since 2000 included in this chapter.

2.2.1.1. Studies in chicken and quail embryos (in ovo)

The available studies used different frequencies; most studies dealt with the frequency range of 100 KHz to 2.45 GHz (Table 2.1). Part of the available studies examined effects of high frequency fields on embryo mortality. Other test parameters examined include the incidence of congenital malformations, hatching rate, sex ratio, developmental stages as well as duration of embryonic development, water balance, and various blood parameters. Fertility, laying rate, and food consumption of the hen as well as egg weight were also examined. One study dealt with body growth, body weight and histological tests on brain ontogenesis with the chicks being exposed to a high frequency field during embryonic development.

Table 2.1 presents the available studies, which dealt with high EMFs radiation and their potential effects on chicken embryonic development. The great majority of the studies involved partially considerable differences in test design (i.e. flux density, field parameters, duration and time of exposure). Therefore, we abstain from comparing results. Furthermore, different biological parameters were examined. In two studies using 428 MHz field on chicken embryos, Saito et al., (1991) reported very high embryo mortality in EMF exposed embryos (62% *vs.* 15.8% in controls) as well as a incubation time lengthening by up to three days. Additionally, in 89% of the chicks hatched from EMF exposed eggs serious malformations of the legs (so-called splay legs) were detected; in control animals this malformation was not observed. Further evidence for this stems from the fact that many of the chicks died towards the end of breeding when fully developed (Saito et al., 1991). In the second study by Saito and Suzuki (1995) used SAR of 5.5 mW/cm^2 for the first 48 h of embryogenesis, and reported that embryonic development of the EMF exposed embryos lagged behind that of the control group by about 6 to 16 h.

However, tests applying a 900 MHz field (GSM signal) performed by Youbicier-simo et al. (1998a,b,c) in a total of 3 test series, 60 chicken eggs each were exposed over the whole duration of embryonic development. Compared to controls, EMF-exposed group showed an increase in embryo mortality. During the first test series, 59.3% of the embryos in the EMF-exposed group died prematurely (controls 11.9%); in the second 57.6% (controls 10.9%), and in the third group 100% compared to 15.8% of controls. Closer inspection of the data for all tests of Youbicier-simo et al. (1998a,b,c) showed that the main part of exposed embryos died during the third week of incubation, shortly

before hatching. The death of chick embryos occurring at this time suggests faulty incubation conditions (i.e. too high breeding temperatures) rather than teratogenic effects of the examined 900 MHz field, even more so as apparently no congenital malformations were detected in the dead embryos (Table 2.1). The embryo mortality 100% increased at exposure to a 1.25 GHz field and at certain power flux densities reported by Varga (1992). Whereas the range of 0.1 to 1.0 mW/cm² showed a percentage of dead embryos of 10% to 18%, at a power flux density of 1.5 mW/cm² and above embryo mortality in EMF-exposed groups increased to 100% (controls 11.7%). All embryos of the EMF-exposed groups died as early as the first 5 days. This study also suggests that the described increase in embryo mortality does not result from the examined high-frequency field, but from faulty incubation (Varga, 1992).

The most thoroughly examined high frequency range was that of 2.45 GHz (Table 2.1). Two (McRee et al., 1975; Braithwaite et al., 1991) of a total of four studies could not find any effect of the applied 2.45 GHz fields on test parameters. During a study performed in quails (Inouye et al., 1982), embryos were exposed to a 2.45 GHz field with a power flux density of 5 mW/cm² (SAR 4.03 mW/g) from 1st through to 12th day of incubation (you should correct this everywhere). On the 12th, 13th and 14th day of incubation some of the exposed eggs (11, 9 and 10 exposed, respectively; 11 sham-exposed controls each) were removed from the incubator and dissected to histologically determine the developmental status of the cerebellum. The result for exposed embryos was a delayed development of the cerebellum on the 12th to 14th day of incubation. The differences between exposed and sham-exposed groups were significant. In contrast, hatched chicks eight weeks of age from the same test series showed no difference between exposed and sham-exposed animals. This can be seen as evidence for developmental disturbances found in certain phases of embryonic development in exposed embryos apparently being compensated during later periods of embryogenesis or ontogenesis (Inouye et al., 1982). Moreover, Byman et al. (1985) exposed quail eggs two times per day for 30 min to a 2.45 GHz field (CW) and a power flux density of 25 and 50 mW/cm² over the 17-days breeding. During EMF exposure, breeding temperature was decreased from about 35.5° C to 21-23° C. At a power flux density of 25 mW/cm² no disturbances in embryonic development could be detected. In contrast, at 50 mW/cm² the hatching rate of exposed embryos (31.6%) decreased compared to that of controls (73.9%). In the view of the authors, the temperature increase caused by the 2.45 GHz field in exposed eggs was responsible for this effect. Postnatal tests in the hatched chicks did not show any disadvantageous effects for either of the used power flux densities. However, results show that detected effects of 2.45 GHz fields are caused by thermal influences.

The results of available studies reported that the examined high frequency fields led to considerable development disturbances in exposed embryos. However, it must be pointed out that to date there is no evidence at all for an actual teratogenic potential of these fields (Thalau, 2002).

Table 2.1
Studies on effects of high frequency EMF (from100 kHz to 2.45 GHz) on chicken and quail embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Juutilainen & Saali 1986	magnetic fields: **100 kHz**; 0.1, 1, 10, 100 A/m exposure duration: 52 h additional tests with frequencies of 1 Hz to 10 kHz (see corresponding tables)	chickens	malformations and developmental stage in embryos 52 h of age	At 1, 10 & 100 A/m EMF-exposed embryos showed a statistically significant increase in malformations (no precise data).
Krueger et al. 1975	VHF field: **260 MHz**; 125 μW/cm^2 exposure duration: 12 weeks further tests with electric and/or magnetic 60-Hz fields, 915 MHz, 2.435 GHz (see corresponding tables)	chickens	adult animals: fertility, laying rate, food consumption, egg weight embryos and/or chicks: hatching rate, malformations, sex ratio, embryo mortality	Across the different tests, laying rate of EMF-exposed hens sank to 59% (controls 78 to 87%) during 13th to 16th week. None of the other test parameters showed influences of the examined field. * Eggs were removed from the cages immediately after laying.
Saito et al. 1991	**428 MHz**; 0.05 mW/cm^2 to 0.42 mW/cm^2, 5.5 mW/cm^2 (very unprecise data) SAR: 3.1 to 47.1 W/kg exposure duration: 21 days (total breeding time)	chickens	malformations, embryo mortality, breeding duration Increase in embryo mortality: EMF-exposed eggs: 62%, controls: 15.8%.	Malformation of legs in 89% of hatched EMF-exposed chicks. Breeding duration was lenghtened in EMF-exposed eggs by up to 2 days.
Saito & Suzuki 1995	**428 MHz**; 5.5 mW/cm^2 exposure duration: the first 48 h of embryogenesis	chickens	embryonic development during the first 48 h(developmental stages, number of somites)	Embryonic development of the EMF-exposed embryos lagged behind that of the control group byabout 6 to 16 h.
Youbicier-Simo et al. 1998a	**900 MHz** (mobile phone, GSM signal)	chickens	embryo mortality, 3 test series	All three test series showed a statistically significant increase of embryo mortality in the EMF-exposed group compared to controls. Test 1: EMF-exposed: 59.3%, control: 11.9% Test 2: EMF-exposed: 57.6%, control: 10.9% Test 3: EMF-exposed: 100%, control: 15.8%
Youbicier-Simo et al. 1998b	**900 MHz** (mobile phone, GSM signal) with "shielding antenna" exposure duration: 21 days (total breeding time)	chickens	embryo mortality	Mortality rate in EMF-exposed embryos was 57.6% compared to 10.9% in controls. When exposed to a mobile phone equipped with a shielding antenna, the eggs showed an embryo mortality of only 29.3%.
Youbicier-Simo et al. 1998c	**900 MHz** (mobile phone, GSM signal) with "shielding antenna" exposure duration: 21 days (total breeding time)	chickens	embryo mortality	Mortality rate in EMF-exposed embryos was 61% compared to 11.9% in controls. When exposed to a mobile phone equipped with a shielding antenna, the eggs showed an embryo mortality of only 29.3% (see publication 1998b).
Krueger et al. 1975	**915 MHz**; 1000 μW/cm^2 (1st to 17th day) 18th to 25th day: no field, followed by 200 μW/cm^2 until the end of 12th week exposure duration: 12 weeks further tests with electric and/or magnetic 60-Hz fields, 260 MHz, 2.435 GHz (see corresponding tables)	chickens	*adult animals*: fertility, laying rate, food consumption, egg weight *embryos and or chicks*: hatching rate, malformations, sex ratio, embryo mortality	Over the course of the study the laying rate of EMF-exposed hens varied from 78% to 68% (controls 78% to 87%). None of the remaining test parameters showed influences of the examined field. * The eggs were removed from the cages immediately after laying.
Varga 1992	**1.25 GHz**; 0.1 to 3 mW/cm^2 exposure duration: 8 h per day up to 5th day of breeding breeding temperature reduced for compensation	chickens	embryo mortality	At 0.1 to 1 mW/cm^2 there was no difference between controls and EMF-exposed embryos. At 1.5 to 3 mW/cm^2 all EMF-exposed embryos died (controls 11.7%).

Table 2.1
Studies on effects of high-frequency EMF (from100 kHz to 2.45 GHz) on chicken and quails embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Krueger et al. 1975	**2.435 GHz**; 1000 μW/cm² exposure duration: 11 weeks further tests with electric and or magnetic 60-Hz fields, 260 MHz, 915 MHz (see corresponding tables)	chickens	adult animals: fertility, laying rate, food consumption, egg weight embryos and/or chicks: hatching rate, malformations, sex ratio, embryo mortality	Over the course of the study the laying rate of EMF-exposed hens varied from 74% to 61% (controls 78% to 87%). None of the other parameters showed influences of the examined field. * The eggs were removed from the cages immediately after laying.
Braithwaite et al. 1991	**2.45 GHz** (cw); 3.6 mW/cm² SAR: 0.8 mW/kg exposure duration : total breeding time (21 days)	chickens	embryo mortality	There was no evidence for effects on embryo mortality. Hatching rate: controls 97.7%, EMF-exposed eggs 82.9%.
Byman et al. 1985	**2.45 GHz** (cw); 25 & 50 mW/cm² SAR: 0.5 W/kg (calculated for 92 mW/cm²) exposure duration: 2 x 30 min per breeding day, over total breeding time (17 days) breeding temperature during exposure: 21° to 23° C breeding temperature of controls: 37.4° to 37.5° C breeding temperature of sham-exposed animals: about 20° to 24° C	quails	embryo mortality, water balance, egg weight, body growth of chicks	25 mW/cm²: no disturbances in hatching rate and/or of growth in chicks hatched from EMF-exposed eggs. 50 mW/cm²: decreased hatching rate in EMF-exposed embryos Controls: 73.9%, EMF-exposed animals: 31.6%. The difference was statistically significant.
Inouye et al. 1982	**2.45 GHz** (cw); 5 mW/cm² SAR: 4.03 mW/g exposure duration: constant, from 1st to 12th day of breeding (also chicks 8 weeks of age)	quails	embryos (12, 13 & 14 days of age) and chicks (8 weeks) body weight, brain weight, cerebellum (cortex): development of grain layer (stratum granulosum cerebelli), and of the molecular layer (stratum moleculare cerebelli), development of Purkinje cells	In EMF-exposed controls, body weight as well as brain weight were below those of controls. In embryos 12 days of age (body weight; brain) and 14 days of age (brain) differences were statistically significant. In EMF-exposed embryos, the development of different structures of the cerebellum (cortex) lagged behind that of controls. In chicks 8 weeks of age no differences between controls and EMF-exposed chicks could be detected.
McRee et al. 1975	**2.45 GHz** (cw); 30 mW/cm² SAR: 14 mW/g 6 test groups with EMF exposure only on 1st, 2nd, 3rd, 4th or 5th day of breeding + 1 group with EMF exposure on all 5 days exposure duration: 4 h per day each breeding temperature during exposure: 24° C	quails	embryo mortality, malformations, different blood parameters (f.e. hemoglobin, leukocytes, lymphocytes, hematocrite value)	No increase of embryo mortality (hatching rate: exposed animals: 67.5%; controls: 64.3%), no increase of malformation rate. No EMF effects on: leukocyte percentage, hemoglobin, hematocrite value, lymphocytes. Differences observed on some test days between controls and EMF-exposed embryos were within known variability.

2.2.1.2. Studies in mammalian embryos (in utero)

The studies used different frequencies in the range between 10 MHz and 36.11 GHz; some tested animals in the UWB (ultra-wide band) range from 0.1 to 1.0 GHz (Cobb et al., 2000) and in the RF and UHF range (88.5 to 950 MHz, Magras and Xenos, 1997). In addition to embryos, fetuses and mother animals, some studies also examined the F1 and F2 generation for possible effects due to the applied high-frequency fields. Most frequently examined parameters in embryos and fetuses were the incidence of congenital malformations, the number of dead embryos and fetuses, and the body weight of fetuses. For mother animals being exposed to a high-frequency field during pregnancy, the focus of the studies were histological tests in the ovaries, the placenta, the brain, as well as a number of other organs. Further study foci included fertility, weight gain during pregnancy, as well as the body weight of mother animals. In young animals, also histological studies, body weight as well as different behavioural patterns were the most frequent parameters (Tables 2.2 to 2.4).

As was the case with the studies performed in chicken and quail embryos, with respect to varying frequencies, there were partially considerable differences in test design for mammals. Studies (including UWB & RF/UHF range) could detect an influence of the examined field at least on one test parameter. Only few studies did not show statistically significant differences between EMF-exposed test groups and controls (Tables 2.2 to 2.4). However, at closer inspection of single studies this high percentage is highly relative. In embryos and fetuses studies were performed on single biological parameters for all frequency ranges. A number of the studies provided evidence for statistically significant differences between EMF-exposed embryos/fetuses and controls. Other studies showed no influence of the applied fields on test parameters.

The parameter most frequently examined in embryos and/or fetuses was the incidence of congenital malformations. A statistically significant increase in malformation rate was observed. In part of these studies performed in rats, the embryos or fetuses were exposed to a 10 MHz field (Nelson et al., 1991, 1994), at 27.12 MHz field (Dietzel, 1975; Lary et al., 1982, 1986, Table 2.2) and at 2.45 GHz (CW) on mice (Nawrot et al., 1981, Table 2.3a). All the studies showed an increase of the rectal temperature in mother animals during exposure from normally 38° C to values between 40- 43° C. Therefore, results conclude that the increase of congenital malformations found in these studies within the EMF-exposed test group results from thermal effects of the examined high-frequency fields.

Tables 2.3a,b present the studies on the effect of 2.45 GHz on rats and mice embryo mortality, the number of dead fetuses, and the incidence of congenital malformations. Further test parameters included the body weight of fetuses, skeletal formation (ossification), and histological tests on various other organs. Two studies examined only young animals of the F1 generation, as well as their mothers (Inouye et al., 1983; Berman et al., 1984).

Effects determined in rats were: smaller body weight, delayed ossification (Berman and Carter, 1984), a smaller body weight in the F1 generation and smaller weights of liver and brain in mother animals (Jensh, 1997). In nearly all cases, the authors attributed the described effects as being caused by thermal influences of the examined fields (e.g. Berman et al., 1981).

It could be concluded that, to date there is scarcely conclusive data on non-thermal effects of high frequency fields on embryonic development and ontogenesis of mammals. Nearly all described disturbances in embryogenesis and ontogenesis with high probability are caused by thermal effects, independently of the examined frequency range (Thalau, 2002).

Whatever the examined frequency or test animals, most of the studies showed statistically significant differences between controls or sham exposed and EMF exposed groups with regard to embryos malformation rate as well as to development stage. However, studies on the embryonic development and teratogenic effects of high frequency EMFs low dosing are still needed.

Table 2.2
Studies on effects of high-frequency EMF (from 10 MHz to 1 GHz) on mammalian embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Nelson et al. 1991	**10 MHz** (cw); SAR: 6.6 W/kg Due to the applied 10-MHz field the rectal temperature of exposed mother animals increased from 38° C (normal) to 42° C over 30 min. Total exposure duration was 60 min. During an additional test series, EMF-exposed mother animals were injected with 150 mg/kg 2-metoxy-ethanol. EMF exposure as well as the application of 2-metoxy-ethanol took place on the 13th day of pregnancy.	rats	*embryos*: mortality *fetuses*: sex ratio, weight, malformations (skeleton, organs) Fetuses were examined on 20th day of embryonic development. *mother animals*: fertility, mortality, food and water consumption, weight gain	In the EMF exposed group (without 2-metoxy-ethanol), 30% of the fetuses of 56% of the litters were malformed. When 2-metoxy-ethanol was applied without exposure to the 10-MHz field, 14% of the fetuses of 56% of the litters were malformed. When the 10-MHz field was applied in combination with injections of 150 mg 2-metoxy-ethanol, malformations could be detected in 76% of the fetuses from all exposed litters. In controls, no malformations could be found. For all other test parameters and for the examined mother animals as well no effects of the examined 10-MHz field (with or without 2-metoxy-ethanol) could be observed.
Nelson et al. 1994	**10 MHz** (cw); SAR: 5.3 to 6.6 W/kg Caused by the applied field there was an increase of rectal temperature in exposed mother animals from 38° C for 10, 20 or 30 min to 42° C. Exposure duration of the different test series was 30, 40, 50 or 60 min. During further tests EMF exposed mother animals additionally were treated with 2-metoxy-ethanol (75, 100, 125 or 150 mg/kg). Treatment and/or EMF exposure of mother animals was performed on 9th and/or 13th day of pregnancy.	rats	*embryos*: mortality *fetuses*: sex ratio, weight, malformations (skeleton, organs) Fetuses were examined on 20th day of embryonic development. *mother animals*: fertility, mortality, general behavior, weight gain *Fetuses*: Independent of the application of 2-metoxy-ethanol, EMF exposure (30 min at 42° C) on 9th day of embryonic development led to an increase in malformation rate (fore/hind extremities, tail).	Additionally, EMF-exposed fetuses had smaller body weight than controls. When the 10-MHz field was applied on the 13th day of embryonic development (together with 2-metoxy-ethanol), an increase in malformations of fore and hind extremities could be detected. Moreover, the weight of treated fetuses was smaller than that of controls. All differences between controls and EMF exposed fetuses were statistically significant. *Mother animals*: 20% of EMF-exposed animals with a rectal temperature increased to 42° C for 30 min died 1 to 2 days after exposure. In addition, short-time effects as f.e. lethargy could be observed.
Brown-Woodman & Hadley 1988	**27.12 MHz**; pulse rate/SAR/exposure duration: 10 Hz/2.8 W/kg/60 min 20 Hz/4.2 W/kg/45 min 30 Hz/5.6 W/kg/30 min 15 Hz/no data/60 min 26 Hz/no data/45 min 35 Hz/no data/30 min EMF exposure on 9th day of pregnancy	rats	*embryos*: mortality *fetuses*: weight, malformations Fetuses were delivered and examined on 20th day of embryonic development. *mother animals*: fertility	At a pulse rate of 10 Hz and an exposure duration of 60 min the EMF-exposed group showed an increased percentage of died (resorbed) embryos (20.4%) compared to controls (7.4%; no statistical data availaible). None of the other examined parameters showed an influence of the examined 27.12-MHz field.

Table 2.2
Studies on effects of high-frequency EMF (from 10 MHz to 1 GHz) on mammalian embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Dietzel 1975	**27.12 MHz** (CW) three test groups (55, 70 & 100 W) duration of EMF exposure: 5 and 10 min At 55 W (5 min) a rectal temperature of 39° C was measured (normal = 38° C), at 75 W (10 min) the temperature was 40.5° C, and at 100 W (10 min) 42° C. The animals were exposed to the EMF between the 1st and the 16th day of embryonic development. (Note: very unprecise EMF exposure parameters!)	rats	*embryos*: resorptions, implantations *fetuses* (20 days of age): malformations, mortality	Embryos (blastocytes): In the EMF exposed groups the number of embryos having died before or after implantation was increased compared to controls. *Fetuses*: An increase in mortality rate could be observed in EMF-exposed groups. In addition, malformation rate was increased compared to controls. Controls: 0.26%; 55 W: 0.27%; 70 W: 12.4%; 100 W: 46.1%. Data on statistical processing of results are lacking. The author explains observed effects by the decreased DNA synthesis rate.
Lary et al. 1982	**27.12 MHz** (electric field: 300 V/m, magnetic field; 55 A/m); SAR: 11-12.5 W/kg 8 test groups: exposure on 1st, 3rd, 5th, 7th, 9th, 11th, 13th or 15th day of embryonic development over 20 to 40 min until a rectal temperature of 43° C was reached	rats	*embryos*: implantations, resorptions *fetuses*: mortality, malformations, weight, sex ratio, body size *mother animals*: corpus luteum, mortality	EMF exposure on the 1st, 3rd or 5th day of embryonic development had no effect on fetus and/or embryo mortality (before implantation). When the 27.12-MHz was applied on 7 (29%) or on 9 day (49%) of embryonic development, the increase in the number of died EMF-exposed fetuses (29 & 49%) was statistically significant compared to controls (12 & 6%) and sham-exposed fetuses (22 & 16%). EMF exposure on 11th, 13th or 15th day did not lead to a statistically significant increase in mortality rate. Both body size (EMF on 1st to 15th day) as well as weight (EMF on 7th to 15th day) were below values of controls and/or sham-exposed animals (statistically significant). All test series showed a statistically significant increase of malformation rate (extremities, skeletal formation, inner organs, etc) in EMF-exposed embryos and/or fetuses compared to controls and sham-exposed animals. Here, malformation rate in EMF-exposed animals rose to 87%-94% (controls 0 to 24%) depending on the day of EMF application. 26 (11%) of EMF-exposed mother animals died during or shortly after EMF exposure. There was no loss of animals in controls and/or the sham-exposed group.
Lary et al. 1986	**27.12 MHz**; SAR: 10.8 (±0.3) W/kg magnetic field: 55 A/m, electric field: 300 V/m exposure on 9th day of embryonic development over 10 to 40 min (depending on the intended rectal temperature) In mother animals, the following values of rectal temperature were measured: 41° C, 41.5° C, 42° C, 42.5° C and 43° C. Normal are 38° C.	rats	*embryos*: resorptions, implantations *fetuses*: (20th day of embryonic development) mortality, malformations mother animals: mortality	At a rectal temperature of 41° C and above there was an increase in mortality rate (embryos/fetuses) and in the number of malformations. At 43° C a number of mother animals died. Results were comparable to those of the study of Lary et al 1982 (see above).

Table 2.2
Studies on effects of high-frequency EMF (from 10 MHz to 1 GHz) on mammalian embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Smialowicz et al. 1981	100 MHz (cw); power flux density: 46 mW/cm²; SAR: 2.8 mW/g (±1.5) exposure: 4 h per day, from 6th day of embryonic development Mother animals as well as their offspring were exposed up to the 97th day after birth of the F1 generation.		ratsweight gain of mother animals during gestation different tests on postnatal development of young animals (opening of eyes, muscle reflexes, locomotorial activity) on 35th and 84th day different blood parameters: f.e. lymphocytes, erythrocytes, leukocytes, hematocrite value, hemoglobin in F1 generation rats 22 and 42 days of age different immunologic and toxicologic tests (mitosis, mutageneity, antibodies) neurochemical tests in rats 22, 40 and 97 days of age (striatum, cerebellum, hippocampus, cortex, hyhothalamus, medulla, mesencephalon)	In rats 22 days (striatum, medulla) and 40 days (mesencephalon) of age (prenatal EMF exposure) acetyl-choline-sterase activity temporarily was smaller than that of controls (no effect on 97 day). The difference was statistically significant. The exposed young animals opened their eyes earlier than controls. None of the other test parameters provided evidence for statistically significant differences between EMF-exposed animals and controls.
Bornhausen & Scheingräber 2000	**900 MHz** (217 Hz); power flux density: 0.1 mW/cm² SAR: 17.5 to 75 mW/kg duration of exposure: 1st to 20th day of embryonic development	rats	behavioral tests in rats after prenatal EMF-exposure 2 behavioral tests in Skinner box: 1. differential reinforcement of high rate (DRH) = this test requires a high lever activation rate of test animals 2. differential reinforcement of low rate (DRL) = test demands a low lever activation rate both test procedures reward the animals by giving them food in addition to behavioral tests, the following parameters were examined: number of young animals per litter, development of body weights in young and old animals	None of the test parameters showed an influence of the examined 900-MHz field.
Jensh et al. 1982	**915 MHz**; power flux density: 10 mW/cm² SAR: 3.57 W/kg exposure: 6 h per day, 1st to 21st day of embryonic development	rats	embryos: embryo mortality (resorptions, implantations) fetuses: number of died fetuses, malformations, weight (22th day of embryonic development) mother animals: histological tests in brain, liver, kidneys, placenta, ovaries, body weight	None of the test parameters showed statistically significant differences between controls and EMF-exposed embryos, fetuses and mother animals.
Jensh 1997	**915 MHz** (cw); power flux density: 10, 20 or 30 mW/cm² exposure: 6 h per day, 1st to 21st day of pregnancy additional tests using 2.45 & 6 GHz (see corresponding tables)	rats	*embryos*: embryo mortality (resorptions, implantations) *fetuses*: number of died fetuses, malformations, weight (22th day of embryonic development) F1 generation: ontogenesis, behavior, fertility, histological tests in brain, liver, kidneys, ovaries and testes on 100th day psycho-physical tests: among others negative geotaxis, reflexes, swimming, locomotorial activity, blood parameter (see below) *F2 generation*: teratologic tests (f.e. weight, size of litters, malformations) *mother animals*: histological tests in brain, lever, kidneys, placenta, ovaries, on body weight, fertility; blood parameters: leukocytes, erythrocytes, lymphocytes, monocytes, hemoglobin, hematocrite value	The body weight of the EMF-exposed F1 generation lay above that of controls (stastically significant). Further, statistically significant differences could be observed in some behavioral tests (reflexes). None of the other test parameters showed statistically significant differences between EMF-exposed fetuses and/or young animals and controls. To the main part, this study is a review of earlier studies performed by the author.

Table 2.2
Studies on effects of high-frequency EMF (from 10 MHz to 1 GHz) on mammalian embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Berman et al. 1992	**970 MHz**; SAR values: 0.07, 2.4 & 4.8 W/kg exposure: 22 h per day, 1st to 19th day of embryonic development	rats	*embryos/fetuses*: embryo mortality (implantations, resorptions), number of dead and living fetuses, weight of fetuses, skeletal formation (ossification of sternum), malformations *mother animals*: body weight, fertility, number and size of litters, corpus luteum, weight gain	SAR 4.8 W/kg: The EMF-exposed mother animals showed a smaller weight gain during tests than controls over the same. period. The weight of EMF-exposed fetuses was 12% below that of controls. Both differences were statistically significant. None of the other test parameters provided evidence for statistically significant differences. Tests with 0.07 & 2.4 W/kg could not demonstrate any statistically significant differences. The differences observed at 4.8 W/kg are explained by hyperthermia caused by EMF.
Magras & Xenos 1997	different frequencies in the RF and the UHF range (88.5 to 950 MHz) power flux densities from 168 to 1053 nW/cm² SAR value: 1.935 mW/kg constant exposure over the whole duration of test	mice	*embryos:* mortality *fetuses:* mortality, malformations, body size, body weight, skeletal formation (ossification) 2 test groups *mother animals*: number of litters & young animals	In the EMF-exposed test groups the number of litters as well as that of living fetuses and young animals were smaller than in control. None of the other parameters provided evidence for effects caused by EMF. Note: data presentation is flawed; statistical data lack altogether.
Cobb et al. 2000	UWB (ulta wideband), 0.1 to 1 GHz SAR: 45 mW/kg (calculated for whole body) absorption rate per pulse: 0.45 mJ/kg electric field: 55 kV/m peak pulse duration: 300 ps pulse duration: 1.8 ns pulse rate: 1000 pps duration of exposure: 2 min, from 3rd to 18th day of embryonic development, and in one group additionally from 1st to 10th day after birth positive control with application of lead acetate (2 g/l, from 3rd to 18th day after birth)	rats	*mother animals*: body weight, water consumption, fertility, number of young animals, mortality *F1 generation*: course of juvenile development, histological tests in hippocampus, different behavioural tests (locomotorial activity, 'Morris Water-Maze'-Test, food consumption), body weight at birth, weight gain, fertility of male animals, malformations in F2 generation, ultrasound noise (as a consequence of strain), sex ratio In UWB-exposed animals of the F1 generation the following statistically significant differences compared to sham-exposed controls could be detected:	The UWB-exposed animals produced more (stress-related) ultrasound noise, and had a bigger (medial-lateral) hippocampus than controls. Moreover, in UWB-exposed males (F1 generation) a smaller coupling rate was observed. None of the other test parameters showed statistically significant differences between controls and UWB-exposed animals.

Table 2.3a

Studies on effects of high-frequency EMF (2.45 GHz) on mice embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Berman et al. 1978	**2.45 GHz** (cw); power flux density: 3.4, 13.6 & 14 mW/cm² SAR value: 2.1, 8.1, 8.3 & 22.2 W/kg duration of exposure: 100 min per day, 1st to 17th day of embryonic development test with 28 mW/cm² from 6th to 15th day of embryonic development	mice	*embryos*: mortality *fetuses*: mortality, malformations, weight (on 18th day of embryonic development)	At exposure with a SAR of 22 W/kg the body weight of EMF-exposed fetuses was smaller than that of controls. The difference was statistically significant. None of the other tests could find statistically significant differences between controls and EMF-exposed embryos and/or fetuses. In total (all data taken together), there was a statistically significant increase in the number of malformations in the head (craniochisis) of exposed fetuses.
Berman et al. 1982	**2.45 GHz** (cw); power flux density: 28 mW/cm² duration of exposure: 100 min per day, from 6th to 17th day of embryonic development	mice	*embryos*: mortality *fetuses*: mortality, malformations, weight, skeletal formation (ossification) *F1 generation*: body weight of young animals 7 days of age, mortality	Both EMF-exposed fetuses (on 18th day of embryonic development) as well as (prenatally) EMF-exposed F1 generation showed a smaller body weight compared to controls. The differences were statistically significant. None of the other test parameters provided evidence for statistically significant differences.
Berman et al. 1984	**2.45 GHz** (cw); power flux density: 28 mW/cm² SAR: 16.5 W/kg duration of exposure: 100 min per day, from 6th to 15th day of embryonic development	mice	young animals (1, 5, 10, 12, 15 & 17 days of age) after prenatal exposure: body weight, urine, brain and bone development	In young animals 1 day of age of the EMF-exposed group, body weight was statistically significant below that of sham-exposed controls. The brains of (prenatally) EMF-exposed young animals 10, 12 and 17 days of age had a smaller weight compared to controls. The differences were statistically significant. None of the other test parameters provided evidence for statistically significant differences.
Chazan et al. 1983	**2.45 GHz** (cw); power flux density: 10 & 40 mW/cm² SAR: 4 to 5 and/or 16 to 18 W/kg duration of exposure: 2 h per day, from 1st to 7th, 8th to 18th and/or 1st to 18th day of embryonic development	mice	*embryos*: embryo mortality (implantations, resorptions) *fetuses*: mortality, malformations *F1 generation*: resistance towards infections	At exposure to a power flux density of 40 mW/cm² (rectal temperature of mother animals increased by about 2° C) an increase in embryo and fetal mortality was determined. In EMF-exposed fetuses, bleedings in the abdomen as well as in the skull were observed. After prenatal EMF exposure, the young animals of the F1 generation were more susceptible to viral or bacteria infections. All differences compared to controls were statistically significant. Tests using 10 mW/cm² could not show any statistically significant differences between EMF-exposed animals and controls.
Inouye et al. 1982	**2.45 GHz** (cw); power flux density: 9 & 19 mW/cm² SAR: 11.7 & 24.7 W/kg exposure over 3 h on 2nd or 3rd day of embryonic development additional tests with heat strain (holding of mother aninals at 38° C)	mice	embryo mortality, embryonic stages and malformations in embryos 4 days of age	No verified statistically significant effects of the examined 2.45-GHz fields (9 & 19 mW/cm²) could be proved.

Table 2.3a
Studies on effects of high-frequency EMF (2.45 GHz) on mice embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Nawrot et al. 1981	**2.45 GHz** (cw); power flux density: 5, 21 and 30 mW/cm² SAR: 6.7, 28.14 or 40.2 W/kg duration of exposure: at 5 mW/cm²: 8 h per day from 1st to 15th day of embryonic development at 21 & 30 mW/cm²: 8 h per day from 1st to 6th or 6th to 15th day of embryonic development At 21 and/or 30 mW/cm² the rectal temperature of exposed animals increased by 1 and/or 2.3° C.	mice	*embryos*: number of implantations, mortality (resorptions) *fetuses* (18 days of age): number of living and/or dead fetuses, sex, weight, malformations *mother animals*: weight gain during pregnancy	At exposure to a power flux density of 30 mW/cm² on the 1st to 6th day of embryonic development, the number of implantations in the EMF-exposed group showed a statistically significant decrease compared to controls. Additionally, EMF-exposed fetuses were (statistically significant) lighter than controls. EMF exposure from 6th to 15th day of embryonic development (30 mW/cm²) led in the EMF-exposed group to a statistically significant increase of malformations (mainly cleft palate). None of the other test parameters could prove verifiable statistically significant effects of the examined 2.45-GHz fields.
Nawrot et al. 1985	**2.45 GHz** (cw); power flux density: 30 mW/cm² SAR: 40.2 W/kg duration of exposure: 8 h per day from 1st to 6th and/or 6th to 15th day of embryonic development	mice	*embryos*: number of implantations, mortality (resorptions) *fetuses* (18 days of age): number of living and dead fetuses, sex, weight, malformations (skeletal formation, inner organs), cholinesterase activity in the brain, histological tests in the brain, the tongue, teeth, eyes, ears and palate *mother animals*: weight gain during pregnancy, number of pregnancies	In female animals being exposed to the 2.45-GHz field during the first 6 days, a smaller percentage of pregnancies could be found compared to control group animals. The difference was statistically significant. None of the other tests provided evidence for verifiable statistically significant effects of the examined 2.45-GHz field.

Table 2.3b
Studies on effects of high-frequency EMF (2.45 GHz) on rats embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Berman & Carter 1984	**2.45 GHz** (cw); power flux density: 40 mW/cm² SAR value: 2 & 4 W/kg duration of exposure: 100 min per day, 6th to 15th day of embryonic development	rats	*fetuses*: number of living or dead fetuses, malformations, skeletal formation (ossification), weight *mother animals*: fertility	The fetuses within the EMF-exposed group were 9% lighter than those of the control group. In EMF-exposed fetuses, ossification in the sternum was less developed compared to controls. Both differences were statistically significant. Caused by the applied 2.45-GHz field during exposure the temperature in the colon of the animals increased by 2° C to about 40° C. Observed alterations are explained by a thermal effect of the examined 2.45-GHz field.
Berman et al. 1981	**2.45 GHz** (cw); power flux density : 28 mW/cm² SAR: 4.2 W/kg duration of exposure: 100 min per day from 6th to 15th day of embryonic development	rats	*fetuses*: mortality, malformations (extremities, innards, skeleton), body weight *mother animals*: pregnancy rate	None of the test parameters could provide evidence for a statistically significant difference between EMF-exposed fetuses and controls.
Inouye et al. 1983	**2.45 GHz** (cw); power flux density: 10.34 mW/cm² SAR (mother animals): 1.76 W/kg brain SAR: Animals 2 days of age: 13.95 W/kg 15 days: 19.18 W/kg 20 days: 10.05 W/kg 30 days: 9.72 W/kg 40 days: 9.52 W/kg duration of exposure: 3 h per day from 4th to 21th day of embryonic development as well as from 2nd to 40th day of life (postnatal)	rats	*young animals* (postnatal): brain: weight and size, histological tests in the cerebellum; malformations, body weight, sex ratio *mother animals*: number of pregnancies, number of young animals per litter	There was no evidence for verifiable statistically significant effects of the examined 2.45-GHz field.
Jensh et al. 1983	**2.45 GHz** (cw); power flux density: 20 mW/cm² SAR: 2 to 4 W/kg exposure: 6 h per day over the whole duration of embryonic development The mother animals were sacrificed on the day of birth and examined like the fetuses.	rats	*embryos*: embryo mortality (resorptions, implantations) *fetuses*: mortality, weight, malformations *mother animals*: weight gain during pregnancy, weight of liver, brain, kidneys and ovaries	None of the other test parameters provided evidence for statistically significant differences between controls and EMF-exposed embryos/fetuses and/or mother animals.
Jensh 1997	**2.45 GHz** (cw); power flux density: 10, 20, 30 mW/cm² exposure: 6 h per day, 1st to 21st day of embryonic development further tests with 915 MHz & 6 GHz (see corresponding tables)	rats	*embryos*: embryo mortality (resorptions, implantations) *fetuses*: number of dead fetuses, malformations, weight (22nd day of embryonic development) *F1 generation*: ontogenesis, behavior, fertility, histological tests in brain, liver, kidneys, ovaries and/or testes on 100th day Psycho-physical tests: among others, negative geotaxis, reflexes, swimming, locomotorial activity, blood parameters (see below), weight *F2 generation*: teratologic tests (f.e. weight, litter size, malformations) *mother animals*: histological tests in brain, liver, kidneys, placenta, ovaries, body weight, fertility, blood parameters: leukocytes, erythrocytes, lymphocytes, monocytes, hemoglobin, hematocrite value	In EMF-exposed mother animals, the weight of the liver was increased compared to that of control animals. The difference was statistically significant. None of the other test parameters could show statistically significant differences between EMF-exposed fetuses and/or young animals and controls. To the main part, the study is a review of earlier tests performed by the author.

Table 2.4
Studies on effects of high-frequency EMF (frequency range: 6 to 40 GHz) on mammalian embryonic development

Authors	EMF exposure	test animals	examined parameters	result
Jensh 1984	**6 GHz** (cw); power flux density: 35 mW/cm² SAR: 7 W/kg exposure: 8 h per day, 1st to 22nd day of embryonic development	rats	*embryos*: embryo mortality (implantationations, resorptions) *fetuses*: number of dead fetuses, malformations, weight and developmental stage *mother animals*: body weight, weight of different organs (brain, liver, kidneys, ovaries), blood samples (hematocrite, hemoglobin, leukocytes, monocytes)	*Fetuses*: Delayed growth in EMF-exposed fetuses. The difference compared to controls was statistically significant. *Mother animals*: The number of monocytes was smaller in EMF-exposed animals compared to controls. The difference was statistically significant. None of the other test parameters provided evidence for statistically significant differences between controls and EMF-exposed fetuses and/or mother animals.
Jensh 1997	**6 GHz** (cw); power flux density: 10, 20 and 30 mW/cm² exposure: 6 h per day, 1st to 21st day of embryonic development additional tests with 915 MHz & 2.45 GHz (see corresponding tables)	rats	*embryos*: embryo mortality (resorptions, implantations) *fetuses*: number of died fetuses, malformations, weight (22nd day of embryonic development), developmental stages *F1 generation*: ontogenesis, behavior, fertility, histological tests in brain, liver, kidneys, ovaries and/or testes on 100th day psycho-physical tests: among others, negative geotaxis, reflexes, swimming, locomotorial activity, blood parameters (see below) *F2 generation*: teratologic tests (f.e. weight, litter size, malformations) *mother animals*: histological tests in brain, liver, kidneys, placenta, ovaries, body weight, fertility, blood parameters; leukocytes, erythrocytes, lymphocytes, monocytes, hemoglobin, hematrocrite value	*Fetuses*: Delayed growth in EMF-exposed fetuses.The difference compared to controls was statistically significant. *F1 generation*: Statistically significant differences in some of the psycho-physiscal tests as well as in weight proportions of some organs between EMF-exposed animals and controls. *Mother animals*: The number of monocytes was smaller in EMF-exposed animals than in controls. None of the other test parameters provided evidence for difference was statistically significant. In addition, weight proportions of brain, liver and kidneys statistically significant differences were found compared to non-exposed animals. statistically significant differences between controls and EMF-exposed fetuses and/or mother animals.performed by the author. To the main part, this study summarises earlier studies
Zhao et al. 1997	**36.11 GHz** (cw); power flux density: 10 mW/cm² exposure: 2 h per day from 6th to 15th day of embryonic development	mice	*fetuses*: body size (birth), birth weight *F1 generation*: development of body weight, different behavioural patterns and/or reflexes *mother animals*: weight gain during pregnancy, body weight, weight of placenta *fetuses/F1 generation/mother animals*: different neurochemical tests (postnatal) in the brain: number of M-choline receptors (M-R), concentration of arginine vasopressin (AVP), and somatostatin (SS) in the hypothalamus and hypophysis, DOPAC (3.4-dihydroxy-phenyl-aceic-acid), dopamine (DA), HVA (homovanilin-acid)	*Fetuses*: On the day of birth, body size and body weight of EMF-exposed fetuses lay below those of controls. *F1 generation*: During the first eight weeks of life, the body weight of prenatally EMF-exposed young animals was below that of controls. Additionally, disturbances in behavioural patterns could be observed. *Mother animals*: Both body weight gain during gestation as well as body weight at the time of birth were smaller in EMF-exposed mother animals compared to control group. *Neurochemical tests*: The concentration of AVP (hypophysis of fetuses; hypothalamus of F1 generation) and DOPAC (brain of F1 generation) decreased in EMF-exposed animals compared to controls. In contrast, the concentration of SS (hypophysis and hypothalamus of fetuses and F1 generation), M-R (hippocampus of F1 generation) & HVA (brain of mother animals) was increased compared to controls. This study originally was an abstract written for a congress; more precise data and/or statistics are not available.

2.2.2. Biochemical Changes

Biochemical alterations have been reported to result from exposure to RF energies. Such effects generally appear to be reversible and no well-defined characteristic response pattern has been determined, nor is it known whether the changes are direct or indirect effects of exposure (Michaelson et al., 2007).

Effects on mitochondria isolated from exposed rat have been reported (Dumansky and Rudichenko, 1976) but there was no effect on rat liver mitochondria exposed *in vitro* to 2.4 GHz, 1 to 4 W/kg, or 10 to 12 GHz, at a maximum of 20 W/m^2. No effect of microwave exposure has been found on a number of enzymes and proteins irradiated *in vitro* (Belkhode et al., 1974; Allis, 1975; Ward et al., 1975; Bini et al., 1978). Albert et al. (1974) exposed Chinese hamsters at 2.45 GHz, 500 W/m^2 for 0.5 to 4.5 h over a period of 1 to 21 days and found no change in liver ATP.

The limited number of studies on oxidative enzyme systems has yielded mixed results. Exposure of suspension of the membrane-bound enzyme cytochrome oxidase to sinusoidally modulated 2.45 GHz MW energy at an SAR of 26 W/kg did not significantly affect its activity during exposure. On the other hand, Sanders et al. (1980), exposed the rat brain surface *in vivo* to 591 MHz continuous wave (CW) RF, at 50 or 138 W/m^2 under conditions of negligible systemic temperature change and reported that increases in NADH and decreases in ATP concentration occurred supporting a hypothesis of radio frequency radiation (RFR) inhibition of electron transport in brain mitochondria. These observations were later extended to effects of pulsed and sinusoidal amplitude-modulated microwaves on brain energy metabolism where similar changes were found (Sanders et al., 1985).

Baranski (1972) found inconsistent changes in cholinesterase activity in both rabbit and guinea pig brains following 3 months of exposure to 1 h/d, with pulsed fields of 250 W/m^2. The author also found a decrease in cholinesterase activity in the brains of guinea pigs after a single 3-h exposure to 35 W/m^2 of pulsed 2.45 GHz MWs. Increase to 250 W/m^2 caused a further decrease in activity. Pulsed energy was found to produce a more severe effect than CW exposures of the same average power density, suggesting that these effects are due to peak fields. Nikogosyan (1962) found an increase in blood cholinesterase activity after a single 90-m in exposure to 3 GHz waves at 400 W/m^2. However, Revutsky and Edelman (1964) also reported an increase in specific cholinesterase activity in rabbit blood exposed *in vitro* to 2.45 GHz MWs. It should be noted that what is measured in blood is pseudocholine esterase, which has no neural-related activity. On the other hand, Olcerst and Rabinowitz (1978) found no effect on aqueous cholinesterase exposed to 2.45 GHz CW up to 1.25 kW /m^2 for half an hour or 250 W /m^2 for 3 h. No effect was found on cholinesterase activity in defibr-inated rabbit blood exposed for 3 h to 210, 350, or 640 W/m^2, 2.45 GHz, CW or pulsed. Under similar exposure conditions, there was no effect on release of bound calcium or magnesium from rabbit red blood cells.

Ho and Edwards (1977, 1979) studied oxygen consumption in mice exposed to 2.45 GHz radiation at various SARs in a waveguide exposure system and found that the animals compensated homeostatically to SARs of 10.4 W/kg or greater by a decrease in metabolic rate to compensate for thermal loading. Normal metabolic activity was resumed following cessation of exposure.

Moreover, oxidative stress has been proposed as a possible mechanism for the biological effects of EMF MW exposure (Lai and Singh, 1995, 1996, 1997) and some experimental support for such a mechanism has been reported. Other experimental work, however, has not fully supported this proposal. Induction of stress proteins was investigated in HeLa and CHO cells following 2 h of RF exposure at 2450 or 2700 MHz, respectively (Cleary et al., 1997). The study was conducted under isothermal conditions at an SAR 25 W/kg (HeLa cells) or 27 W/kg (CHO cells). Exposures had no detectable effect on stress protein induction with comparisons made to both sham and positive control cells. Reports of decreased binding in rat olfactory tissue that potentially could indicate oxidative stress effects in exposed samples did not show correlation with SAR (Bruce-Wolfe and Adair, 1985; Philippova et al., 1988, 1994). High levels of EMF MW, which generated elevated temperatures in liposomes (*in vitro*), showed no effect on peroxidation (Logani and Ziskin, 1996). However, in a series of studies in which the RF levels resulted in considerable temperature elevations, changes could be demonstrated in membrane fluidity, permeability, and protein shedding in a way that might be related to oxidative stress (Liburdy and Penn, 1984; Liburdy and Magin, 1985; Liburdy and Vanek, 1985, 1987; Liburdy et al., 1988).

In addition, Zmyslony et al. (2004) have exposed rat lymphocytes *in vitro* to 930 MHz continuous wave (CW) radiation inside a GTEM cell SAR =15 W/kg. 5 min and 15 min of acute exposure resulted in no change in reactive oxygen species production in living cells. Yurekli et al. (2006) used a GHz transverse electromagnetic (GTEM) cell as an exposure environment for plane wave conditions of far-field free space EM field propagation at the GSM base transceiver station (BTS) frequency of 945 MHz, and effects on oxidative stress in rats were investigated. When EMF at a power density of 3.67 W/m^2 (SAR 113 mW/kg), which is well below current exposure limits, as applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly. Additionally, there was a less significant increase in SOD (superoxide dismutase) activity under EMF exposure.

Interest has grown in possible effects of EMF radiation on circadian function, mediated through the pineal hormone melatonin. In a number of recent reports, nighttime levels of melatonin have been measured in response to RF exposure in both animals and humans (Michaelson et al., 2007). Burch et al. (2002) reported a marginal increase in melatonin in humans associated with mobile phone use. This report however, is countered by a number of human provocation studies in which no effects in melatonin levels or secretion was observed following RF exposures (de Seze et al., 1998, 1999; Mann et al., 1998; Radon et al., 2001a,b). Indeed, no changes were observed in exposed humans for a range of hormones as well, including growth hormone, luteinizing

hormone, cortisol, melatonin, and others. Research in rats has shown results consistent with these general findings in that no demonstrable effects on hormones, including melatonin, have been reported with low-level RF exposure at 435 MHz (Bonasera et al., 1988; Toler et al., 1988) or at 900 MHz (Vollrath et al., 1997; Heikkanen and Juutilianen, 1999).

Mann et al. (1998) reported that pineal and serum melatonin levels were not altered by 6 h exposure to a 900 MHz EMF in rats. Another study reported that exposure to a 900 MHz EMF with whole body averaged SAR of 0.06 – 0.36 W/kg had no short term effect on pineal serotonin N-acetyl transferase (NAT) activity, or serum melatonin level (Vollrath et al., 1997). In that study, the whole bodies of the rats were exposed to 900 MHz TDMA signals (Global System for Mobile Communication, GSM).

Hata et al. (2005) attempted to clarify the effects on melatonin synthesis in rats after short-term exposure to a 1439 MHz time division multiple access (TDMA) EMF. The average SAR of the brain was 7.5 W/kg, and the average SARs of the whole body were 1.9 and 2.0 W/kg for male and female rats, respectively. After acclimatization to a 12 h light – dark (LD) cycle, serum and pineal melatonin levels together with pineal serotonin level under a dark condition (less than 1 lux) were examined by radioimmunoassay. No significant differences in melatonin and serotonin levels were observed between the exposure, sham, and cage control groups. These results suggest that short-term exposure to a 1439 MHz TDMA EMF, which is about four times stronger than that emitted by mobile phones, does not alter melatonin and serotonin synthesis in rats.

A few human studies regarding the effects of high frequency EMF on melatonin synthesis have been reported. (Radon et al., 2001b) conducted a human randomized controlled study and conformed that salivary melatonin was not affected by 4 h exposure to 900 MHz TDMA signals. Bortkiewicz et al. (2002) examined effects of short-term exposure to a 900 MHz EMF (SAR 1.23 W/kg) on urine 6-hydroxymelatonin sulfate in healthy young men. de Seze et al. (1999) examined the effects of long term exposure to 900 or 1800 MHz EMF (SAR 0.1 – 0.3 W/kg) on serum melatonin profile. Neither of them showed significant differences with exposure to high frequency EMF.

However, more studies are necessary to ascertain the effects of high frequency EMF on melatonin synthesis. Further investigations on the effects of long-term exposure are warranted.

2.2.3. Immune System

Studies of immune responses, summarized by WHO (1993) and ICNIRP (2009), were mostly conducted using 2.45 GHz continuous wave EMF. In general, the changes that have been reported with any consistency were usually transient and resulted from acute, thermally-significant exposures. For example, changes in natural killer cell and macrophage activity were reported by several studies after the acute exposure to 2.45 GHz of hamsters at SARs of about 13 W/kg or of mice at whole-body SAR of around 21 W/kg (Smialowicz et al., 1983; Rama Rao et al., 1983; Yang et al., 1983). An

increase in the primary antibody response of B-lymphocytes has been associated with the exposure of mice to 3.0 GHz at whole-body SAR above 4-5 W/kg and hamsters to 2.45 GHz at SARs of 8 W/kg and above (Shao and Chiang, 1989; Rama Rao et al., 1985). However, an enhanced mitogenic response was reported in lymphocytes from rhesus monkeys following exposure to 10.5, 19.27 or 26.6 MHz EMF radiation between 0.4 and 2 W/kg (Prince et al., 1972). The effects in these studies were associated with transiently increased rectal temperatures (WHO 1993).

With regard to effects on the developing immune system, two studies conducted by the same group of the prenatal and postnatal exposure of rats to 2.45 GHz at whole-body SARs of 1-5 W/kg (Smialowicz et al., 1979) or to 425 MHz at 3-7 W/kg (Smialowicz et al., 1982) also reported an increased lymphocyte responsiveness to mitogen stimulation at thermogenic levels. In contrast, a lifetime exposure study in which rats were exposed to pulsed 2.45 GHz at whole-body SAR of up to 0.4 W/kg between 2 and 27 months of age did not reveal any effects on immunological parameters except for a transient change in the responsiveness of B- and T-lymphocytes to specific mitogens after 13 months exposure (Chou et al., 1992).

In some studies using 900 MHz EMF, Radon et al. (2001a,b) found that mobile phone EMF radiation had no effect on immune function on eight healthy young men. The men were exposed to 900 MHz fields; pulsed with 217 Hz, pulse width 217 µs. An antenna was positioned 10 cm behind the subject's head. The power-flux density was approximately 1 W/m^2, and the maximum local SAR in the head (averaged over 10g tissue) was 0.025 W/kg. The study was designed to assess the effects of the RF fields on salivary levels of melatonin, cortisol, neopterin, and IgA. Neopterin and IgA are substances that are part of the immune system. Moreover, Gatta et al. (2003) found that 900 MHz GSM-modulated radiation for 1, 2, and 4 weeks (2 h/day) in a TEM cell to a SAR of 1 or 2 W/kg had no substantial effects on immune function in mice. Black and Heynick (2003) reviewed the subject and concluded: "Lifetime studies of RF radiation exposed animals show no cumulative adverse effects in their endocrine, hematological, or immune systems." Moreover, two studies evaluated the effects of EMF radiation on mouse peripheral lymphocytes and on B-cell peripheral differentiation and antibody response in mice (Gatta et al., 2003; Nasta et al., 2006). Mice were exposed or sham-exposed to GSM900 at whole body SARs of 1 or 2 W/kg for up to 4 weeks. The first study investigated the effects on T- and B-lymphocyte frequencies, expression of activation markers (CD28, CD69), cytokine (IL2 and IFNγ) production and T- and B-cell proliferation (Gatta et al., 2003). The second study investigated B-cell peripheral differentiation in spleen, and antibody (IgM and IgG) production in response to polyclonal or antigen-specific stimuli (Nasta et al., 2006). The authors concluded that T- and B-lymphocytes were not substantially affected by exposure to EMF.

In summary, most studies indicate that the most consistently observed EMF induced changes in immune function and hematology are transient and associated with temperature rise of 1 ºC or more (ICNIRP, 2009).

2.2.4. Behaviour and Cognition

This chapter presents an overview of the interaction of non-ionizing electromagnetic fields (EMFs) as external stimuli to the nervous systems and the behaviour of laboratory animals. This overview of the scientific literature specifically includes the detection of EMFs and effects of EMFs on behavioral performance and cognition. This chapter extends the reviews of the animal and human research literature (Hermann and Hossmann, 1997; D'Andrea, 1999; Cook et al., 2002; D'Andrea et al., 2003a,b; Hossmann and Hermann, 2003; Barnes and Greenebaum, 2007; Johnston and D'Andrea, 2007; Nittby et al., 2008).

Biological effects of MWs occur due to absorption of energy in the body. Microwave absorption by water molecules has a resonant frequency in the region of 2.45 GHz that causes heating by molecular vibration and is the result of the interaction of MWs and tissue of the body. Water is the molecular component of tissue known to have a resonant frequency that could contribute to the absorption of MW energy (Barnes and Greenebaum, 2007). Heating (absolute temperature rise) is the best understood mechanism for the effective transfer of MW energy into tissue.

Several studies have shown that the disruption of ongoing behaviour during acute MW exposure is generally associated with 1ºC or greater increase of body temperature. This is associated with whole body SARs in a narrow range between 3.2 and 8.4 W/kg, with different frequencies (225 MHz to 5.8 GHz), of rodents and rhesus monkeys (see Barnes and Greenebaum, 2007). The changes in behaviour are particularly associated with the thermal increase produced by the MW exposures and have proved robust in replication studies.

2.2.4.2. Microwave Cognitive Performance Disruption

The animal studies of cognitive performance disruption that have been conducted to-date have not confirmed the implied concept of more being sensitive to MW exposure than performance disruption of a simple task (D'Andrea, 1999). For example, Mickley et al. (1994) and Mickley and Cobb (1998) investigated memory deficits in rats exposed to MWs and determined that the threshold for memory effects was 10 W/kg. Similarly, Luttges (1980) evaluated the effects of MW exposure on learning and memory in mice and found an enhancement of performance (estimated whole body SAR 13 W/kg). The MW memory facilitation was found in both automated active avoidance testing and in single trial, passive avoidance tests. Beel (1983) repeated the study by Luttges (1980) and again found significant enhancement of learning and memory following 15 min exposure, both with five consecutive days of multiple trial, active avoidance training and with single trial, passive avoidance training.

Raslear et al. (1993) exposed rats to high peak power pulsed MWs produced by the TEMPO (Transformer Energized Megavolt Pulsed Output) virtual cathode oscillator at a whole body average SAR of 0.072 W/kg. The authors concluded that significant effects on cognitive function in rats were observed, particularly in the decision-making process. But in a subsequent experiment, Seaman et al. (1994) reported that the

microwave pulse inhibition and enhancement of startle were similar to previously reported effects of sensory stimuli delivered at similar lead times, indicating the possibility that action was mediated by sensory stimulation (Seaman et al., 1994).

From the descriptions of the studies described above, it appears that measures of cognitive-based performance disruptions in animals are not more sensitive to MW exposure (D'Andrea, 1999) than simple performance tasks. However, when considering the different cognitive and simple performance tasks, different exposure systems, modulation parameters, and differences in irradiation frequency make a definitive conclusion difficult. Additionally, support for this conclusion comes from basic neuroscience. The results are in line with the demonstrated conservation of the memory mechanisms across simple tasks and more complex tasks such as spatial memory (Kandel et al., 2000). Thus, neurophysiologically simple and complex memory mechanisms are similar, and as such would expect them to be similarly disrupted by the same rises in temperature induced by similar levels of SAR (Barnes and Greenebaum, 2007).

Nittby et al. (2008) investigated in a rat model the long-term effects of protracted exposure to Global System for Mobile Communication-900 MHz (GSM-900) radiation for 2 h each week for 55 weeks to radio-frequency electromagnetic radiation at different SAR levels (0.6 and 60 mW/kg at the initiation of the experimental period) emitted by a (GSM-900) test phone. Effects on exploratory behaviour were evaluated in the open-field test, in which no difference was observed. Effects on cognitive functions were evaluated in the episodic-like memory test. In the study, GSM exposed rats had impaired memory for objects and their temporal order of presentation, compared to sham exposed controls. Detecting the place in which an object was presented was not affected by GSM exposure. The results suggest significantly reduced memory functions in rats after GSM microwave exposure.

2.2.4.3. Spatial Memory Replications and Confirmations

Effects of MW exposure on spatial memory have been investigated in animal replication and confirmation experiments. These experiments were undertaken to establish scientific evidence on the effects of MW exposure on spatial memory (Table 2.5).

The MW dosimetry of the experiments of Lai et al. (1989, 1994), Cobb et al. (2004), Cassel et al. (2004), and Cosquer et al. (2005a,b) are similar. They used the circular waveguide (Guy et al., 1979) to expose the whole body of rats to MWs at 0.6 W/kg. The circular waveguide (Guy et al., 1979) was designed initially for experiments on animals in the MW oven frequency range (2450 MHz) with whole body exposures like those in MW ovens. The exposure is from two sources of ''circularly polarized, guided waves that provide a relatively constant and easily quantifiable coupling of microwave energy to each animal, regardless of their position, posture or movement'' (Guy et al., 1979). For the same average incident power density, the average SARs in the heads of these rats were about two times higher in the circular waveguide (0.6 W/kg) than for other

exposures, such as in an anechoic chamber (Chou et al., 1985). In the circular waveguide, Guy et al. (1979) and Cassel et al. (2004) have estimated the brain average SAR to be 0.8 W/kg when the whole body SAR is 0.6 W/kg in rats of 250–300g. Wang and Lai (2000) and Lai (2004) also used the circular waveguide (Guy et al., 1979) to expose the whole body of rats to MWs, but used a whole body SAR of 1.2 W/kg. As well, Cosquer et al. (2005c) used the same exposure chamber but used a whole body SAR of 2 W/kg.

This circularly polarized wave, dual source exposure used in the experiments above is in contrast to the plane wave exposure from a single source, typical of the mobile telecommunications industry, used to expose animals in the other spatial memory experiments (Sienkiewicz et al., 2000 (GSM 900 MHz average whole body SAR of 0.05 W/kg); Jones et al., 2005 (900 MHz GSM whole body exposure SARs of 0.1, 0.6, or 3 W/kg, or exposure mainly of the head, local SARs of 0.1, 0.4, or 2.2 W/kg); Dubreuil et al., 2002, 2003a (GSM 900 MHz, head only exposure, SAR 1 and 3.5 W/kg); Yamaguchi et al., 2003 (pulsed 1439 MHz time division multiple access (TDMA) brain average SAR 7.5 W/kg or 25 W/kg)).

The MW exposure experiments on spatial memory used the 12-arm radial maze (Lai et al., 1989, 1994) and the water maze (Wang and Lai 2000; Lai, 2004) they have consistently reported positive results. Lai et al. (1989, 1994) investigated the effects of MW exposure (circular waveguide paradigm, 2450 MHz, 2 s pulses, 500 pulse per second (pps), whole body average SAR 0.6 W/kg) on learning in the 12-arm radial arm maze. Rats were trained in the maze to obtain food reinforcements immediately after 20 or 45 min of MW exposure. Lai et al. (1994) did not report on the blindedness of their procedures. Exposure to MWs for 20 min prior to training had no significant main effect on maze learning but affected the shape of the learning curve, whereas 45-min exposure significantly retarded the performance of the rats. Wang and Lai (2000) and Lai (2004) found that acute exposure to pulsed 2450 MHz MWs affected water maze performance of rats. Lai et al. (1989, 1994) and Wang and Lai (2000) concluded that maze studies have statistical problems and the exposed and sham animals exhibited no apparent interaction between testing days and treatment (IEGMP, 2000; Sienkiewicz, 2002; Dubreuil et al., 2002).

Cobb et al. (2004) attempted to replicate the results of Lai et al. (1994) that showed a working memory deficit in rats exposed to 2450 MHz pulsed MW fields (2 ms, 500 pps, whole body average SAR of 0.6 W/kg) and also attempted to reverse this deficit by a pretreatment with physostigmine, an acetyl-cholinesterase inhibitor, or with naltrexone hydrochloride, an opioid antagonist that acts on both CNS and peripheral receptors, but not with the peripheral opioid antagonist, naloxone methiodide. Cobb et al. (2004) study was conducted with a double blind procedure. Cobb's analyses of error rates revealed no significant exposure effect, no significant drug effect, and no significant interaction between the two main factors. There was a significant difference in test days, as expected, with repeated test–trial days, which indicates that learning was accomplished. Lai et al. (1994) concluded that there is no evidence from their current study that

exposure to MWs under parameters examined caused decrements in the ability of rats to learn the spatial memory task.

Replication studies by Cassel et al. (2004) and Cosquer et al. (2005b) failed to confirm the MW exposure effects of Lai et al. (1994) on spatial learning in the 12-arm radial maze. Cosquer et al. (2005a) reported that similar MW exposure did not alter anxiety responses assessed in the elevated plus maze. These elevated plus maze, anxiety responses are hypothesized to be associated with changes in benzodiazepine receptors (Lai et al., 1992: ‘‘benzodiazepine receptors in the brain are responsive to anxiety and stress’’). Cosquer et al. (2005a) failed to confirm the work of Lai et al. (1992) about 2450 MHz exposure altering anxiety and the hypothetically associated benzodiazepine receptors in the rat brain.

Yamaguchi et al. (2003) reported that pulsed TDMA fields (1439 MHz, brain average 7.5 and 25 W/kg) affected performance of rats in a T-maze task only when body temperature was elevated (at 25 W/kg). Sprague-Dawley rats were exposed for either 1 h daily for 4 d or for 4 weeks to a pulsed 1439 MHz TDMA field in a carousel-type exposure system. When the brain, average SAR was 7.5 W/kg, the whole body average SAR was 1.7 W/kg. Other subjects were exposed at the brain average SAR of 25 W/kg with the whole body average SAR of 5.7 W/kg for 45 min daily for 4 d. Learning and memory were evaluated by reversal learning in a food-rewarded T-maze, in which rats learned the location of food (right or left) by using environmental cues. The animals exposed to MW with the brain average SAR of 25 W/kg for 4 d showed statistically significant decreases in the transition in number of correct choices in the reversal task, compared to sham-exposed or cage control animals. However, rats exposed at the brain average SAR of 7.5 W/kg for either 4 d or for 4 weeks showed no T-maze performance impairments. Intraperitoneal temperatures as measured by a fiber optic thermometer, increased in the rats exposed to the brain average SAR of 25 W/kg but remained the same for the brain average SAR of 7.5 W/kg. These results suggest that the exposure to a TDMA field at levels about four times stronger than emitted by cellular phones (2 W/kg limit) does not affect the learning and memory processes when there is no whole body temperature rise.

Hancock and Vasmatzidis (2003), on reviewing the literature on the effects of heat stress on cognitive performance, found two trends: ‘‘first, heat stress affects cognitive performance differentially, depending on the type of cognitive task. Secondly, it appears that a relationship can be established between the effects of heat stress and deep body temperature.’’ Hancock and Vasmatzidis concluded, ‘‘that much remains to be understood before a (temperature rise) limit becomes universally acceptable.’’

Dubreuil et al. (2003) reported that mobile phone RF radiation (45 minutes head-only exposure to 900 MHz GSM at densities between 1 and 3.5 W/kg) had no effect on spatial and non-spatial memory of rats.

In summary, some studies suggested that some aspects of cognitive functions and measures of brain physiology may be affected while others do not. These include

changes in memory tasks, response patterns, normal sleeping EEG patterns, and other brain functional changes, although a few studies have demonstrated improved cognitive functions in volunteers exposed to RF radiation in the frequency range of mobile phones.

TABLE 2.5

Effects of MW Exposure on Spatial Memory Performance

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm^2)	Duration	Ref.
No overall effect for 20-min exposure but more errors each day in a 12-arm radial maze at 45-min exposure	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	20 min/d and 45 min/d exposure for 10 d	Lai et al. (1989)
Deficit in spatial working memory function reversed by pretreatment with physostigmine or naltrexone	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	45-min exposure	Lai et al. (1994)
These results show that acute exposure to pulsed MWs caused a deficit in the spatial ''reference'' memory in the rat	SD Rats	Avg. whole body 1.2 W/kg	2450 MHz	Pulsed width 2 μs, 500 pps	Avg. power density 2 mW/cm^2	For 1 h in a circular waveguide system	Wang and Lai (2000)
Low-level exposure to pulsed 900 MHz MW radiation does not cause deficits in the performance of a spatial learning task in mice	Male mice C57BL/6J	Whole body 0.05 W/kg	900 MHz	Pulsed at 217 Hz		45 min/d for 10 d	Sienkiewicz et al. (2000)
No deficits in spatial learning after ''head-only'' exposure of rats to GSM electromagnetic fields in the two spatial learning tasks	SD Rats	Head only 1 or 3.5 W/kg	900 MHz EMF	GSM modulation at 217 Hz, 1/8 duty factor		45 min over 10–14 d	Dubreuil et al. (2002)
Head-only exposure to GSM 900 MHz electromagnetic fields does not alter rat's memory in spatial and nonspatial tasks	SD Rats	Head only 1 and 3.5 W/kg	900 MHz EMF	GSM modulation at 217 Hz, 1/8 duty factor		45 min	Dubreuil et al. (2003a)
No significant effects on memory performance were found due to RF, avg. SAR within the brain was 7.4 W/kg and the whole body average SAR was 1.4 W/kg	SD Rats	1: Brain (B) 7.4 W/kg and whole body (WB) 1.4 W/kg, 2: Brain 25 W/kg and WB 4.5 W/kg	1439 MHz	PDC (Japan)		I: 1 h/day for 4 d; II: 45 min/day for 4 d	Yamaguchi et al. (2003)

TABLE 2.5

Effects of MW Exposure on Spatial Memory Performance

EMF Effect	Species	SAR (W/kg)	Frequency (MHz)	Modulation	Intensity (mW/cm^2)	Duration	Ref.
The results included no significant exposure effect, no significant drug effect, and no significant interactions between those two factors	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	45 min/d for 10 d	Cobb et al. (2004) (Lai et al., 1994 replication)
Response of MWs and temporally incoherent 60 mG on spatial learning in a water maze was similar to that of the sham-exposed animals	SD Rats	Avg. whole body 1.2 W/kg	2450 MHz	CW circular polarized	2 mW/cm^2	1 h CW; 1 h CW + 60 mG	Lai (2004)
Whole body exposure to 2.45 GHz electromagnetic fields does not alter radial maze performance in rats.	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	45 min	Cassel et al. (2004) (Lai et al., 1994 replication)
Whole body exposure to 2.45 GHz does not alter anxiety responses in rats: a plus maze study including test validation	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	45 min before testing	Cosquer et al. (2005a)
Whole body exposure to 2.45 GHz electromagnetic fields does not alter 12-arm radial maze with reduced access to spatial cues in rats	SD Rats	Avg. whole body 0.6 W/kg	2450 MHz	Pulsed circular polarized 2 μs pulses, 500 pps	1 mW/cm^2 but very high peak power	45 min before testing	Cosquer et al. (2005b)
MW exposure had no effects on learning in a radial arm maze.	Adult male mice C57BL/6J mice	1. Whole Body 0.1, 0.6 or 3 W/kg 2. headmainly exposure 0.1, 0.4 or 2.2 W/kg	1. 900 MHz	1. GSM talk		1.1 h/d over 15 days	Jones et al. (2005)

2.3. Thermoregulation

Thermoregulation, or the maintenance of a fairly steady body temperature even under a variety of external conditions, is important to animals because each body has a preferred temperature at which functioning is optimal. These external conditions can include changes in temperature, vapour pressure, air velocity, exposure to radiation including RF fields, and insulation among other factors that affect the temperature of the skin (Krewski et al., 2007). Previously, Adair et al. (1999) measured thermoregulatory responses of heat production and heat loss in human adult volunteers. Subsequently, Adair et al. (2001) exposed two different groups of volunteers to 2450 MHz continuous wave (CW) (2 females, 5 males) and pulsed wave (PW) (65 seconds pulse width, 104 pulse per second (pps); 3 females, 3 males) RF fields. They measured thermo-physiological responses of heat production and heat loss under a standardized protocol (30-min baseline, 45 min-RF or sham exposure, 10-min baseline), conducted in 3 ambient temperatures (24, 28, and 31°C). At each temperature, average power densities studied were 0, 27, and 35 mW/cm^2 (SAR = 0, 5.94, and 7.7 W/kg). Mean data for each group showed minimal changes in core temperature and metabolic heat production for all test conditions and no reliable differences between CW and PW exposure. Local skin temperatures showed similar trends for CW and PW exposure that were power density-dependent; only the skin temperature of the upper back (facing the antenna) showed a reliably greater increase during PW exposure than during CW exposure. Local sweat rate and skin blood flow were both temperature and power density-dependent and showed greater variability than other measures between CW and PW exposures; this variability was attributable primarily to the characteristics of the two subject groups. Similar results were obtained by Adair et al. (2003).

Recently, Adair and Black (2003) reviewed the current literature concerned with physiological thermoregulatory responses of humans in the presence of RF fields. They stated: “The conclusion is inescapable that humans demonstrate far superior thermoregulatory ability over other tested organisms during RF exposure at, or even above current human exposure guidelines”.

2.4. Authoritative Reviews

The widespread use of wireless telecommunications devices, particularly mobile phones, has resulted in increased human exposure to radiofrequency (RF) fields. Although national and international agencies have established safety guidelines for exposure to RF fields, concerns remain about the potential for adverse health outcomes to occur in relation to RF field exposure. A number of authorities have conducted detailed reviews of the potential health risks associated with exposure to RF fields. Krewski et al. (2007) provided the summaries of each of these reviews below.

2.4.1. American Cancer Society (2001)

The American Cancer Society conducted a review of the research on mobile phone technology and cancer and presented the findings in March, 2001. The review concludes: "There is now considerable epidemiological evidence that shows no consistent association between cellular phone use and brain cancer. The lack of ionizing radiation and the low energy level emitted from cell phones and absorbed by human tissues makes it unlikely that these devices cause cancer."

2.4.2. British Medical Association (2001)

The British Medical Association published a report on mobile phones and health that both summarizes available knowledge about mobile phones and health, and outlines on-going and planned research in this area. The report concludes: "The most recently published reviews of the literature have concluded that whilst there are small physiological effects within the existing guidelines, there are no definite adverse health effects from mobile phones or their base stations. However, all the main professional organizations have called for more research to be conducted, since the possibility that RF radiation may cause adverse effects cannot be ruled out on the currently available data. Clearly, there are large gaps in the knowledge that need to be addressed."

2.4.3. Director General of Health of France (Zmirou, 2001)

An expert group led by Dr. Denis Zmirou prepared a report to the Director General of Health of France concerning state of knowledge and recommendations about mobile phones, base stations and health. The report concluded: "Scientific data indicates, with comparative certainty, that due to RF exposure from a mobile phone, a variety of biological effects occur (e.g., EEG profile, reaction time, etc.) at energy levels that do not cause any local increase in temperature. However, in the current state of knowledge of these nonthermal effects, it is not yet possible to determine whether they represent a health hazard." The expert group recommended a risk management approach based on the precautionary principle, aimed at reducing public exposure to RF associated with mobile telephony to the lowest possible level compatible with service quality and justified by current scientific data.

2.4.4. European Commission's Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001)

The European Commission's Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) was requested to prepare an update of the opinion of the Scientific Steering Committee (SSC) on health effects of EM fields dated 1998, which endorsed the guidelines published by the ICNIRP. The CSTEE appointed a Working Group (WG) to evaluate the scientific findings resulting from new investigations. The WG concluded in its report: "The additional information which has become available on carcinogenic and other non-thermal effects of RF and microwave radiation frequencies in the last years does not justify a revision of exposure limits set by the Commission on the basis of the conclusions of the 1998 opinion of the SCC. In particular, in humans, no evidence of carcinogenicity in either children or adults has resulted from epidemiological studies."

2.4.5. Health Council of the Netherlands (2002)

The Health Council of the Netherlands prepared a report on the potential risks of EM fields from mobile telephones. The report concluded: "The EM field of a mobile telephone does not constitute a health hazard, according to the present state of scientific knowledge." However, the Council recommends conducting more research in the Netherlands on the influence of EM fields on cognitive functions. In a significant departure from the conclusions of the Independent Expert Group on Mobile Phones report (IEGMP, 2000), the Council does not recommend the application of the precautionary principle concerning non-thermal effects and finds no justification to recommend restriction in the use of mobile phones by children.

2.4.6. Institute of Electrical Engineers (IEE) Position Statement (IEE, 2002)

Every two years the IEE publishes on the worldwide web its Position Statement on the biological and health effects of low-level EM fields and radiation principally attributable to power lines, mobile phones and base stations. In 2002, the IEE Policy Advisory Group on the Biological Effects of Low Level Electromagnetic Fields has concluded that there is still no convincing scientific evidence that shows harmful effects of low-level EM fields on humans. This conclusion is the same as that reached in its previous position statement, being in May 2000.

2.4.7. Swedish Radiation Protection Authority (Boice and McLaughlin, 2002)

The Swedish Radiation Protection Authority conducted a review of all published epidemiology studies of cellular phone use and cancer since 1996. The authors concluded "in our view, a consistent picture has emerged from these studies that appear to rule out, with a reasonable degree of certainty, a causal association between cellular telephones and cancer to date. While the current state of the science is reassuring, ongoing case-control studies being conducted in 13 countries using a shared protocol, and continued follow-up of cohorts of cellular phone users, should provide further evidence regarding any possible carcinogenic effect associated with long-term cellular telephone use."

2.4.8. World Health Organization (2002)

In response to public concerns, the World Health Organization (WHO) established the International Electromagnetic Fields (EMF) Project to assess the scientific evidence of possible health effects of EM fields. Specific studies have been identified to address the problem of localized exposure. The project has established a formal mechanism for reviewing the research results and conducting risk assessments of EM exposure. It is also developing public information materials, and bringing together standards groups worldwide in an attempt to harmonise international exposure standards.

WHO is also conducting a large-scale epidemiology study being coordinated in over 13 countries through the International Agency for Research on Cancer (IARC), an agency of WHO, to identify if there are links between use of mobile phones and head and neck cancers. Further details of the study are described by Cardis and Kilkenny (1999). Fieldwork for the study is expected to be completed by the end of 2003 (WHO, 2000), with final results to be reported following a careful assessment of the data from this important international investigation.

2.4.9. Australian Government (2003)

The Australian government conducted an inquiry into the safety of mobile phone technology. The inquiry found no substantiated scientific evidence of health effects from mobile phones and their base stations. The inquiry reiterated that mobile phones must comply with strict safety guidelines established by the government.

Overall, some studies reported effects of EMF exposure on the embryo development, behaviour, biochemical, and immunity systems in animals and/or humans. These studies indicated that the observed defects of EMF exposure are associated with a thermal effect (1 ºC or more). Additionally, to-date all expert reviews on the health effects of exposure to RF fields have reached the same conclusion (ICNIRP, 2007; SCENIHR, 2007; WHO, 2007): there have been no adverse health consequences established from exposure to RF fields at levels below the international guidelines on exposure limits published by ICNIRP (1998)(the 900 MHz exposure guidelines limit level reported as maximum SAR of 2 W/kg). According to ICNIRP (1998) the threshold for biological effects is observed at SAR values above 4 W/kg. A SAR value of 4 W/kg is associated with temperature increase of more than 1 ºC. However, whether these guidelines of exposure apply during the embryogenesis period, is not clear. The present research aims to provide a more thorough knowledge to this crucial issue.

Part B
Experiments

Part B: Experiments

In this chapter the biological effect of 900 MHz EMF exposure, during the first five days of embryonic development, studied by four experiments. First experiment was to evaluate the effect on embryonic development. The second was to assess the effects on the hatched chicks' behaviour, following two protocols to study; a) the effect on the daily behaviour pattern, the feeding and drinking as well as the activity, b) the effects on the spatial learning and memory. Third experiment was to examine the effects on the immune system response ability. This chapter included the general materials required, radiation dosimetry calculation, and then the detailed experiments information (experimental protocol, statistical analysis, results and discussion) of the present study.

3. General Materials and Methods

3.1. Equipments

3.1.1. Incubators

Two FIEM MG Model breeding/hatching incubators were used. Each incubator was kept in a different room so that the treated incubator was far away from the other. The overall outside incubator dimensions were 55×58×52 cm (height × length × width), each incubator hatched 60 eggs placed in two trays. The trays move continuously around their axes with a maximum rotation angle of ±45º completing a full round per hour. The incubators were especially constructed to ensure exclusion of interfering metal structures, except for the motor, turning axis and the fan (Figure 3.1). The walls were made of wood (MDF) covered by plastic layer (0.1 cm), and the eggs trays were made of plastic material.

Figure 3.1.1. The FIEM MG Model breeding/hatching incubator

3.1.2. Radiation transmitter and dosimetry calculation

The output of a transmitter operating at 900 MHz was fed into an amplifier to result into a signal of 1 W (+30dbm), used as input to a Yagi antenna (6.2 dBi gain) at horizontal polarization. The incubator was placed in the far field of the antenna (1.5 m from the antenna brim), which was pointing at the middle of it. Electric field measurements were performed in front of the incubator with an SRM-3000 selective radiation meter (Narda Safety Test Solutions, Pfullingnen, Germany) and at three heights, namely at the lower and upper edges, and in the middle of the incubator's side. The spatial average value measured for the electric field strength at 900MHz was 1.79±0.17 V/m, whereas the spatial average background value (when the transmitter was off) from the same positions was less than 0.013 V/m. This means that there was an exposure ratio (exposed compared to non-exposed) of more than 135. The above values refer to the peak of the electric field strength intensity. Taking into account the maximum extended uncertainty (k=2) reported by the measuring equipment manufacturer (Eskerski and Braach 2007), which at the frequency of 900 MHz is 53% for isotropic measurements, the exposure ratio still remains higher than 40.

The numerical dosimetry of the exposure was performed with the software SEMCAD X (version 14.0 Aletsch, Schmid & Partner Engineering AG, Zurich, Switzerland). The software implements the Finite-Difference Time-Domain (FDTD) method (Taflove, 1995) for solving the electromagnetic problem. The dielectric properties for egg white and yolk were taken from (Ragni et al. 2007). In the model a harmonic plane wave source was used, scaled at the above measured spatial average value of the peak electric field.

The numerical model of the incubator (Figure 3.1.2) was build with the CAD tools of the software, to reflect the realistic experimental setup. Special care was given to include the metallic parts of the device and the conducting materials (e.g. the water dish used for humidification), which are important and can alter the field distribution because of reflections. Table 3.1.1 contains the parts of the incubator and the materials assigned to them, together with their assumed electrical properties at 900MHz, i.e. the frequency of radiation. It should be noted that, although several parts of the incubator are made of various plastic materials (different polymers) in the simulations the same value was used for the dielectric constant and the conductivity.

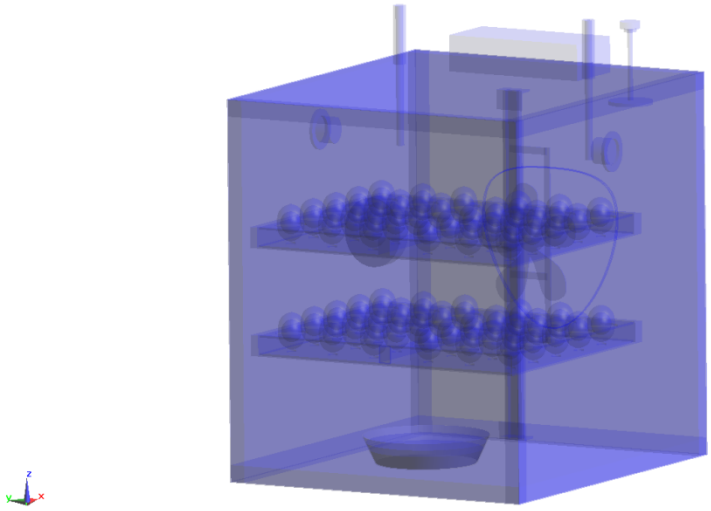


Figure 3.1.2. Numerical model of the incubator, fully loaded with the eggs

Table 3.1.1. Incubator model

Name of part	Material	Dielectric constant (ε_r)	Conductivity (σ) [S/m]
incubator housing	plastic	2	0.0005
egg tray	plastic	2	0.0005
water dish	plastic	2	0.0005
water	water	79	0.18
glass meters	glass	3	0.0005
egg white [2]	albumen	70	1.08
egg yellow [2]	yolk	30	0.5
metallic parts (fan, motor)	PEC*	1	∞

**perfectly electric conductor*

A plane wave source was used for the illumination of the incubator from its side. The simulation time was set at 20 periods, which was enough for reaching steady-state, as it can be seen with a field sensor placed inside an egg (Figure 3.1.3). The grid consisted of 28.3 million cells with edge size of 1/100 to 1/10 of the minimum wavelength in the model, in order to avoid numerical dispersion.

The two trays of eggs (lower and upper) were modeled inside the incubator at their horizontal position. However, the horizontal position was assumed a good proxy of exposure, since it is the average position in time. The numbering of the eggs in the trays is shown in Figure 3.1.4.

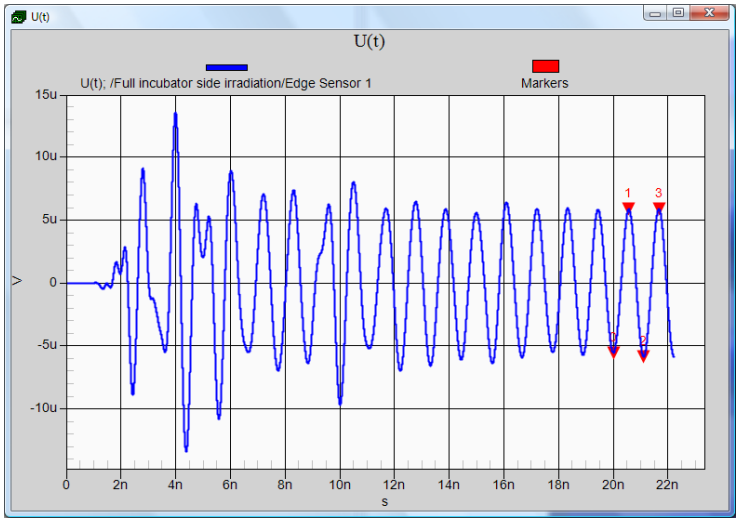


Figure 3.1.3. Field sensor result. The difference in the peak-to-peak values of the electric field is less than 3.0% for the last two periods, as indicated by the markers

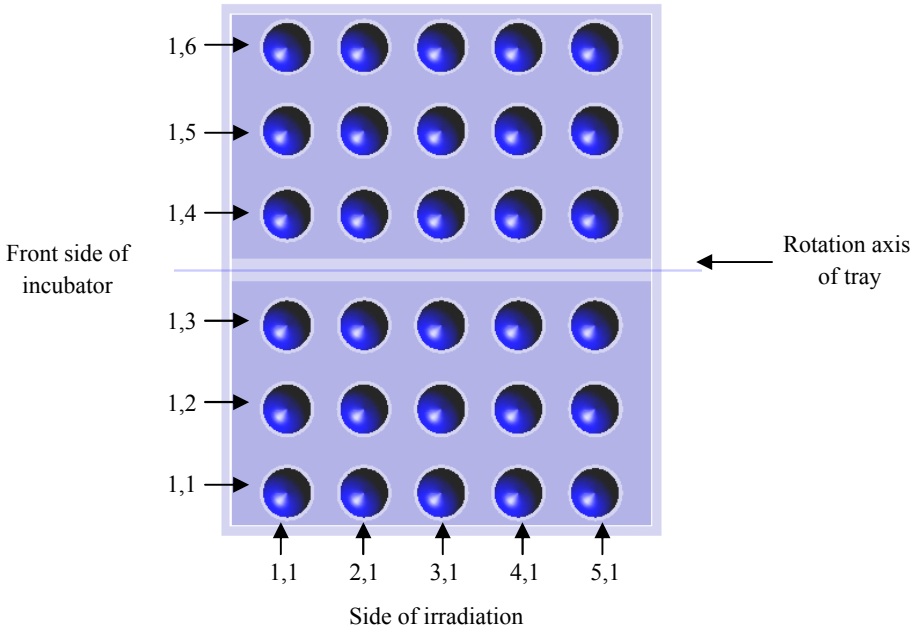
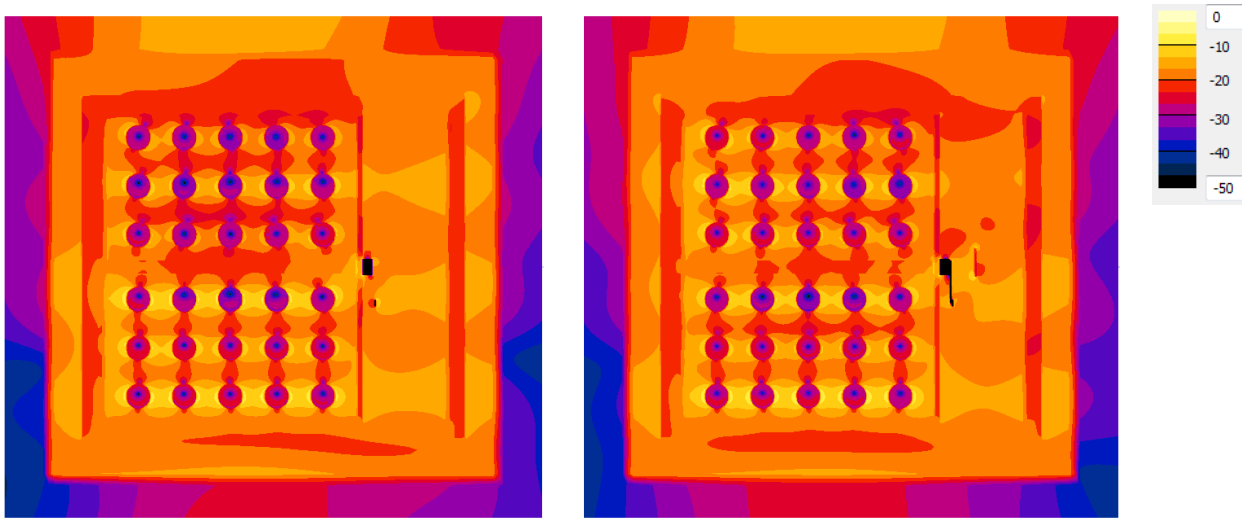


Figure 3.1.4. The first index in the numbering of eggs denotes the column and the second denotes the row (as seen from the side of irradiation)

The numerical model of the egg falls in the class of medium eggs (53-63 g), since the average numerical mass is 56.4±0.1 g, with the yolk and the albumen occupying 32.1% and 67.9% of this mass. The shell of the egg (less than 0.5 mm in thickness) was not modeled, because it would increase dramatically the required computational resources, although, due to its calcium carbonate content (>90%) it has a dielectric constant different from air, in its mineral form the dielectric constant of calcium carbonate is 6.1 (CCA-EU, 2009).

The results of dosimetry are expressed in term of intra-individual and inter-individual variations of the SAR for illumination electric field strength of 1 V/m (peak value). However, it is useful to show first the electric field (rms) distribution in the computational domain at the level of the lower (Figure 3.1.5a) and the upper (Figure 3.1.5b) egg trays. The actual dosimetry of the exposure setup can be deduced by scaling the results according to the measurements.

Table 3.1.2 gives the individual egg dosimetry for the lower tray and Table 3.1.3 the dosimetry for the upper tray. The quantities included in the tables and given separately for the albumen and the yolk are the total electromagnetic power absorbed, the mean SAR and its standard deviation, as well as the minimum and maximum SAR values found in single voxels. The last column of the table gives the percentage of volume which falls differs up to 50% from the mean SAR value; it is obvious that the larger this volume, the more uniform the exposure.



(a) (b)

Figure 3.1.5. Electric field distribution (rms value) inside the incubator at the height of (a) the lower, and (b) the upper tray. The color scale is logarithmic (dB) and its maximum (0dB) corresponds to 5V/m

A first analysis of the results shows that in the egg the exposure is equally uniform in the egg white and the egg yolk. Since the 3dB volumes are all larger than 71.4% there appears to be good uniformity of exposure within each egg. The mean SAR of all eggs is 1.28 E-04W/kg (±34%), therefore the inter-individual egg exposure is also good. The overall maximum voxel SAR found is 2.22e-4W/kg in the albumen of egg (1, 6) of the upper tray, which seems to be the most exposed egg, judging also from the mean values of SAR.

It is interesting to calculate the maximum temperature rise in the highest exposed egg under adiabatic conditions, using the formula

$$\Delta T = \frac{SAR \cdot t}{c} \qquad (1)$$

The heat capacity value of 3425J/(kg ^{o}C) for the egg is taken from (Coimbra at al., 2006) and corresponds to the volume ratio of egg white and yolk assigned to the numerical model. If we assume an exposure time of 5 days, then the temperature rise for the maximum SAR and the mean SAR value in the egg would be 0.028 ^{o}C and 0.016 ^{o}C, respectively.

Table 3.1.2 Dosimetry for the incubator lower tray

Egg	Total Power Loss (W)	Mean SAR (W/kg)	Std. Deviation SAR (W/kg)	Minimum SAR (W/kg)	Maximum SAR (W/kg)	Volume within ±3dB in %
egg 1_1/albumen	2.78E-06	2.33E-04	3.53E-05	1.21E-07	1.68E-04	80.8
egg 1_1/yolk	6.15E-07	1.09E-04	1.84E-05	3.70E-07	7.52E-05	76.1
egg 1_2/albumen	3.22E-06	2.69E-04	4.06E-05	1.63E-07	2.06E-04	80.5
egg 1_2/yolk	6.91E-07	1.22E-04	2.08E-05	1.61E-07	8.66E-05	76.4
egg 1_3/albumen	2.09E-06	1.76E-04	2.68E-05	2.04E-07	1.19E-04	80.7
egg 1_3/yolk	4.86E-07	8.60E-05	1.50E-05	3.84E-07	6.17E-05	74.9
egg 1_4/albumen	2.58E-06	2.16E-04	3.32E-05	1.40E-07	1.49E-04	80.3
egg 1_4/yolk	5.48E-07	9.70E-05	1.64E-05	3.15E-07	6.62E-05	75.5
egg 1_5/albumen	1.24E-06	1.05E-04	1.56E-05	1.92E-07	8.13E-05	81.3
egg 1_5/yolk	2.82E-07	4.95E-05	8.26E-06	8.22E-08	3.27E-05	76.9
egg 1_6/albumen	1.76E-06	1.47E-04	2.25E-05	8.44E-08	1.16E-04	80.6
egg 1_6/yolk	3.80E-07	6.72E-05	1.12E-05	2.37E-07	4.59E-05	76.4
egg 2_1/albumen	2.74E-06	2.30E-04	3.43E-05	2.11E-07	1.64E-04	81.2
egg 2_1/yolk	6.09E-07	1.08E-04	1.82E-05	5.13E-07	7.41E-05	76.7
egg 2_2/albumen	2.99E-06	2.50E-04	3.75E-05	1.34E-07	1.90E-04	80.9
egg 2_2/yolk	6.36E-07	1.13E-04	1.94E-05	4.19E-07	7.83E-05	76.1
egg 2_3/albumen	1.20E-06	1.01E-04	1.54E-05	6.41E-08	6.97E-05	80.0
egg 2_3/yolk	3.03E-07	5.35E-05	9.63E-06	3.04E-07	3.90E-05	73.2
egg 2_4/albumen	1.70E-06	1.43E-04	2.18E-05	5.23E-08	9.81E-05	80.7
egg 2_4/yolk	3.59E-07	6.35E-05	1.06E-05	8.45E-08	4.30E-05	76.3
egg 2_5/albumen	8.42E-07	7.05E-05	1.03E-05	1.34E-07	5.56E-05	82.0
egg 2_5/yolk	1.91E-07	3.39E-05	5.62E-06	7.62E-08	2.28E-05	77.3
egg 2_6/albumen	1.53E-06	1.28E-04	1.95E-05	6.59E-08	1.04E-04	80.7
egg 2_6/yolk	3.27E-07	5.79E-05	9.74E-06	2.06E-07	3.94E-05	75.6
egg 3_1/albumen	3.00E-06	2.51E-04	3.77E-05	2.99E-07	1.77E-04	81.0
egg 3_1/yolk	6.59E-07	1.17E-04	1.99E-05	5.40E-07	8.28E-05	77.0
egg 3_2/albumen	2.74E-06	2.29E-04	3.42E-05	9.52E-08	1.68E-04	81.0
egg 3_2/yolk	5.81E-07	1.03E-04	1.76E-05	5.35E-07	7.31E-05	76.5
egg 3_3/albumen	7.34E-07	6.15E-05	9.66E-06	4.92E-08	4.61E-05	78.4
egg 3_3/yolk	2.03E-07	3.59E-05	6.62E-06	4.66E-08	2.61E-05	71.4
egg 3_4/albumen	1.89E-06	1.58E-04	2.42E-05	1.24E-07	1.08E-04	80.3
egg 3_4/yolk	3.92E-07	6.96E-05	1.16E-05	6.39E-08	4.66E-05	76.6
egg 3_5/albumen	8.83E-07	7.39E-05	1.08E-05	2.51E-07	5.57E-05	82.0
egg 3_5/yolk	2.01E-07	3.56E-05	6.01E-06	9.58E-08	2.41E-05	76.5
egg 3_6/albumen	1.18E-06	9.86E-05	1.50E-05	1.46E-07	7.73E-05	80.6
egg 3_6/yolk	2.56E-07	4.53E-05	7.75E-06	2.47E-07	3.20E-05	76.0
egg 4_1/albumen	2.78E-06	2.32E-04	3.51E-05	2.44E-08	1.67E-04	81.0
egg 4_1/yolk	6.18E-07	1.10E-04	1.89E-05	3.12E-07	7.90E-05	75.0
egg 4_2/albumen	2.62E-06	2.19E-04	3.27E-05	1.52E-07	1.70E-04	81.0
egg 4_2/yolk	5.56E-07	9.85E-05	1.68E-05	2.14E-07	6.86E-05	75.8
egg 4_3/albumen	9.67E-07	8.12E-05	1.24E-05	2.51E-07	5.67E-05	80.3
egg 4_3/yolk	2.51E-07	4.43E-05	7.88E-06	2.07E-07	2.87E-05	74.9
egg 4_4/albumen	2.55E-06	2.13E-04	3.23E-05	2.29E-07	1.40E-04	80.8
egg 4_4/yolk	5.32E-07	9.41E-05	1.58E-05	1.98E-07	6.44E-05	76.6
egg 4_5/albumen	1.11E-06	9.34E-05	1.39E-05	1.09E-07	7.10E-05	81.4
egg 4_5/yolk	2.56E-07	4.49E-05	7.67E-06	8.45E-08	3.05E-05	75.7
egg 4_6/albumen	1.22E-06	1.02E-04	1.55E-05	1.57E-07	6.87E-05	80.6
egg 4_6/yolk	2.67E-07	4.70E-05	8.02E-06	1.30E-07	3.41E-05	76.0
egg 5_1/albumen	2.20E-06	1.84E-04	2.80E-05	2.61E-07	1.36E-04	80.8
egg 5_1/yolk	5.01E-07	8.87E-05	1.53E-05	2.10E-07	6.11E-05	75.7
egg 5_2/albumen	2.94E-06	2.46E-04	3.76E-05	1.24E-07	1.87E-04	80.5
egg 5_2/yolk	6.27E-07	1.11E-04	1.86E-05	3.78E-07	8.19E-05	76.7
egg 5_3/albumen	1.88E-06	1.58E-04	2.39E-05	1.76E-07	1.11E-04	80.7
egg 5_3/yolk	4.31E-07	7.62E-05	1.32E-05	3.44E-07	5.15E-05	76.2
egg 5_4/albumen	2.84E-06	2.38E-04	3.59E-05	5.00E-07	1.73E-04	80.5
egg 5_4/yolk	6.00E-07	1.06E-04	1.78E-05	3.90E-07	7.53E-05	76.7
egg 5_5/albumen	1.23E-06	1.03E-04	1.56E-05	1.22E-07	7.66E-05	80.7
egg 5_5/yolk	2.84E-07	4.98E-05	8.51E-06	7.08E-08	3.45E-05	75.5
egg 5_6/albumen	1.79E-06	1.50E-04	2.31E-05	6.33E-08	1.04E-04	80.3
egg 5_6/yolk	3.86E-07	6.81E-05	1.14E-05	4.06E-07	4.58E-05	76.7

Table 3.1.3. Dosimetry for the incubator upper tray

Egg	Total Power Loss (W)	Mean SAR (W/kg)	Std. Deviation SAR (W/kg)	Minimum SAR (W/kg)	Maximum SAR (W/kg)	Volume within ±3dB in %
egg 1_1/albumen	2.47E-06	2.06E-04	3.12E-05	4.11E-07	1.47E-04	80.6
egg 1_1/yolk	5.54E-07	9.89E-05	1.68E-05	7.94E-07	6.79E-05	76.0
egg 1_2/albumen	3.41E-06	2.85E-04	4.33E-05	6.53E-08	2.20E-04	80.4
egg 1_2/yolk	7.19E-07	1.28E-04	2.13E-05	4.47E-07	9.32E-05	76.7
egg 1_3/albumen	2.18E-06	1.83E-04	2.74E-05	3.42E-07	1.34E-04	81.2
egg 1_3/yolk	4.82E-07	8.53E-05	1.44E-05	2.29E-07	5.94E-05	76.8
egg 1_4/albumen	2.91E-06	2.43E-04	3.71E-05	2.93E-07	1.63E-04	80.3
egg 1_4/yolk	6.30E-07	1.12E-04	1.93E-05	7.94E-08	7.91E-05	75.4
egg 1_5/albumen	2.16E-06	1.81E-04	2.71E-05	5.97E-07	1.31E-04	80.8
egg 1_5/yolk	4.89E-07	8.66E-05	1.46E-05	3.98E-07	5.92E-05	76.9
egg 1_6/albumen	3.25E-06	2.71E-04	4.14E-05	1.21E-07	2.22E-04	80.4
egg 1_6/yolk	6.91E-07	1.23E-04	2.08E-05	1.61E-07	8.60E-05	75.6
egg 2_1/albumen	2.43E-06	2.03E-04	3.05E-05	2.24E-07	1.42E-04	80.7
egg 2_1/yolk	5.55E-07	9.86E-05	1.70E-05	4.55E-07	6.82E-05	75.5
egg 2_2/albumen	3.03E-06	2.53E-04	3.82E-05	2.54E-07	1.90E-04	80.7
egg 2_2/yolk	6.37E-07	1.13E-04	1.90E-05	4.93E-07	8.23E-05	77.3
egg 2_3/albumen	1.24E-06	1.04E-04	1.54E-05	3.38E-07	7.62E-05	81.5
egg 2_3/yolk	2.91E-07	5.15E-05	8.79E-06	1.24E-07	3.68E-05	76.2
egg 2_4/albumen	2.47E-06	2.07E-04	3.13E-05	6.25E-08	1.34E-04	80.5
egg 2_4/yolk	5.37E-07	9.52E-05	1.64E-05	7.84E-08	6.71E-05	74.6
egg 2_5/albumen	1.84E-06	1.54E-04	2.29E-05	2.31E-07	1.13E-04	81.0
egg 2_5/yolk	4.15E-07	7.35E-05	1.24E-05	1.81E-07	4.92E-05	76.8
egg 2_6/albumen	2.54E-06	2.12E-04	3.22E-05	3.43E-08	1.70E-04	80.5
egg 2_6/yolk	5.42E-07	9.61E-05	1.63E-05	1.54E-07	6.82E-05	75.8
egg 3_1/albumen	2.61E-06	2.18E-04	3.27E-05	1.26E-07	1.50E-04	80.8
egg 3_1/yolk	5.90E-07	1.05E-04	1.82E-05	4.41E-07	7.40E-05	74.8
egg 3_2/albumen	2.68E-06	2.24E-04	3.37E-05	3.53E-07	1.72E-04	81.0
egg 3_2/yolk	5.63E-07	9.98E-05	1.68E-05	3.38E-07	7.36E-05	76.6
egg 3_3/albumen	7.97E-07	6.66E-05	9.93E-06	1.15E-07	4.90E-05	81.3
egg 3_3/yolk	2.00E-07	3.53E-05	6.10E-06	4.37E-08	2.41E-05	75.6
egg 3_4/albumen	2.47E-06	2.06E-04	3.13E-05	6.81E-08	1.34E-04	80.6
egg 3_4/yolk	5.28E-07	9.35E-05	1.60E-05	3.52E-07	6.56E-05	75.1
egg 3_5/albumen	1.55E-06	1.29E-04	1.93E-05	1.02E-07	9.32E-05	81.2
egg 3_5/yolk	3.50E-07	6.20E-05	1.06E-05	1.82E-07	4.10E-05	76.1
egg 3_6/albumen	1.81E-06	1.51E-04	2.29E-05	1.46E-07	1.20E-04	80.7
egg 3_6/yolk	3.89E-07	6.89E-05	1.18E-05	1.91E-07	4.86E-05	75.3
egg 4_1/albumen	2.38E-06	1.98E-04	3.00E-05	2.27E-08	1.39E-04	80.8
egg 4_1/yolk	5.47E-07	9.71E-05	1.71E-05	4.40E-08	6.84E-05	74.2
egg 4_2/albumen	2.59E-06	2.16E-04	3.27E-05	1.19E-07	1.67E-04	80.7
egg 4_2/yolk	5.47E-07	9.72E-05	1.63E-05	1.33E-07	7.02E-05	76.3
egg 4_3/albumen	1.13E-06	9.43E-05	1.40E-05	1.21E-07	7.05E-05	81.6
egg 4_3/yolk	2.70E-07	4.78E-05	8.13E-06	2.47E-07	3.06E-05	77.0
egg 4_4/albumen	2.53E-06	2.11E-04	3.20E-05	2.13E-07	1.44E-04	80.7
egg 4_4/yolk	5.35E-07	9.49E-05	1.61E-05	4.40E-07	6.62E-05	76.3
egg 4_5/albumen	1.21E-06	1.01E-04	1.52E-05	1.43E-07	7.53E-05	81.0
egg 4_5/yolk	2.80E-07	4.96E-05	8.46E-06	1.03E-07	3.32E-05	76.4
egg 4_6/albumen	1.73E-06	1.44E-04	2.20E-05	8.54E-08	9.83E-05	80.7
egg 4_6/yolk	3.67E-07	6.49E-05	1.11E-05	9.12E-08	4.48E-05	75.6
egg 5_1/albumen	2.04E-06	1.70E-04	2.60E-05	2.74E-07	1.22E-04	80.7
egg 5_1/yolk	4.69E-07	8.37E-05	1.45E-05	2.76E-07	5.78E-05	75.3
egg 5_2/albumen	3.00E-06	2.50E-04	3.84E-05	2.76E-07	1.90E-04	80.1
egg 5_2/yolk	6.38E-07	1.13E-04	1.87E-05	5.88E-07	8.37E-05	76.8
egg 5_3/albumen	2.06E-06	1.72E-04	2.61E-05	1.54E-07	1.22E-04	80.5
egg 5_3/yolk	4.60E-07	8.13E-05	1.39E-05	3.32E-07	5.63E-05	76.6
egg 5_4/albumen	2.62E-06	2.18E-04	3.34E-05	3.42E-07	1.58E-04	80.5
egg 5_4/yolk	5.49E-07	9.74E-05	1.65E-05	2.06E-07	6.75E-05	76.4
egg 5_5/albumen	9.64E-07	8.04E-05	1.21E-05	2.09E-07	6.43E-05	80.9
egg 5_5/yolk	2.27E-07	4.00E-05	6.68E-06	6.42E-08	2.58E-05	77.5
egg 5_6/albumen	2.22E-06	1.86E-04	2.87E-05	2.15E-07	1.31E-04	80.1
egg 5_6/yolk	4.68E-07	8.26E-05	1.40E-05	3.40E-07	5.91E-05	76.2

3.2. Eggs treatment

After the eggs were delivered to the laboratory, they were stored for 4-5 hours at room temperature (18-21ºC). They were numbered and measured (weight, length, and diameter), before they were placed into the two incubators. Each time (batch) 120 eggs were randomly assigned to two groups of 60 eggs, placed in the control and the exposure unit. The eggs were incubated at 37 ºC and a relative humidity of 55-60%.

The eggs were placed on a movable rack 1.5 m far of the antenna brim (Figure 3.2.5). At the beginning of each experiment, the exposure protocol included 900 MHz continuous wave (CW) electromagnetic radiation during the first five days of embryonic development. As mentioned above, the average (±SD) specific absorption rate (SAR) in the exposed eggs was determined numerically at 0.13±0.03 mW/kg.

Figure 3.2.5. The experimental room with the incubator and radiation transmitter

3.3. Animal treatment

For the further experiments, after hatching, each treatment group was randomly divided into two subgroups. Chicks were marked directly after hatching with leg clips for individual identification when used in the spatial learning and memory experiment. One day after hatching, the chicks were moved and randomly assigned to four separate identical straw-bedded solid floored rooms (~2 x 1.5m) with heating lamps. The feed was provided in red bowls from the second day of hatching. Commercial feed and water were offered *ad libitum*. The feeders and drinkers were reloaded with feed and water daily between 09:00 and 10:00 am. The room light was on 24 h.

4. Experiments

4.1. Effect of Radiation on Embryo Development

Numerous studies have evaluated the developmental effects of electromagnetic fields on mammals, birds, and other species. In all species studied, EMF is reported to be teratogenic at exposure levels that cause significant increase of the maternal and/or embryo body temperature (for a detailed review, see Heynick and Merritt, 2003 and Juutilainen, 2005). In avian species, in particular, EMF exposure may markedly increase the egg temperature and thus cause subsequent hyperthermia to the exposed embryos (Clarke and Justesen, 1983). The distinction between thermal and non-thermal interactions is important, particularly in the interpretation of biological studies (see Glaser, 2005 and Foster and Glaser, 2007, for a detailed review). Examining the effect of exposure itself without the confounding effect of the temperature is not always straightforward, unless the internal egg temperature rise could be compensated or low intensity electric field is used that is not expected to result in a significant temperature rise.

Related to thermal or non-thermal effects, EMFs are generally reported to be associated with increased embryonic mortality and/or abnormality rates at a wide range of frequencies, e.g. extremely low (ELF) (Martin 1988; Ubeda et al., 1994 and Farrell et al., 1997), 428 MHz (Saito et al., 1991), 900 MHz (Youbicier-simo et al., 1998a,b) and 1.25 GHz (Varga, 1992) or 2.45 GHz (Carpenter et al., 1960 and Hills et a1., 1974). In some of the above studies, however, obviously exposures beyond the currently recommended reference levels have been applied.

Research on the biological effects of high intensity EMF (at 900 MHz) within or close to international guidelines limit level [maximum SAR of 2 (ICNIRP 1998) or 1.6 W/kg (IEEE 1992)] has already been carried out on rats examining effects on spatial learning, hormones and brain structures (Dubreuil et al., 2002, 2003a,b; Koyu et al., 2005 and Odaci et al., 2008). Among non-mammalian species, chicken embryos are considered to be a more suitable animal model for the detection of the adverse effects of EMF radiation. Major advantages of using this animal model include: self-sufficiency, considerable existing knowledge of the embryogenesis and observation of the embryonic process over a short time of period. Moreover, any response of the chicken embryo to external agents, such as magnetic field, may be attributable to only direct action of the stimulus since no maternal protective mechanism is involved (Ubeda et al., 1994). There are only a few studies using the chicken as an animal model to study the effect of 900 MHz (GSM signal) on mortality and abnormality rates during the 21 days of incubation (Youbicier-simo et al., 1998a,b,c). Examination of early exposure i.e. 24-52 h on chicken development is limited to only ELF [Juutilainen et al., 1986, Martin, 1988, Berman et al., 1990, Ubeda et al., 1994, Farrell et al., 1997]. ELF EMFs has presumably a different mode of interaction with biological matter, while early embryonic examination is not a good indicator whether teratogenesis has really occurred (Brent, 1999).

Studies using the 900 MHz and exposure for the first crucial stage of development (e.g. 5 days) with low intensity fields are scarce in the literature. Given the lack of relevant data in the literature, we have elaborated the present research. The objective of the study was to investigate whether 900 MHz CW low intensity exposure of chicken embryos during early embryogenesis affects mortality, abnormality rates and embryo development.

4.1.1. Experimental protocol

Three batches of eggs of a commercial chicken layer line (ISA Brown) were in total used to ensure statistical power resulting in an overall number of 360 eggs. After 5 days of incubation, eggs were opened and the excised embryos were placed on a Petri dish. Infertile eggs, as well as, embryos that died during the first 2 days of incubation were excluded from the evaluation. Staging of the embryos was performed according to Hamburger and Hamilton (1951) and the embryos were classified as dead or alive.

Before performing the final experiments, two series of pre-experiments by following the same protocol were carried out in an attempt to gain experience with the embryo treatment and evaluation.

4.1.2. Evaluation of embryonic stage and abnormalities

The excised embryos which were classified as alive, were subsequently immersed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.5 for 1 h at room temperature, and left overnight in 7% sucrose in 0.1 M PBS and 0.1% sodium azide.

To assess the morphology of the embryos in conjunction with the development of the nervous system the acetyl-holinesterase histochemical reaction was carried out using a variation of the Karnovsky and Roots (1964) method as proposed by Baker et al. (1986).

The embryos were washed in PBS for 5 min and incubated in 10 ml soda-vials in PBS containing hyaluronidase (0.33 mg per 100 ml; H3506, SIGMA) and iso-OMPA (tetraisopropylpyrophosphoramide; final concentration 10-4; T1505, SIGMA) at 4 ºC for 30 min. Iso-OMPA at the final concentration of 10^{-4} specifically inhibits non-specific or pseudo-cholinesterase (butyrylcholine esterase; Cochard and Coltey, 1983). Hyaluronidase is used to increase the permeability of the tissue (Baker et al., 1986). The embryos were then washed in PBS for 5 min and incubated in the reaction medium overnight, in rotation. The reaction medium consists of two parts which, when mixed, form the working solution.

Part A: 0.1 M acetate buffer pH 5 with 1.5% Triton X-100 (65 ml)
0.15 M sodium citrate (10 ml)
30 mM copper sulphate (10 ml)
5 mM potassium ferricyanide [$K_3Fe(CN)_6$] (10 ml)

Part B: 1 mM iso-OMPA (10 ml)
Acetylthiocholine iodide (50 mg; A5751, SIGMA).

The embryos were washed in distilled water for one hour, fixed in 4% paraformaldehyde in PBS for 30 min. The omission of acetylthiocholine iodide in control experiments, revealed no reaction product in the embryos examined.

Embryos' body weight were also measured and recorded after staining, prior to mounting with 70% glycerol in PBS. Following histochemical staining, the embryos were examined under a stereo-microscope (ZEISS Sterni 200-C) fitted with a SONY CCD-IFIS color video camera.

Embryos were classified according to two criteria. First, according to morphological anomalies of the head and limbs or grossly abnormal (two categories: normal, abnormal) and second according to anatomical malformations of the neural spinal tube (degree of curvature). In the latter case there were three categories built: normal, light and strong (curvature). Some embryos displayed malformations and anomalies jointly. Thus, a new category (embryos with combined problems) was formed using the number of normal and abnormal embryos in total. Note, that embryos with combined problems were accounted twice.

4.1.3. Statistical Analysis

A 2x2 contingency table analysis was performed for the number of infertile, dead embryos, used and embryos with combined problems using both the chi-square and a two sided Fisher's exact test. This analysis was carried out at group, tray and batch level. A 2x4 contingency table analysis was employed to examine effects of radiation on anomalies as well as malformations, simultaneously, using a Fisher's exact test. Embryo weight was subjected to two-way analysis of variance using the group, the batch and the batch by group interaction as the fixed effects. A Bonferroni adjustment of the p-values during multiple comparisons of means was employed here. A logistic regression analysis using the exposure levels obtained from the dosimetry analysis as the independent variable and abnormalities as the dependent variable(s) was also employed in an attempt to estimate linear and nonlinear dose dependent effects. Results are presented as number of cases for abnormalities and least squares means (with standard errors) for the embryo weight. All analyses were carried out by SAS (2002).

4.1.4. Results

There were significant differences in the fertility rates between the radiated and the control eggs (p=0.04, two-sided Fisher's exact test); more infertile eggs were obtained in the controls (Table 4.1.1). The number of dead embryos remained very low (1-2 cases per batch) and did not discriminate between the two groups (p=0.72, two-sided Fisher's exact test, Table 4.1.1). This was also the case for the total number of embryos used (p=0.48). A detailed presentation of the above numbers per batch is shown in Table 4.1.2.

Table 4.1.1. Number of infertile, dead (within the first two days of incubation) and evaluated embryos per group (in parentheses: p-values of the Fisher's exact test)

	Total	Infertile (p=0.04)	Dead embryos (p=0.72)	Embryos used (p=0.48)
Control	180	30	5	145
Radiated	180	15	3	162

Table 4.1.2. Number of infertile eggs, number of dead embryos (within the first two days of incubation) and total number of evaluated (used) embryos per group and batch

	Batch 1 P-values for infertility=0.053 and dead embryos=0.59		Batch 2 P-values for infertility=0.56 and dead embryos=1.0		Batch 3 P-values for infertility=0.53 and dead embryos=0.24	
	Control	Radiated	Control	Radiated	Control	Radiated
Infertile	15	6	8	5	7	4
Dead Embryos	2	1	1	2	2	0
Embryos used	43	53	51	53	51	56

Table 4.1.3 displays the number of normal embryos and the number of embryos with spinal problems and/or morphological anomalies in the two groups (pooled data). Radiation resulted in less numbers of normal embryos (75 *vs.* 93) and increases in both the cases of embryos with light (55 *vs.* 43) but most notably the number of embryos with strong spinal problems (27 *vs.* 7). This trend was also valid for the morphological anomalies (in head or limbs) resulting in an overall p-value of <0.001. In total, more multiple abnormalities i.e. combined cases were observed in the exposed embryos when contrasted to controls (10 *vs.* 2). A detailed description of all cases per batch and tray along with level of statistical significance is shown in Tables 4.1.4 and 4.1.5, respectively. Briefly, in one and two out of the three batches there were significant results obtained for multiple abnormalities and spinal or morphological anomalies, respectively, while, there was no tray effect detected (Table 4.1.5). Typical embryos with normal and defective morphology are displayed in Figure 4.1.1.

Table 4.1.3. Number of normal embryos and number of embryos with spinal problems (S) as well as with morphological abnormalities (A) per group (p1: chi-square value). Embryos with combined problems are also given (p2: Fisher's exact test)

	Normal	Light curved	Strong curved	Abnormal	p1	Combined cases	p2
Control	93	43	7	4	<0.001	2	0.014
Radiated	75	55	27	15		10	

Table 4.1.4. Number of normal embryos and number of embryos with light (S2), strong spinal (S3) curvature and morphological abnormalities (A) as well as embryos with multiple abnormalities (AS) per group and batch

	Batch														
Group	1 (p1=0.33, p2=1)					2 (p1=0.016, p2=0.011)					3 (p1=0.03, p2=0.20)				
	Normal	S2	S3	A	AS	Normal	S2	S3	A	AS	Normal	S2	S3	A	AS
Control	24	16	2	3	2	33	15	2	1	0	36	12	3	0	0
Radiated	21	23	7	4	2	26	16	9	8	6	28	16	11	3	2

p1: p-value of Fisher's exact test for normal, light, strong and morphological abnormalities
p2: p-value of Fisher's exact test for multiple abnormalities

Table 4.1.5. Number of normal embryos and number of embryos with light (S2), strong spinal (S3) curvature and morphological abnormalities (A) as well as embryos with multiple abnormalities (AS) per group and tray

	Control (p1=0.78, p2=0.50)					Radiated (p1=0.12, p2=0.10)				
	Normal	S2	S3	A	AS	Normal	S2	S3	A	AS
Upper tray	47	20	3	3	2	44	21	13	6	3
Lower tray	46	23	4	1	0	31	34	14	9	7

p1: p-value of Fisher's exact test for normal, light, strong and morphological abnormalities, p2: p-value of Fisher's exact test for multiple abnormalities

Figure 4.1.2 shows results of the dosimetry analysis in the exposed unit. SAR values ranged from 0.049 to 0.207 mW/kg, the maximum SAR value found was in the egg (1, 6) of the upper tray. The dosimetry analysis has shown that the exposure is equally uniform in the egg white and the yolk. Since the 3dB volumes are all larger than 71.4% there appears to be good uniformity of exposure within each egg. The mean SAR was 0.128 mW/kg (±34%), therefore the inter-individual egg exposure was also good. The heat capacity value of 3425 J/(kg ^{o}C) for the egg is taken from (Coimbra et al., 2006) and corresponds to the volume ratio of egg white and yolk assigned to the numerical model. If we assume an exposure time of 5 days, then the worst-case temperature rise for the maximum SAR and the mean SAR value in the egg would be 0.028 ^{o}C and 0.016 ^{o}C, respectively, assuming no heat exchange mechanisms with the environment.

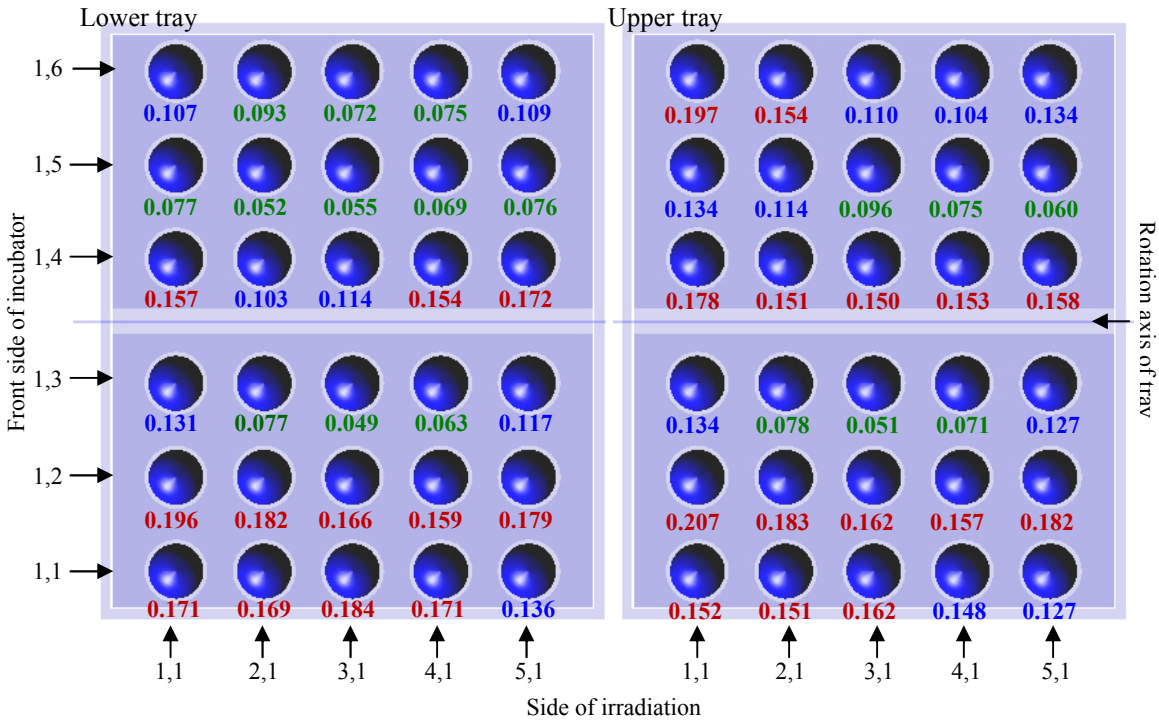


Figure 4.1.2. Calculated mean (of yolk and albumen) SAR values (mW/kg) for individual eggs in the upper and lower tray of the exposed eggs. First and second indices in egg numbering denote column and row, respectively (as seen from the side of irradiation)

The placement of all cases in the exposed and control eggs are depicted in Figures 4.1.3 and 4.1.4, respectively. Logistic regression analysis using the defective cases as the dependent variable (Figure 4) and the SAR values as linear as well as polynomial (up to third degree) covariates did not reveal any dose dependent radiation effect (results not shown).

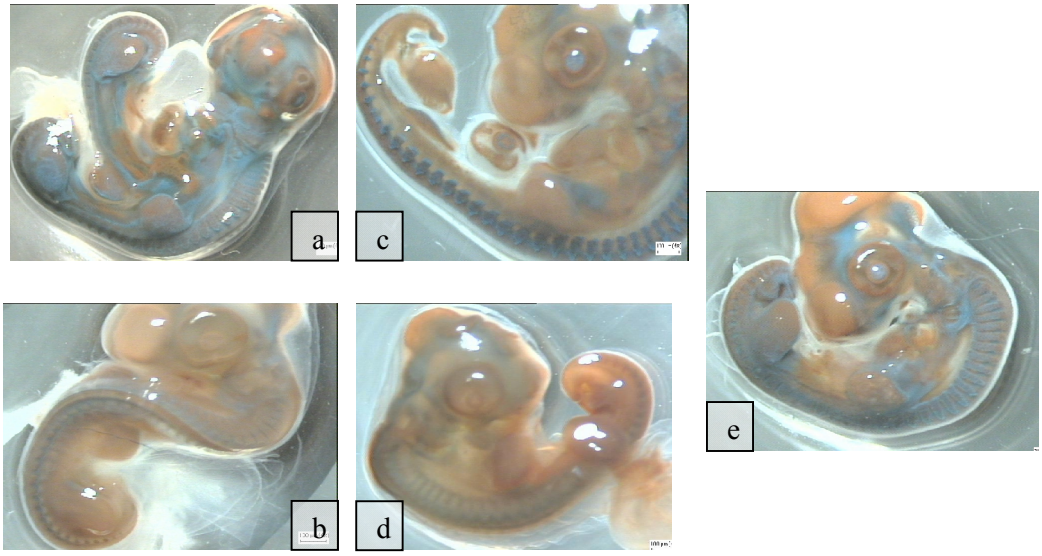


Figure 4.1.1. Chicken embryos photographs at stage 28, according to Hamburger and Hamilton (1951), following acetyl-cholinesterase histochemical staining for the visualization of the nervous system: compare (a,b,c,d) abnormal embryos with (e) normal embryo; a: severe malformation (one head, two tails), b: strongly curved neural tube, c: retarded limb development, d: retarded head development.

Table 4.1.6 shows the egg and embryo weight between groups and batches. As reasonably expected egg weight was the same between groups but increased by batch. Embryo weight clearly discriminated between both batches and groups with radiated embryos always displaying heavier weights (1001 *vs.* 832 mg) than the controls. Table 3 presents body weight of normal and abnormal embryos per group. Abnormal embryos' weight was higher in the exposed group (709 *vs.* 475 mg) than the controls. Due to limited number of cases, this apparent difference was not, however, statistically confirmed. Embryo weight by group and batch is depicted in Figure 4.1.5. Although some level of interaction is apparent as denoted by differences in group means by batch, the weight of the exposed embryos was always higher in the exposed group when compared to the controls.

(Lower tray)

	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
1.6	S 2	S 2	N	S 2	N	S 3	S 2	S 3	N	IF	S 2	N	S 3	N	IF
1.5	S 3	IF	IF	S 2	N	N	S 2	N	S 2	S 2	N	S 2	S 2	A 1 S 2	N
1.4	S 2	N	N	N	A 3 S 3	N	IF	S 2	N	S 3	N	A 1 S 2	A 3	N	S 2
1.3	S 2	S 3	S 2	S 3	S 3	S 3	S 2	A 2	S 3	IF	IF	N	S 2	N	S 2
1.2	N	S 2	N	S 2	N	S 2	N	A 3 S 3	IF	S 2	N	S 2	N	S 2	S 2
1.1	A 2 S 2	IF	N	N	S 2	S 2	N	S 2	S 3	N	N	A 3 S 3	S 2	A 3 S 2	N
		1.1			2.1			3.1			4.1			5.1	

(Upper tray)

	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
1.6	N	A 2	S 3	IF	N	N	N	N	N	IF	S 2	N	S 3	N	N
1.5	A 2 S 2	S 3	N	N	D	N	S 3	N	N	S 2	N	N	N	N	S 3
1.4	N	S 3	S 2	S 2	N	S 2	A 2	S 2	S 2	S 2	IF	N	D	N	N
1.3	N	S 2	A 2	N	N	S 2	N	N	N	S 3	N	N	S 2	S 2	S 2
1.2	N	D	S 3	N	IF	N	S 2	N	S 3	S 2	N	S 3	N	N	IF
1.1	IF	S 2	N	S 2	A 2 S 2	N	N	A 2 S 3	S 3	N	S 2	N	N	S 3	S 2
		1.1			2.1			3.1			4.1			5.1	

Figure 4.1.3. Morphological malformations per tray, position in tray and batch (B1-3) of the exposed embryos. (N: normal, A1: limbs, A2: head and A3: grossly abnormalities, S2: light and S3: strong neural tube curvature, IF: infertile eggs, D: dead embryo)

(Lower tray)

	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
1.6	N	N	IF	IF	S 2	N	N	N	N	N	N	N	N	N	S 2
1.5	IF	N	S 2	IF	N	S 3	A 1	N	N	S 3	S 2	IF	IF	N	N
1.4	D	IF	N	S 2	S 2	IF	N	S 2	S 2	N	N	IF	S 2	N	N
1.3	S 2	S 2	N	IF	S 2	S 2	S 2	N	N	S 2	IF	N	N	S 2	N
1.2	IF	N	S 3	N	IF	N	IF	S 2	N	IF	N	S 2	N	N	S 2
1.1	S 2	S 3	N	N	N	S 2	N	S 2	N	N	S 2	N	N	N	N
		1.1			2.1			3.1			4.1			5.1	

(Upper tray)

	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
1.6	S 2	S 2	N	N	IF	IF	S 2	N	N	IF	N	S 2	IF	S 3	N
1.5	N	N	N	IF	S 2	N	IF	S 2	N	N	S 2	N	N	N	S 2
1.4	IF	S 2	N	D	N	N	S 2	N	N	S 2	IF	N	S 2	IF	S 3
1.3	N	A 2	S 2	N	N	N	N	IF	D	N	N	IF	S 2	N	N
1.2	N	N	N	A 2 S 3	N	N	S 2	IF	N	IF	N	D	S 2	N	S 2
1.1	A 1 S 2	N	S 2	N	D	N	N	N	IF	S 2	N	N	IF	N	N
		1.1			2.1			3.1			4.1			5.1	

Figure 4.1.4. Morphological malformations per tray, position in tray and batch (B1-3) of the control embryos. (N: normal, A1: limbs and A2: head abnormalities, S2: light and S3: strong neural tube curvature, IF: infertile eggs, D: dead embryo)

Table 4.1.6. Least squares means (with standard errors) of egg and embryo weight per batch and group as well as the body weight per group of normal and abnormal embryos

	Group		Batch		
	Control	Radiated	1st	2nd	3rd
Egg Weight (g)	59.61±0.30[a]	59.52±0.28[a]	58.58±0.37[a]	59.55±0.35[ab]	60.56±0.35[b]
Embryo Weight (mg)	832 ± 19[a]	1001 ± 18[b]	740 ± 23[a]	597 ± 22[b]	1413 ± 22[c]
	Control		Radiated		
Normality	Normal	Abnormal	Normal	Abnormal	
Embryo Weight (mg)	849 ± 35[a]	475 ± 210[a]	1042 ± 35[b]	709 ± 108[a]	

[a–c] Means within batches or groups with different letters as superscripts are statistically significantly different (P <0.05).

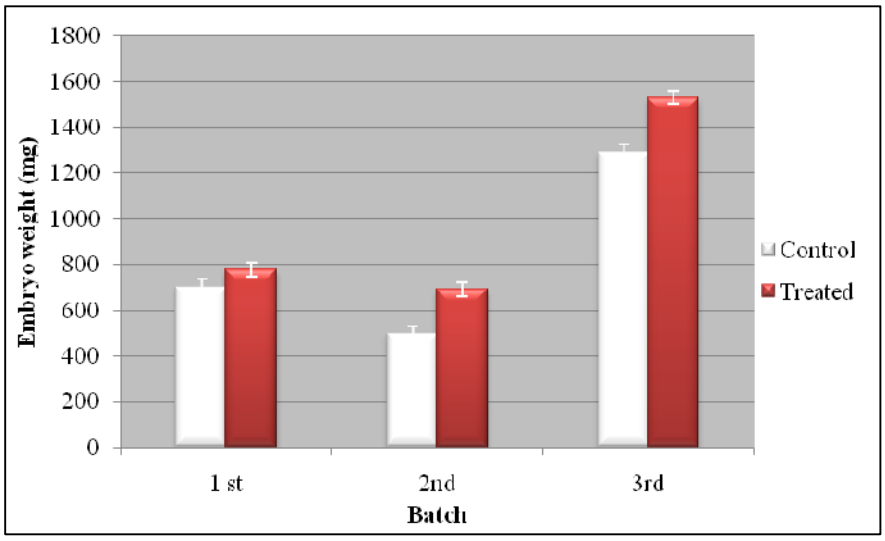


Figure 4.1.5. Least squares means of embryo weight per group and batch

4.1.5. Discussion

This is one of the few studies investigating the biological effects of low intensity 900 MHz radiation on chicken embryos during early development. While no apparent effect of EMF on embryo mortality was observed, exposed embryos clearly exhibited higher rates of morphological and spinal abnormalities. Various exposure scenarios as a result of combinations of frequency, pulse or continuous wave, as well as exposure duration have been employed so far. Most of these works are in accordance with the results obtained in the present study. Using the same frequency of GSM and apparently higher exposure over the whole incubation period, Youbicier-simo et al. (1998a,b,c) observed highest mortality rates especially during the third week of incubation. Using a lower frequency, i.e. 428 MHz, at a power density of 5.5 mW/cm^2 for more than 20 days Saito et al. (1991) also observed highest embryo mortality and/or teratogenic effects as well as delayed hatching in developing chicken embryos exposed to 8 h/day until the 5th day of incubation. At higher frequency (1.25 GHz) and power densities ranging from 0.1 to 1 mW/cm² Varga (1992),

cited by Thalau (2002) found no increase of chicken embryo mortality rates when the incubation temperature was reduced for compensation; however, all exposed embryos died when the power increased up to 1.5 to 3 mW/cm². Higher mortality rates in exposed chicken and turkey embryos were also observed by Hills et a1. (1974) using either 6 GHz at 0.05 and 0.2 mW/cm^2 or 2.45 GHz at an incident power density of 0.246-1.02 W/cm^2. Carpenter et al. (1960), cited by Durney et al. (1978) reported increased morphological abnormalities and mortality rates in 96-hr-old chicken embryos that were irradiated at 48 h *in ovo* with 2.45 GHz continuous wave (CW) microwave radiation at power densities of 200, 280 and 400 mW/cm^2 for 1-15 min. In contrast to these findings, Braithwaite et al. (1991) found no increase in embryo mortality for irradiated chicken eggs to 2.45 GHz (CW) at a SAR of 0.8 mW/kg and a power density of 3.6 mW/cm² for the total incubation period (21 days).

Findings of the present study also seem to be confirmed when using ELF pulsed magnetic field as well. Martin (1988) found more abnormalities in chicken embryos exposed to an extremely low frequency pulsed magnetic field during the first 24 h of incubation. Similar results are obtained during the first 48-49 h of incubation by Ubeda et al. (1994) and Farrell et al. (1997). Juutilainen and Saali (1986) found significantly high percentage of neural tube malformations and abnormal torsion embryos exposed at 16.7 Hz to 100 kHz during the first 48 h of development. On the contrary, Fargeix et al. (1988) exposed fertilized quail eggs to a 50 Hz 500μT magnetic field for the first 4–7 incubation days and observed no increase in mortality rate and/or number of abnormalities.

Although incubation temperature is one of the most crucial factors for successful embryonic development, there is a considerable lack of data with regard to temperature conditions of embryonic tissues exposed to EMF. As a consequence, it is often difficult to interpret any teratogenic effects caused by EMF, adequately (Talau et al., 2003). The latter authors suggested that hyperthermia during the early stages of chicken embryo development as a result of high exposure (SAR values between 1.45 and 10.44 W/kg) may have teratogenic and embryo-lethal effects. There is no clear evidence that using low intensity radiation, like that of the present study (average SAR values of 0.13 mW/kg), is expected to be associated with any marked rise of embryos' body temperature. This hypothesis was confirmed by dosimetry analysis where maximum temperature rise during radiation was calculated as high as 0.028 ºC. No actual measurements of the temperature conditions were, however, performed during experimentation. Whether this, theoretically, slight increase in temperature has any biological effect on developing embryos remains unclear. This issue is of particular importance, because if the adverse effects of EMF observed in the present study are not associated with thermal effects, another teratogenic mechanism might be involved. Apart from temperature measurements, another important point of dosimetry to be noted is that a full numerical analysis would require the simulation of egg-bearing trays at various angles between the horizontal and the 45^o position.

However, the horizontal position was only used, assuming that it represents a good proxy of the average exposure condition.

A further interesting finding of the present study was the increased body weight of the exposed embryos, whether normal or abnormal. This trend was observed in all of the three batches. The apparent range of response in embryos weight between batches observed in the present study could be related to various pre-incubation environmental factors such as temperature during transportation. Significant variations of the conditions to which the eggs were exposed before arrival at the laboratory are generally observed (Farrell *et al.,* 1997).

There are no relevant studies, with regard to effects on body weight, which could be directly comparable to our findings. Using ELF on kestrels eggs, Fernie *et al.* (2000) found that exposed embryos were structurally larger and longer than control embryos, under 60 Hz electrical current, a magnetic field of 30 microtesla (μT) and an electric field of 10 kV/m. EMFs used were equivalent to those experienced by wild kestrels when nesting under a 735-kV transmission line running at peak capacity. With regard to possible mechanisms of action, Fernie *et al. (*2000) have suggested involvement of growth factors such as thyroid hormones, growth hormone, and/or insulin-like growth factors. In mice, EMF exposed embryos to a 50 Hz sinusoidal magnetic field at 20 mT (rms) from day 0 to day 17 of gestation were found to be longer and heavier (Kowalczuk *et al.,* 1994). If such a scenario holds, then radiation might have some other effects on development not previously recognized.

Notably, the adverse effects of radiation observed in avian species are not confirmed in other species such as the mammals (rats and mice). A large number of studies have shown no significant effect on embryo mortality (resorptions, implantations), malformations and weight (22^{th} day of embryonic development) for a wide range of frequencies and dose levels. Jensh *et al.* (1982) exposed rats to 915 MHz at a SAR of 3.57 W/kg for 6 h per day. The same results were obtained by Jensh (1997) using 915 MHz (CW) & 2.45 MHz (CW) and a power flux density of 10, 20 or 30 mW/cm² for 6 h per day. Likewise, Magras and Xenos (1997) exposed mice to different frequencies of the RF and the UHF range (88.5 to 950 MHz) and power flux densities from 168 to 1053 nW/cm² at a SAR of 1.935 mW/kg. No detectable adverse effects of radiation in mammal embryos could be attributed to the protective environment provided by the mother during pregnancy as well as the movement of embryos in the field.

In this study, a total number of 360 eggs were used extended in 3 batches to ensure statistical power. Using low intensity exposure and small number of embryos in experimental studies may not always result in statistical significance and, thus, in no detectable adverse effects of EMFs. This implies the need for a more detailed examination of the power of the studies examining the effects of EMFs. Results of the present study have furthermore established no dose dependent effect on abnormality rates. This rather unexpected finding could be attributed to the relatively homogenous field applied here as

implied from the small variation in SAR values (~35%). Further studies are needed to elucidate the actual mechanisms causing the adverse effects on the development observed in the present study. However, there are indications from similar studies that it is not possible to make a direct extrapolation of these results to early human, or even mammalian, development.

4.2. Effect of Radiation on Behaviour

4.2.1. Effect on Feeding, Drinking Behaviour and Activity

The prenatal period is critically important for normal development. During this period the central nervous system (CNS) rapidly grows and is highly sensitive to environmental changes (Sun et al., 2010). Since there is tremendous electrical activity in neural transmission, the nervous system may be the most sensitive to the electromagnetic field exposure (D'Andrea et al., 2003b). Prenatal EMF exposure and subsequent assessment of postnatal neural or behavioral effects can be considered as one of the most sensitive systems for investigating possible EMF exposure effects (ICNIRP 2009). Studies have shown that prenatal exposure to magnetic fields (MF) may affect dopamine levels of mice offspring (Lee et al., 2001) and increase norepinephrine levels in chick embryos (Rajendra et al., 2004). Moreover, the exposure of rats to mobile phone EMF resulted in neuronal damage in the brain cortex, hippocampus, and basal ganglia (Salford et al., 2003). However, it is not clear whether effects on the biochemical level and/or damaged brain structures are also manifested at the behavioral level. Studies on rats and mice have shown that different levels of EMF exposure may affect learned behaviours (Dubreuil et al., 2002, 2003; Heynick and Merritt 2003; Cassel et al., 2004; Cosquer et al., 2005c; Juutilainen, 2005a,b) in the presence of hyperthermia.

Studies considering the effects of EMF during embryo development on later behavioral daily patterns such as the general activity as well as feeding and drinking are scarce in the literature. Studying typical daily behaviours under radiation within a context of an experimental set may reflect possible defects of the CNS. This is the first study designed to investigate whether prenatal exposure to EMF of 900 MHz affect typical broilers' activity as well as their feeding and drinking behaviour. A total number of 236 chicks and 8285 observations were used to ensure statistical significance.

4.2.1.1. Experimental protocol

Three batches of eggs of red broiler chickens were totally used to ensure statistical power resulting in an overall number of 360 eggs.

After hatching, the behaviour of 236 chicks was recorded 24 h/day, using four Panasonic PV120 video cameras fitted with a 3.6 mm lens. Each camera was placed in a fixed position in order to record the behaviour patterns in each room. The recorded data were stored in a digital video recorder equipped with a hard disk (TX168, Telexper Inc, USA), placed in the control room.

4.2.1.2. Behavioral observations

The following behaviour components from the day after hatching to day 7 were measured: a) number of birds feeding and drinking per room, in photos taken every 10 min, defined as any bird standing over a feeder or a drinker with its head towards the trough and b) the

activity of the birds per room, defined as the number of birds moving in the cells (not eating or drinking) in photos taken every 10 min. For clarity of exposition, the time was grouped into 4-h intervals. A total number of 8285 observations on 236 (110 radiated and 126 controls) chicks were obtained.

4.2.1.3. Statistical Analysis

Behavioural data were analyzed using a suitably tailored multivariate generalized linear mixed model. In particular, we used a trivariate logistic regression with correlated random effects. Specifically, we assumed the F_i~Bin (n, P_i^f), W_i~Bin (n, P_i^d) and M_i~Bin (n, P_i^m), where F_i, W_i and M_i is the number of birds feeding, drinking and for moving respectively, n the total number of birds per cell. $P_i^{f,d,m}$ the probability of a random bird feeding, drinking, moving respectively and Bin denotes the binomial distribution. The basic model was as follows:

$$\text{logit}\,(P_{ijklm}) = \mu + C_i + G_j + T_k + R_l + B_m + A_n + E_{ijklm}$$

Where logit (P) is the log (p/1-p) transformation, P_{ijklm} is the probability of a chicken feeding, drinking or moving, μ the overall mean, C_i the effect of the cell (i=1,…,4), G_j the effect of age (j=1,…,6), T_k the effect of the time of the day (k=1,…,6) where k is given in 4-hours interval, R_l the effect of the treatment (l=1,2), B_m the effect of the batch (m=1,…,3), A_n the random (animal) effect (n=1,…,236) and E_{ijkl} the residual error. We assumed different random effects for drinking, feeding and moving. In addition, we allowed for the within treatment random effects to be correlated in order to capture the dependence in the chicks' behaviour. Statistical analyses were performed using the WINBUGS 1.4.3 software (Bayesian inference Using Gibbs Sampling, Lunn et al., 2000).

4.2.1.4. Results

Table 4.2.1.1 shows the numbers of infertile eggs as well as the number of hatched chicks, dead chicks (during the experiment) and the number of chicks used. Chicks' body weight at hatching and at the end of experiment (at the 7th day of age) of the two groups (radiated and controls) is also given.

Note that the increased number of dead chicks in the radiated group was due to reasons not related with treatment. More specifically, one group of chicks (n=23) escaped from an experimentation room (number 1) and was thus lost, in the first day of the first batch. A result, the two groups included 126 and 110 chicks, respectively (Table 4.2.1.1). EMF exposure did not affect the chicks' weights either at hatching (43.6 *vs.* 43.3) or at 7 days of age (136 *vs.* 131).

Table 4.2.1.1. Number of infertile eggs, hatched chicks, dead chicks (during the experiment), total number of chicks used and the least squares means (±SE) of the chicks' weight at day 1 and 7 per treatment

	Total eggs	Infertile eggs	Hatched	Dead chicks	Used chicks	Weight (g) at day 1 (±SE)	Weight (g) at day 7 (±SE)
Control	180	17	140	14	126	43.6 ± 0.2	136 ± 2
Radiated	180	11	147	37	110	43.3 ± 0.2	131 ± 3

Table 4.2.1.2 shows the mean probabilities of the feeding, drinking and moving for the radiated animals and the controls. The two groups displayed the same pattern in all the three behaviours examined (Figure 4.2.1.1). However, radiated animals were visiting the feeders twice as many times as the controls (32% *vs.* 17%) and were captured to moving three and half times as many as the controls (4.3% *vs.* 1.3%). A slight increase in the visiting frequency of the drinkers was also observed (2.6% *vs.* 2.0%). Note that according to the observation protocol a bird not moving, or drinking or feeding is considered as resting. As a result, non radiated animals displayed higher resting times. Behavioural differences of the two groups by age and day-time are shown in Figure 4.2.1.1 where clear differences in particular for moving were manifested.

Highest numbers of feeder visits, for both radiated and non-radiated chicks, were observed during 9 to 12 am while most of the drinker visits followed that of the feeder visits time (at 1 to 4 pm). Chickens' activity started at the time of the feeders visits and ended about 8 pm (Figure 4.2.1.1).

Table 4.2.1.2. Mean probabilities (±SE) of a chick feeding (P_f), drinking (P_d) or moving (P_m) for the non-radiated and radiated group

	P_f	P_d	P_m
Control	0.170±0.004	0.020±0.002	0.013±0.005
	(0.16, 0.18)	(0.017,0.023)	(0.006,0.024)
Treatment	0.316±0.018	0.026±0.005	0.043±0.016
	(0.28, 0.35)	(0.018,0.037)	(0.02,0.08)

Numbers in brackets represent 95% confidence intervals.

Table 4.2.1.3 presents the correlations between the probabilities of observing a random chick feeding, drinking and moving. The three behaviours were positively correlated with correlations being as high as 0.104 between feeding and drinking and 0.31 between feeding and moving. The correlation between drinking and moving was (r=0.27).

Table 4.2.1.3. Correlation coefficients of the probabilities of a chicken feeding (P_f), drinking (P_d) or moving (P_m)

	P_f	P_d	P_m
P_f	1	0.10 (0.06,0.15)	0.30 (0.26,0.35)
P_d		1	0.27 (0.20,0.32)
P_m			1

Numbers in brackets represent 95% confidence intervals.

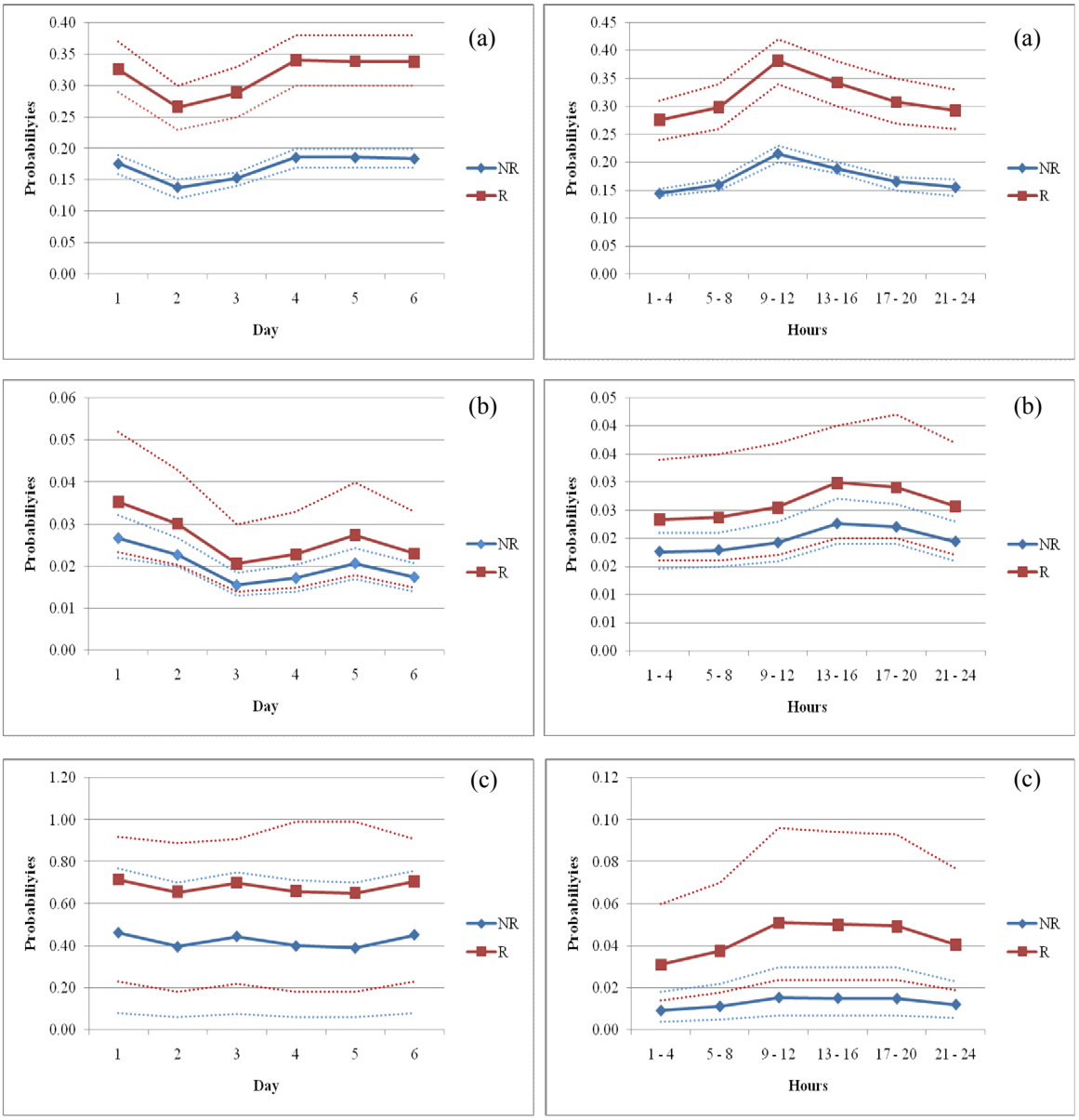


Figure 4.2.1.1. The probabilities (±SD) of the visits to the feeders (a), the drinkers (b) and the moving (c) of the exposed and control chicks by the day-time and the age

4.2.1.5. Discussion

This is the first study reporting effects of low dosing 900 MHz continuous wave on animals' typical behaviours such as feeding, drinking and general activity. The major finding of the study is the two groups of animals follow the same behavioural patterns, however, EMF exposed chicks tend to display higher activity levels (3.5-4 times) and visits to the feeders and drinkers. Given that these differences were observed in every batch it can be concluded that these behavioral differences may be attributed to only the EMF exposure.

Whether differences at the behavioural level were associated with increased feed and water intake cannot be addressed within the current experimental set because no direct measurements of the two parameters in question were undertaken. Higher activity is reasonably expected to result in increased energy expenditure. Animals fed under an *ad libitum* feeding regime are reasonably expected to compensate higher energy expenditure by increasing feed consumption. This is seems to the case here since no significant differences at body weight (at the age of 7 days) were detected.

Additionally, the higher activity of the exposed chicks may also have led to increased feeders and drinkers' visits, since there were positive correlations between the three probabilities calculated (see Table 4.2.1.4). High activity of the exposed chicks is also reasonably expected to result in higher number of drinkers' visits due to increased respiratory rates (panting) to expel surplus heat (Fairchild and Ritz 2009). Moreover, chick feeding and drinking are also expected to be positively correlated, since there is a direct relationship between feeding and drinking in birds (Symeon et al., 2010).

During the latest years the control of the poultry feeding behaviour is increasingly understood and it is clear that parts of the hypothalamus are involved in the initiation and termination of feeding (Appleby et al., 2004) as well as in drinking (Butler and Hodos 2005). Hypothalamus is also reported to be involved in the control of many functions, including voluntary movement, motivation, attention, working memory and learning (Lee et al., 2001). All the above indicate that EMF may affect critical brain structures such as the hypothalamus. Since no examination of the involved brain structure(s) was performed in the present study, no direct inference on the underlying mechanisms can however be made. It should be noted that the present EMF related findings cannot be directly compared with others since there no relevant studies in the literature reported.

The present study also reports interesting results with regard to behavioural patterns of young chicken. Generally, feeding, drinking and moving were found to be age dependent with more intense activity observed during the first day after hatching (see Figure 4.2.1.1). Increased activity for the newly hatchlings may be attributed to the fact that, chickens lack the innate ability to recognize food, but they possess a strong propensity to peck at small particles, both nutritious and non-nutritious (Hogan, 1973). The chick is receiving nutrition from the remains of the yolk sac for the first few days after hatching. As the chicks explore

and learn to respond to the consequences of consuming different items the pecking at food increases (Appleby et al., 2004). As in the case of food, young chicks are initially unable to recognize water, just have a tendency to peck at shiny surfaces (Appleby et al., 2004). Within day-time, highest numbers of visits to the feeders and the drinkers were observed during morning hours (Figure 4.2.1.1). This is probably due to the absence of feeding during the night and the refill of the feeders which occurred daily in the morning hours. Nielsen et al. (2003) found that delivering meals to feed restricted broilers increased both feeding and moving behaviours. Highest numbers of visits to the feeders and the drinkers in the morning hours in broilers are also reported by Symeon et al. (2010). At day 7 (last day of recording) feeders and drinkers visits decreased. This decrease could be attributable to the fact that as the birds grow and get heavier, they spend more time lying or sitting and visiting the feeders and drinkers less often (Savory and Lariviere, 2000; Weeks et al., 2000). Age reduced walking and feeding behaviours in broilers is also reported by Bokkers and Koene (2003) and Kristensen et al. (2007).

Following an observational approach such as that employed here may be not adequate for understanding the control of complex behaviour, particularly because animals do not normally just have one behavioural option available but instead many simultaneously. Since there are no any other reports concerning the effect of EMF radiation on feeding and drinking behaviour and the activity of the chicken, further research is necessary to confirm the results of the present study. In view of the existing data on neuro-physiological and behavioural responses to low-level EMF exposures, thus, animal studies have an important role in determining the influence of exposure on central nervous system function (Repacholi 1998).

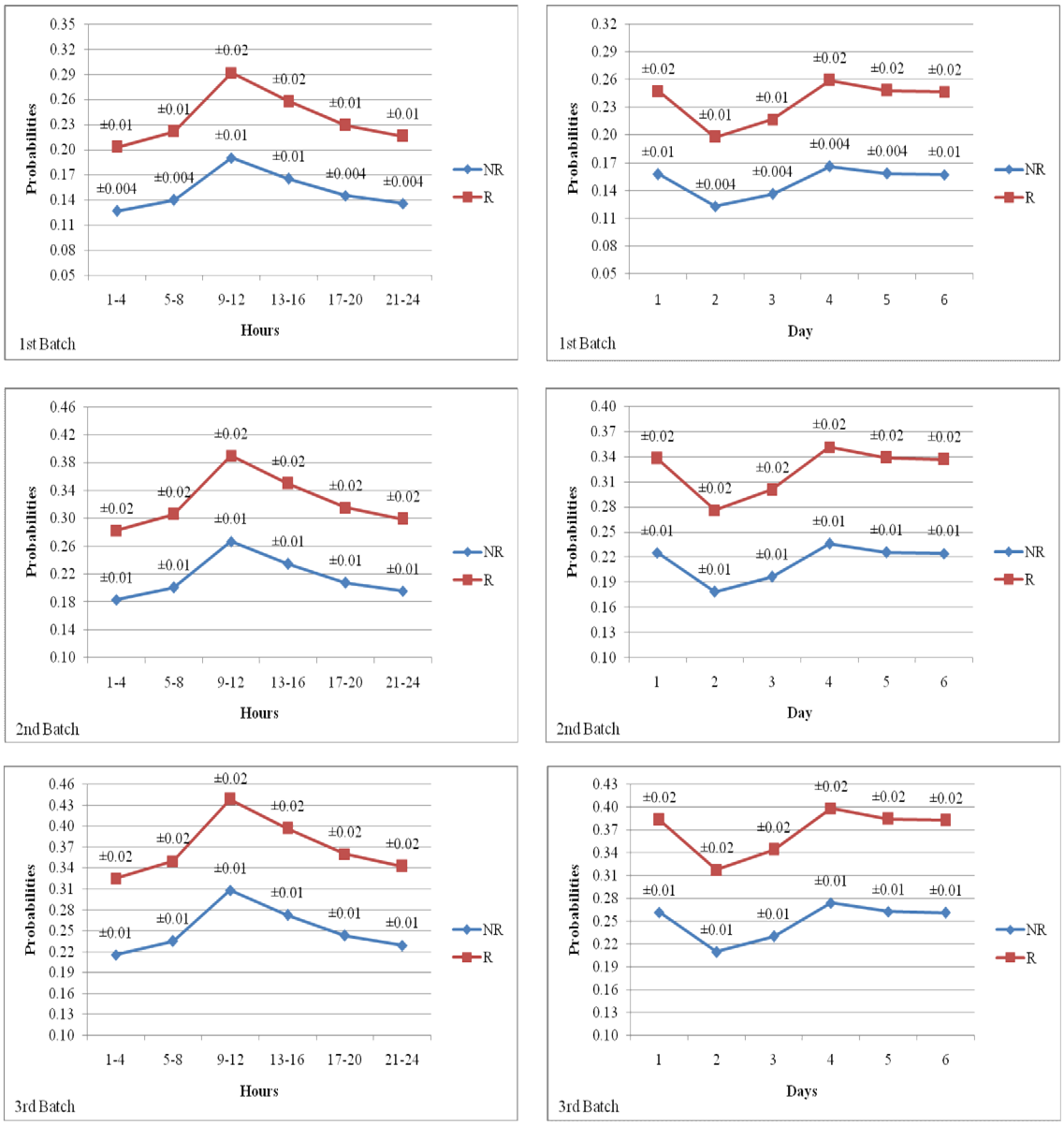


Figure 4.2.1.2. The probabilities (±SD) of the visits to the feeders per batch of the exposed and control chicks by the day-time and the age

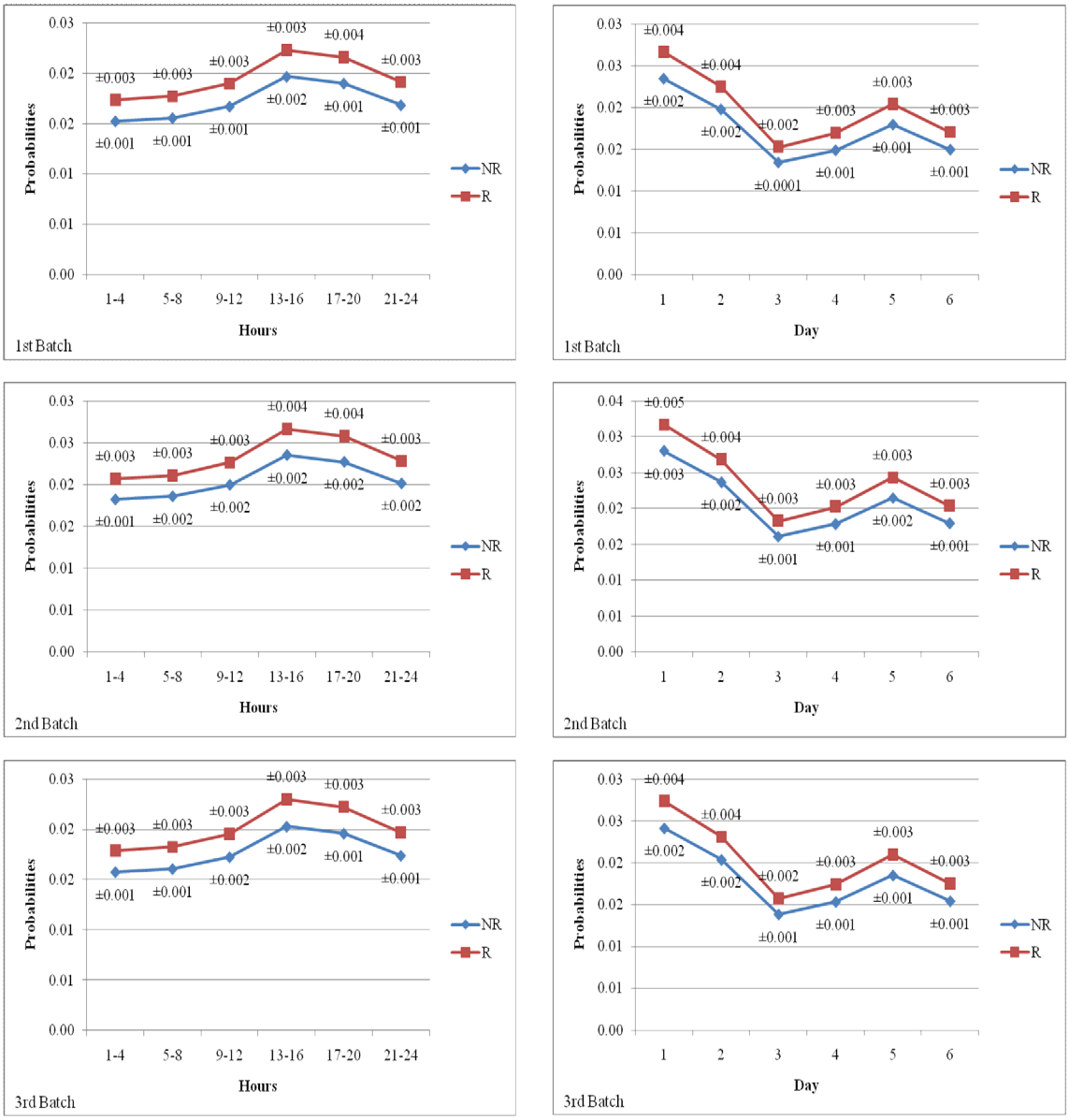


Figure 4.2.1.3. The probabilities (±SD) of the visits to the drinkers per batch of the exposed and control chicks by the day-time and the age

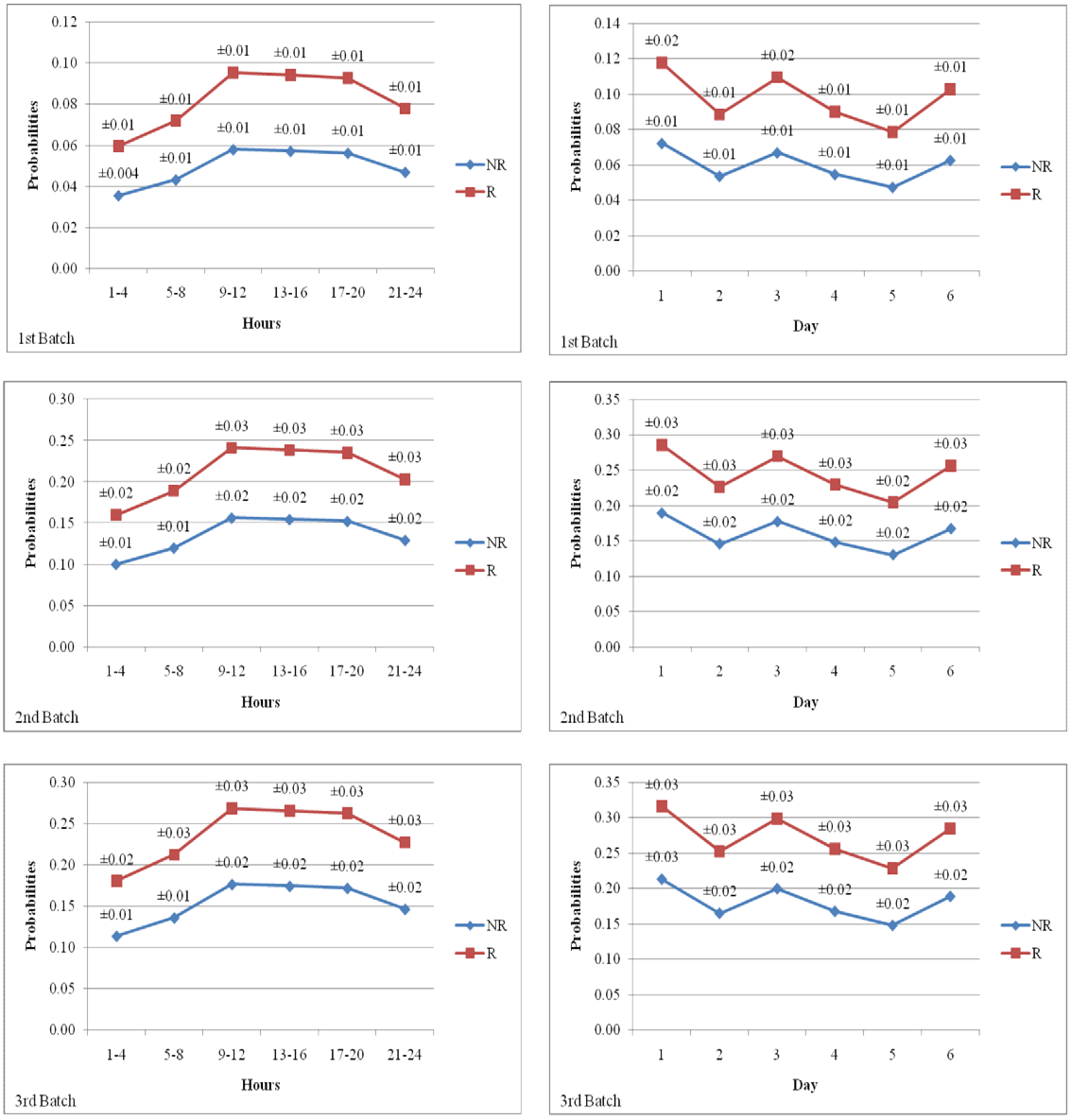


Figure 4.2.1.4. The probabilities (±SD) of the moving per batch of the exposed and control chicks by the day-time and the age

4.2.2. Effect of Radiation on Spatial Learning and Memory

Various maze types such as the radial, T-maze, Y-maze, multi-chamber and the Morris water maze have been extensively used to quantify the effects of various treatment(s) on hippocampal-dependent spatial-based learning and memory of experimental animals. In particular, the Y-maze apparatus is used extensively in preference and spatial learning tasks. The procedure has been used to evaluate the aversiveness of different restraint methods in farm animals such as sheep (Rushen, 1986) and cattle (Grandin et al., 1994; Pajor et al., 2003). With the same apparatus, learning behaviour can be experimentally reinforced using various spatial cues, most frequently food rewards. The food reward may be present at various locations of the arms and the task is for food-deprived subjects to locate it. When repeating the task several times, the changes in time (latency) and distance (path) to reach the target are used as indicators for the learning and memory abilities of the subjects. This procedure has been successfully employed for testing the spatial learning and spatial memory in avian species like chicken (Krause et al., 2006; Boers et al., 2009).

A large body of research has so far been published using various maze architectures and the electromagnetic fields as the treatment factor. Some studies have documented impaired and/or disruptive learning and/or memory as a result of EMF exposure (Nittby et al., 2008; Fragopoulou et al., 2009; Narayanan et al., 2009) while no biological effects of this nature were reported (Sienkiewicz et al., 2000; Dubreuil et al., 2002, 2003a; Cassel et al., 2004; Cosquer et al., 2005b). Given that results of both experimental and epidemiological studies are inconclusive, further studies are needed to investigate possible adverse biological effects of EMF, in particular that of the 900 MHz system, during crucial developmental phases such as early embryogenesis. Therefore, the present study investigates, for the first time, whether 900 MHz exposure during early embryonic development affects later chickens' spatial learning and spatial memory using simple Y-maze tasks.

4.2.2.1. Experimental protocol

In total, 240 eggs (in 2 batches) of red broiler chickens were randomly assigned in two incubators: one serving as the control (no radiation) and the other as the exposure unit.

Before performing the final trials, three series of pre-experiments were carried out following the same protocol in order to gain experience with animal treatment, behaviour and the times required to accomplish the Y-maze tasks.

Two identical Y-mazes were constructed according to Krause et al. (2006) after 50% reduction of the dimensions to meet animals' size (Figure 4.2.2.1). The mazes, the slide door and the start box were made of naturally colored plywood to avoid any unfamiliarity or fearfulness. In the present study the Y-mazes were not covered with wire mesh, since the chicks were not old enough to be able to escape and to be handled easily during the experiment. The arms of the Y-maze were covered with straw instead of wood shaving; since in the pre-experiments we noted that, wood shaving distract the chicks' attention

during the experimentation.

Generally, the experimentation Y-maze protocol of Krause et al. (2006) was followed. At the age of 15 days, 40 chickens were randomly selected in batches of five from each group (radiated and non-radiated). In total, 80 chicks were examined. In each Y-maze trial, five radiated and non-radiated chicks were kept overnight in the two Y-mazes in the same room, excluding the start box. This procedure was applied to habituate animals to the new environment thus reducing stress during the forthcoming trials as well as to allow for free exploration. The experimental room was evenly illuminated so that no shaded areas were present in the Y-maze, to avoid fearfulness. The Y-maze straw was replaced before a test started, and cleaned between chicks.

Commercial feed and water were offered *ad libitum* for the chicks overnight at the neutral position (N) of the maze (Figure 4.2.2.1). During chicks' overnight staying in the maze, feed was provided in the same red bowls, used as feeders during the growing period (from 2nd to the day of the test) and was removed 1-2 hours before the test. Animals were kept out of the Y-maze during the test in the testing room. The experiments started between 10:00 and 10:30 and lasted until 13:00 and 13:30. At the beginning of the test, animals were placed in the start box (Figure 4.3.1) and the slide door was opened immediately during the learning and memory trials. Each chicken was tested individually.

4.2.2.2. Spatial learning task

In total, three different positions (T1 to T3) per arm were used during the learning phase. Subjects were submitted to one acquisition and one retention trial per position. The aim of the acquisition trials was animals to learn to choose the right arm by finding the food reward when this was placed in progressively distant locations in the Y-maze arms (Figure 4.2.2.1). Retention trials were used to assess the ability of animals to retrieve recently acquired spatial information. At the first training position (T1), chicks were able to see the food bowl from the start box. Immediately after a chick pecked the feed, it was transferred back to the start box and started the retention trial with the bowl placed in the same position. After chicks had successfully pecked twice at T1 position, the bowl was transferred to the second position (T2) where the chicks could not see the food bowl from the start box. After chicks had also successfully pecked twice at T2 position, the bowl was transferred to the position T3, where the chicks had to enter the arm of the Y-maze in order to see the bowl (Figure 4.2.2.1). The trial was aborted when the chick did not leave the start box within five minutes, and/or the time in the Y-maze exceeded ten minutes without succeeding. When a chick aborted four trials, it was excluded from further trials. During the whole experimentation, feed was provided in the familiar red colored bowl and the rewarded sides for the Y-maze were randomized across individuals.

4.2.2.3. Spatial memory task

Subjects were submitted to two spatial memory tasks. The first test was carried out directly after completing the learning phase. Here, the food bowl was placed in a hidden place (M, Figure 4.2.2.1), close to T3 position. Animals were required to find the bowl in the M position after having learned the rewarded arm during the previous phase. Since this test was employed immediately after the learning phase, it can be viewed as a short term spatial memory test (M1). After completion of the short term memory test, animals were kept in the test room, out of the Y-maze, for 24 hours until the second memory test (M2). Here, animals were required to find the hidden bowl that was placed in the same position during the M1 test. This test was conducted to assess long term spatial memory performance. In total, two trials per test were carried out in order to avoid effect(s) of chance. During all trials, time to reach target i.e. latency was recorded manually and it was treated as the dependent variable that reflects learning and memory performance. The number of errors i.e. the number of entries of the wrong arm was also recorded during trials and used as indicator(s) of impaired performance.

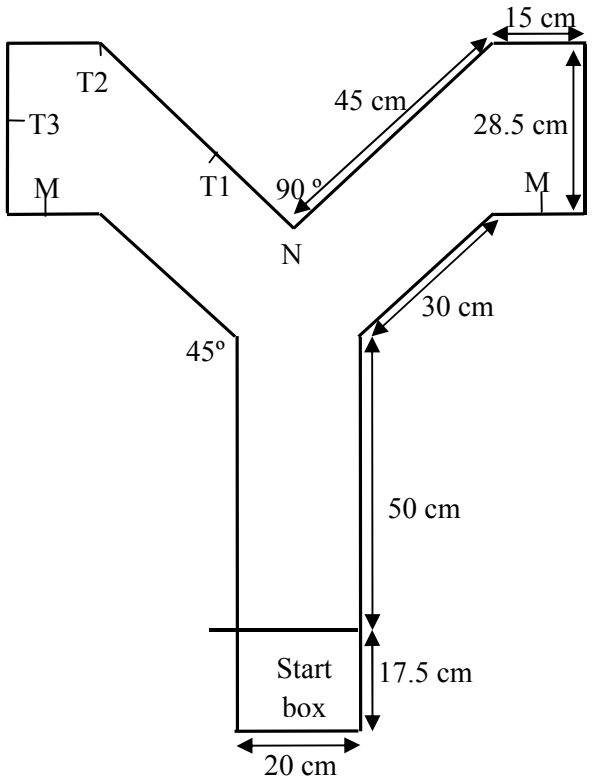


Figure 4.2.2.1. Dimensions of the Y-maze and food bowl positions in the Y-maze during training (T1– T3), memory trials (M), and neutral overnight food position (N)

4.2.2.4. Statistical Analysis

Several plots of latency data showed a highly non-symmetric nature of the observed times. Most time distributions remained non-symmetric even after a variety of transformations. Descriptive statistics of times can be found in Table 4.2.2.1. Because of departure of normality, the medians, rather than the means, are presented. Apart from positional latencies, pooled latency during the learning as well as the memory tasks was also calculated. Since the data are times to an event, such as reaching a destination, survival analysis (often called analysis of time-to-event data, see Collett 2003) was employed. Several different parametric distributions were employed and the Weibull distribution was found to be the most suitable for our data. Therefore, we assumed that each time, say t_i, is distributed as

$$t_i \sim W(r,\mu),$$

where $W(r,\mu)$ denotes the Weibull distribution with shape parameter r and rate parameter μ. Notice that the Weibull reduces to the exponential distribution when $r = 1$, while $r>1$ indicates increasing hazard rates and $r<1$ results in decreasing hazards. The two groups (radiated and non-radiated) were compared using the median latency. In the case of the Weibull distribution the median is given by $(\log_2 e^{-\beta})^{1/r}$ where $\beta = \log(\mu)$. Finally, censoring is very common in survival analysis but there was no censored observations in our experiment. This analysis was performed using the WinBugs ver 1.4 software (Lunn et al., 2000). Numbers of errors were expressed as proportions per group. Estimated proportions were further subjected to power analysis in order to find the minimum number of animals per group needed to detect significant differences. During power analysis, probabilities for the type I (false positive rate) and Type II (false negative rate) errors were set as high as $\alpha=0.05$ and $\beta=0.20$, respectively. This analysis was performed using SAS (2002).

4.2.2.5. Results

4.2.2.5.1. Spatial learning task

Two chicks from the radiated and non-radiated group did not fulfill the learning criteria (see 4.2.2.2) and were thus excluded from analysis. Exposed animals exhibited prolonged latency (40.09 *vs.* 18.3 sec) at the first training position (T1) when compared to controls (Figure 4.2.2.2b). This difference was large due to increased latency of the exposed animals during the acquisition trial and not the retention trial (Figure 4.2.2.3). No statistically significant differences in latency were observed between the two groups for leaving the start-box and for performing the tasks at positions T2 and T3 (Figures 4.2.2.2b and 4.2.2.3). This result was valid for both the acquisition and the retention trials. Pooling latency for leaving the start box (Sall) and the learning phase (Tall) resulted in no statistically significance between groups, despite the fact that radiated subjects displayed delays at completing the learning tasks (110.6 *vs.* 92.4 sec) when compared with the controls (Figure 4.2.2.2a). Overall, when contrasted to controls, exposed subjects were delayed at reaching

the food bowl at any learning position. This delay was, however, not statistically significant. Generally, both groups displayed shorter latencies during the retention trials and this trend was more pronounced for the exposed subjects. It should be, however, noted that the above differences were not statistically significant. The number of errors during the learning phase for the two groups is shown in Table 4.2.2.2. In total, when contrasted to controls, radiated animals made more errors (9 *vs.* 4) especially during the acquisition trials at positions T1 and T2 (Table 4.2.2.2). Considering the number of animals per group (n=40) these error numbers translate to errors proportions as high as 0.23 and 0.10 for the radiated animals and the controls, respectively. Power analysis showed that the minimum sample size per group should be as high as 170.

4.2.2.5.2. Spatial memory tasks

All animals, irrespective of being radiated or not, displayed comparable latencies during the short memory task (M1) (Figures 4.2.2.2b and 4.2.2.3). Radiation resulted, however, in statistically significantly prolonged latency (13.42 *vs.* 8.32 sec) in the long term memory test (M2). This delay was due to significantly delays for the exposed animals during the retention trials (Figure 4.2.2.3). Likewise, exposed subjects exhibited increased latency (13.4 *vs.* 8.3 sec) when pooling memory data (M1 & M2) (Figure 4.2.2.2a). The total number of errors during memory tasks is also shown in Table 4.2.2.2. In total, radiated animals made more errors (13 *vs.* 8) during the memory tasks with most (n=8) of the errors recorded during the retention trials. When errors were expressed as proportions the above numbers were equal to 0.33 and 0.20, respectively. Here, power analysis suggested a minimum number of 180 animals per group.

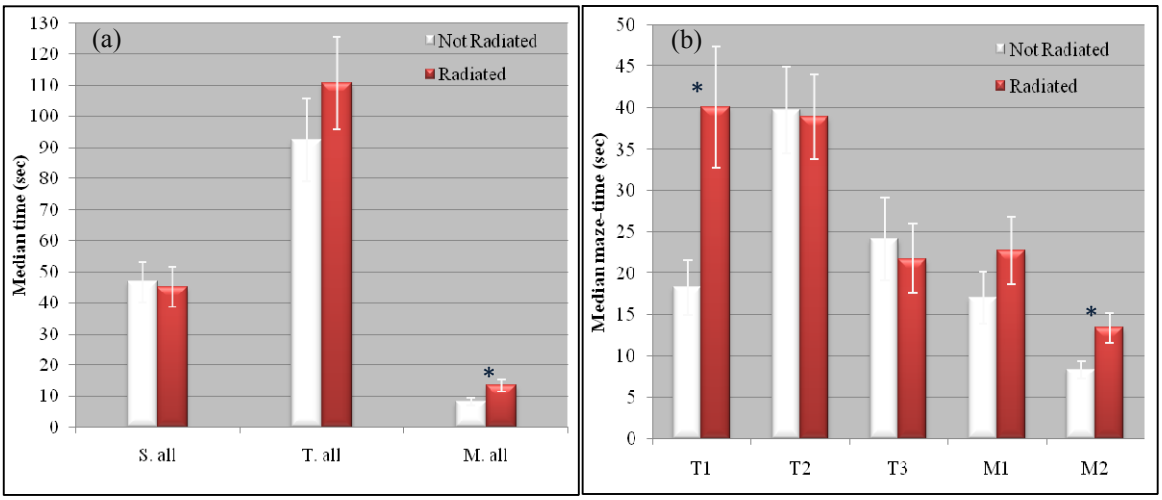


Figure 4.2.2. 2. Median latency ± S.E., per group. (a) leaving the start box (S all), completing the learning tasks (T all) and the memory tests (M all). (b) per learning position (T1-T3) and the two memory tests (M1&M2). See text for details. (* indicates $p<0.05$)

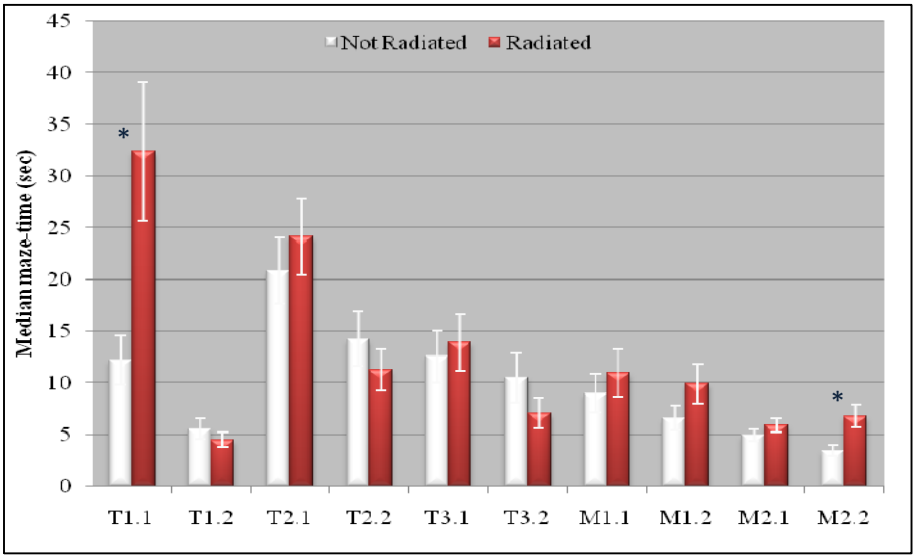


Figure 4.2.2.3. Median latencies ±S.E., per group at the three learning positions (T1-T3) and the two memory tests (M1&M2) per acquisition and retention trial. See text for details. (* indicates $p<0.05$)

Table 4.2.2.1. Descriptive statistics of latencies during the various tasks per group

	Median		1st Quartile		3rd Quartile	
	Control	Radiated	Control	Radiated	Control	Radiated
S1.1	15.63	11.72	2.20	1.99	35.12	33.68
T1.1	9.85	12.61	5.01	7.44	27.67	86.47
S1.2	2.24	3.55	1.35	1.42	5.63	12.86
T1.2	3.63	4.84	2.67	4.27	5.81	6.27
S2.1	2.43	2.89	1.36	1.63	5.66	6.55
T2.1	14.31	29.20	7.83	9.04	39.81	44.36
S2.2	2.13	2.53	1.54	1.34	6.33	5.29
T2.2	7.88	10.26	5.23	6.20	25.55	19.76
S3.1	2.14	2.82	1.58	1.87	5.36	5.91
T3.1	6.93	9.48	4.77	5.84	18.58	24.73
S3.2	1.61	2.54	1.18	1.47	3.32	7.39
T3.2	6.30	6.65	4.79	5.05	8.47	9.22
SM1.1	1.60	2.24	1.11	1.31	4.60	6.08
M1.1	5.48	5.98	4.09	4.42	8.21	15.82
SM1.2	1.38	2.25	1.01	1.32	2.00	5.73
M1.2	5.24	5.76	4.04	4.21	6.28	9.96
SM2.1	1.46	2.22	1.21	1.28	2.34	3.10
M2.1	4.15	5.05	3.69	3.46	5.81	7.68
SM2.2	1.13	1.18	0.79	0.96	1.48	1.43
M2.2	3.56	4.33	2.85	3.07	4.83	6.02

S1.1: start-time during T1 acquisition trial; S1.2: start-time during T1 retention trial; the same applies to S2-S3 for T2-T3 positions and to SM1-SM2 for the memory trails; T1.1: acquisition trial at first learning position; T1.2: retention trial at first learning position. The same applies to T2-T3 positions. M1.1: first trial of the short term memory test; M1.2: retention trial of the short term memory test; M2.1: first trial of the long term memory test; M1.2: retention trial of the long term memory test.

Table 4.2.2.2. Number of errors per group during the learning and memory tasks

	Learning							Memory				
	Total	T1.1	T1.2	T2.1	T2.2	T3.1	T3.2	Total	M1.1	M1.2	M2.1	M2.2
Control	4	0	0	0	3	1	0	8	0	0	4	4
Radiated	9	2	0	2	3	1	1	13	0	2	5	6

T1.1: acquisition trial at first learning position; T1.2: retention trial at first learning position. The same applies to T2-T3 positions. M1.1: first trial of the short term memory test; M1.2: retention trial of the short term memory test; M2.1: first trial of the long term memory test; M1.2: retention trial of the long term memory test.

4.2.2.6. Discussion

The two main results of this study were the demonstration of impairment of exploratory behaviour as well as of the long term memory after EMF exposure. The deficits were manifested in terms of latency and number of errors during the learning and the long term memory tasks. With regard to learning, prolonged latencies for the exposed subjects was only shown during the acquisition trial at the first learning position (T1) and not at any of the subsequent learning trials. Prolonged latency during a spatial learning test may easily be attributable to impaired exploratory behaviour. There are, however, other interpretations as well related to the method(s) used to assess the spatial learning performance. While the Y-maze represents a reliable method for testing spatial learning it investigates various components of exploratory behaviour, simultaneously. The latter behaviour is defined by both cognitive and motivational factors such as neophobia and anxiety. Given that the chicks were offered a whole night to habituate and explore the Y-maze environment, neophobia could reasonably be ruled out since the Y-maze was not a novel environment for animals. Thus the most plausible explanation relates prolonged latencies to the emotional status (such as anxiety, fear, and agility) of birds. There is evidence for that, instance, anxiety affects spatial learning performance in rats (Kucuk et al., 2008). Although not systematically recorded to allow for presentation in results, we have observed that exposed chicks displayed higher number of vocalizations and reduced motivation towards the reward. Both could be indicators of increased anxiety levels in the exposed chicks. More detailed behavioral measurements (bird vocalizations, extreme screaming, number of droppings), suitable apparatus (the elevated plus maze) and reliable indicators (such as hormone levels) that reflect the emotional status are needed to address this central issue in detail. It should be emphasized, here, that special attention was paid to maintain all animals in normal conditions under no stressors or other factors that could have affected their performance and/or emotional status during and between trials.

The suggestion that EMF exposure indirectly impaired the spatial learning performance through modifications of the emotional status of birds could be further verified by closer examination of the rest latencies. Indeed, during all the following learning trials both groups displayed comparable latencies, the T1 retention trial, included. Although exposed

subjects tended to make more errors particularly during the first two acquisition trials, comparable latencies between the groups can be considered as reliable indicators of equally expressed successful learning. This conclusion could be further confirmed after completion of the short term memory trials where animals were challenged to find the hidden bowl for the first time. A further interesting observation obtained herein deals with the learning pattern of animals. On the ground that animals showed shorter latencies during the T3 trials it can be concluded that animals had adequately learned the Y-maze task after completion of the trials at the first two positions.

While the EMF exposure led to no significant systematic impairment of the spatial learning ability it clearly deteriorated the memory function. Memory defects were manifested in only one of the two memory tasks the one that tested for long term memory ability (24 hours after learning). Here exposed subjects showed prolonged latencies (pooled data) mostly due to marked delays in the retention (retrieval) trial. In other words, exposed were able to acquire the bowl position but they were delayed in retrieving this information. The critical brain structures reported to be involved in most types of memory appear to be the cortex and the hippocampus (Martinez and Kesner, 2007). The exact mechanism(s) of the memory deficit reported here could not be investigated as it was beyond the scope of the present study; they could, however, be associated with dysfunction and/or damages in these brain areas.

No direct comparison of the number of errors could be performed here. We have thus calculated the corresponding error rates (proportions) and then performed power analysis. Interestingly, the exposed constantly displayed higher error proportions (at the order of 0.13) during both the learning and the memory tasks. Power analysis showed that this difference becomes statistically significant when the minimum sample size rises up to 170-180 animals (per group). Apparently, this number is well beyond the ones used here implying low study power. This raises the question whether proportion errors could be used as reliable indicators of impaired performance, at least following the present experimentation protocol. Latency and the distance to accomplish the tasks appear to be more attractive indicators for assessing performance especially when simple tasks with the Y-maze are considered.

Detailed examination of the existing literature has shown that some studies are in agreement with the present results while others are not. However, it should be noted that the present EMF related findings cannot be directly compared with others since there are no other studies with chicken and more specifically with chicks radiated during embryogenesis. Using the 900 MHz frequency and various exposure protocols, some authors (Sienkiewicz et al., 2000; Dubreuil et al., 2002, 2003) have shown no effect(s) of radiation on spatial memory, in mice or rats. In contrast, similar findings such as those obtained herein are reported in recent studies (Fragopoulou et al., 2009; Narayanan et al., 2009). Furthermore, Yamaguchi et al. (2003) showed that exposed rats displayed

significantly higher erroneous choices during the T-maze task. In that study, however, higher frequency (1439 MHz) was used, while problems were evident when exposure intensity resulted in elevated body temperature. Heating of critical tissue(s) as a result of EMF exposure seems to be of crucial importance as a number of studies have documented (e.g. D'Andrea et al., 2003; Barnes and Greenebaum, 2007). International guidelines limit the local SAR to a maximum of 2 (ICNIRP, 1998) or 1.6W/kg (IEEE, 1992). Since the SAR calculated in the present study is very low (0.13 mW/kg) it is reasonably not associated with significant temperature rise (the worst-case temperature rise was calculated as high as 0.03ºC). However, this remains unconfirmed due to lack of temperature measurements inside the incubator during irradiation.

All the studies reported above used rats and mice as the experimental animals while their experimental protocol foresaw exposure during postnatal stages. A different approach was followed here: chicks were radiated during early embryogenesis (first quarter) and defects were detected at the age of 2 weeks with no involvement of maternal protective mechanism(s). This approach is entertaining the idea that the EMF related effects are perhaps attributed to the radiation. Whether defects detected at 15 days of age are permanent or not remains unknown since no trials were carried out at later age(s). Although the present study suggests adverse effects of EMF, these results cannot be directly extrapolated to human and/or other mammalian species since different species follow different patterns of learning and memory consolidation. In view of the low-intensity of the radiation used and the lack of dosimetric measurements the results are difficult to interpret; therefore they should be treated with caution and they warrant a further investigation.

4.3. Effect of Radiation on Immune Response Ability

The immune system is a very complex one, built up of a large number of cell types (B- and T-lymphocytes, macrophages, natural killer cells, mast cells, Langerhans cells, etc.) with certain basic defense strategies. It has evolved during an enormously long time-span and is constructed to deal with its known enemies. The modern electromagnetic fields are unknown enemies. However, EMFs disturb immune function through stimulation of various allergic and inflammatory responses, as well as effects on tissue repair processes (for a detailed review, see Johansson, 2009).

For the immune system of the avian, the post-hatching period is crucial, since the chick is abruptly exposed to a wide range of environmental antigens and is not supplemented with further maternal immunity (Davison et al., 2008). The yolk sac produce the first generation of macrophages, since cells with macrophage-like morphology have been found in the yolk sac of embryos incubated for 2.5-4.5 days (Cuadros et al., 1993). In chicken embryos, macrophages with phagocytic activity have been observed as early as embryonic incubation day 12 in the liver and day 16 in the spleen (Jeurissen and Janse, 1989). Elicited macrophages can be obtained in day-old chicks (Qureshi et al., 2000) demonstrating that this part of the innate immune system is functional at hatching. The macrophages play an important role in host defense and immune system. Its major functions include nitric oxide production, phagocytosis, and secretion of cytokines. Moreover, heterophils (neutrophils) represent the first line of cellular defense against invading microbial pathogens. In the present study it was investigated whether exposure of chicken eggs on 900 MHz electromagnetic radiation during the first 5 days of embryonic development affects immune response ability of exposed animals.

4.3.1. Experimental protocol

In total, 240 eggs (in 2 batches) of red broiler chickens were randomly assigned in two incubators: one serving as the control (no radiation) and the other as the exposure unit.

Blood samples were collected at 1(n=16), 6 (n=26) or 10 (n=16) days-old, from 66 chicks that randomly selected after hatching. Blood macrophages and heterophils were isolated and the following parameters were determined: total membrane-bound urokinase plasminogen activator (u-PA) and superoxide (SO) anion production on heterophils (h) and microphages (m) as well as nitric oxide (NO) production by macrophages.

4.3.2. Blood collection, cell isolation

Blood macrophages and heterophils were isolated using published procedures (Scorneaux and Shryock, 1998; Politis et al., 2003). Heparinized venous blood (7.5 ml; 30 IU of heparin per ml) was mixed with an equal quantity of RPMI-1640 medium (Sigma Chemical Co., St. Louis, MI, USA) and layered on to 10 ml of a preconstituted discontinuous Histopack density gradient (specific gravities 1.077/1.119 g/cm^3; Sigma), then centrifuged at 500*g* for 45 min. Cells from the two interface layers (1.077 and 1.119) were collected

separately and washed twice in RPMI-1640. Macrophages and heterophils were respectively the predominant cells in the 1.077 and 1.119 layers (Scorneaux and Shryock, 1998). Contaminating red blood cells were lysed by addition of 20 ml of sterile distilled water, followed by addition of sterile saline solution (2.7% NaCl) to restore isotonicity. Cells were pelleted by centrifugation (200*g*, 10 min) and resuspended in RPMI-1640 medium containing 10% foetal bovine serum (FBS; Sigma) at a concentration of 1 x 10^7 cells/ml. To increase the purity of macrophages obtained from the 1.077 interface layer, cells suspended in RPMI-1640 medium were plated in 100-mm Petri dishes. After 2 h of incubation, media containing the non-adherent cells were removed and fresh media were added. After 24 h of incubation, cells (macrophages) were removed by gentle scraping and used as described below. Heterophils were used immediately after the Histopack density gradient isolation step. Macrophage and heterophil purity and viability were monitored under microscopy and were always high (>95%).

4.3.2.1. Determination of total cell-associated u-PA activity

Total cell-associated u-PA activity (intracellular + membrane-bound) was measured in phorbol myristate acetate (PMA)-activated and resting macrophages and heterophils. Preliminary experiments indicated that maximal stimulation of the u-PA system in chicken-derived macrophages and heterophils was achieved following treatment with PMA (65 μM) for 30 min. Cells (2 x 10^6/ml) were resuspended in 500 μl of Hanks balanced salt solution (HBSS; Sigma) containing HEPES (20 mM) without or with PMA (Sigma; 65 μM). After incubation for 30 min at 37 ºC, cells were washed three times with HBSS. Cells were then lysed by addition of 500 μl of sodium bicarbonate (1 mM), centrifuged at 12000*g* for 3 min, aliquoted, and stored at -80 ºC. Activity of u-PA in aliquots of lysed macrophages and heterophils was determined following previously published procedures (Politis et al., 2001, 2002). The assay system utilizes the enzymatically active u-PA present within the lysed cells (macrophages or heterophils) to convert exogenously supplied plasminogen to active plasmin. Plasmin, so produced, is subsequently allowed to attack the chromogenic substrate Val-Leu-Lys-*p*-nitroaniline adjacent to Lysine and liberate the free chromophore *p*-nitroaniline. In this system, changes in colour are directly related to plasmin concentrations, and, therefore, indirectly to u-PA activity (Politis et al., 2001; 2002).

4.3.2.2. Determination of superoxide production

Superoxide anion production as a direct indicator of respiratory burst activation was measured by use of the superoxide dismutase-inhibitable reduction of ferricytochrome C (Politis et al., 1996). Cells (2 x 10^6/ml) suspended in 1.4ml of HBSS were pre-incubated in the presence or absence of 10 μl of superoxide dismutase (3 mg/ml; Sigma) for 2 min. Then, 0.1ml of type-IV cytochrome C (35 mg/ml; Sigma) was added followed by addition of 1.5 ml of PMA (130 μM). After 10 min, the reaction was terminated by placing tubes on ice, followed by centrifugation at 1500 g for 20 min. The supernatant was collected and the absorbance was measured at 550 nm. The amount of superoxide produced was calculated as

the difference between the amounts reduced in the presence and absence of superoxide dismutase.

4.3.2.3. Determination of nitric oxide production

Nitric oxide production was measured using published procedure (Lee et al., 2009). Briefly, chicken monocytes were cultures in triplicate at 1x 10^7 cells/ml in 96-well plates (100 µl/well) at 41 ºC and 5 % CO_2 for 24 hr with (1.0 µg/ml of recombinant chicken interferon-γ [IFN-γ])(Song et al., 1997) or negative controls (PBS)(Lillehoj et al., 2004). Culture supernatants (100 µl) were transferred to clean 96-well plates, mixed with 100 µl of Griess reagent (sigma), incubated for 15 min at room temperature, optical density at 540 nm (OD_{540}) was measured, and the nitrite concentration was determined using standard curve generated with known concentrations of sodium nitrite.

4.3.2. Statistical analysis

Data were subjected to multivariate analysis of variance (MANOVA) treating group, age at sampling and batch as the class variables, using Wilks' lamda test. Prior to analysis, all parameters were logarithmically transformed [log(x)+1] to meet MANOVA assumptions. This analysis was performed using SAS (2002).

4.3.3. Results

The results are presented as least square means ± SE, log transformed values. Figure 4.3.1 shows the effects of radiation exposure on superoxide anion production by heterophils and macrophages. The radiation resulted in lower values in superoxide anion production by heterophils (1.11 *vs.* 1.2) and macrophages (0.93 *vs.* 1.06) from exposed chicks than from control chicks. The effects of radiation exposure on membrane-bound urokinase plasminogen activator (u-PA) of heterophils and macrophages are presented in Figure 4.3.2. There was a significant decrease in u-PA of heterophils (0.23 *vs.* 0.37) and macrophages (0.08 *vs.* 0.22) of exposed chicks compared to control chicks. The effect of radiation exposure on nitric oxide production by macrophages is presented in Figure 4.3.3. Radiation exposure decreased nitric oxide production by macrophages (1.48 *vs.* 1.71) compared with control chicks. The differences in macrophages and heterophils parameters were statistically significant (Wilks' lamda=0.40, F-value(DF)=9.47(32), $p<0.001$). Figures 4.3.4 and 4.3.6 show the effect of sampling day on superoxide anion production and u-PA of microphages and heterophils. Age (p=0.931) and batch (p=0.858) had no statistically significant effect on the heterophils and macrophages parameters studied.

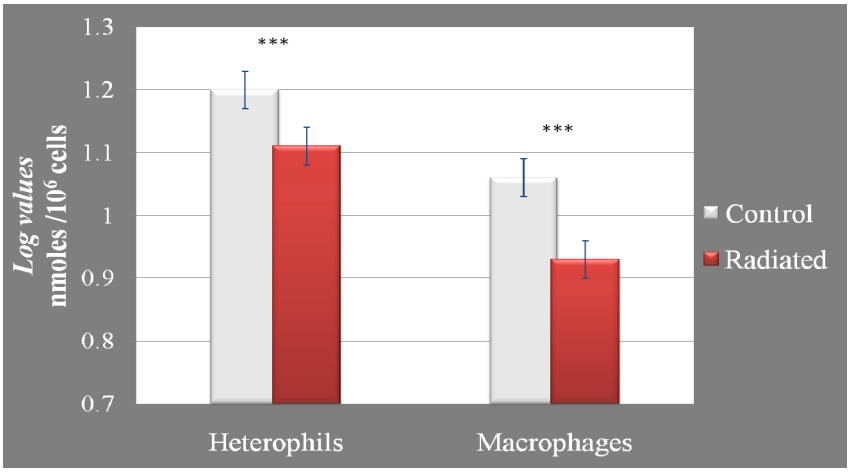


Figure 4.3.1. Effect of radiation exposure on superoxide anion production by heterophils and macrophages (least squares means ±SE, log transformed values)(*** indicates $p<0.001$)

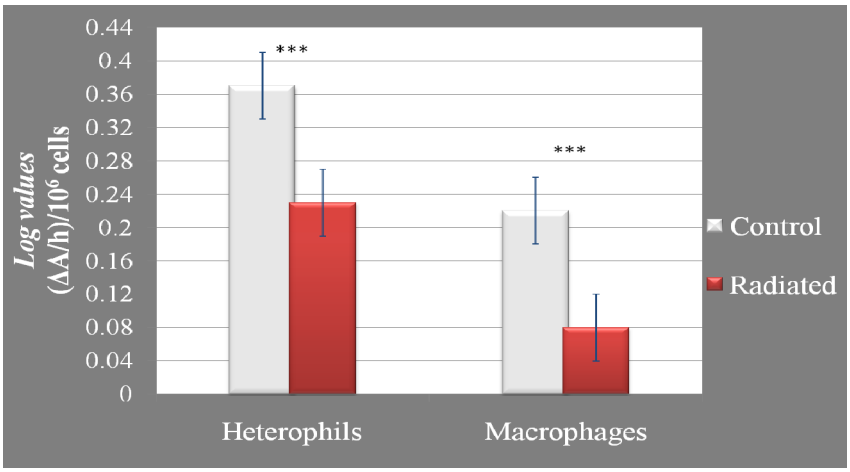


Figure 4.3.2. Effect of radiation exposure on membrane-bound urokinase plasminogen activator (u-PA) of heterophils and macrophages (least squares means ±SE, log transformed values)(*** indicates $p<0.001$)

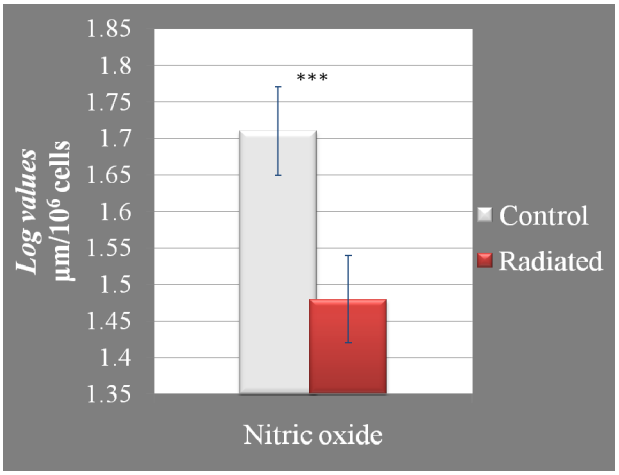


Figure 4.3.3. Effect of radiation exposure on nitric oxide production by macrophages (least squares means ±SE, log transformed values)(*** indicates $p<0.001$)

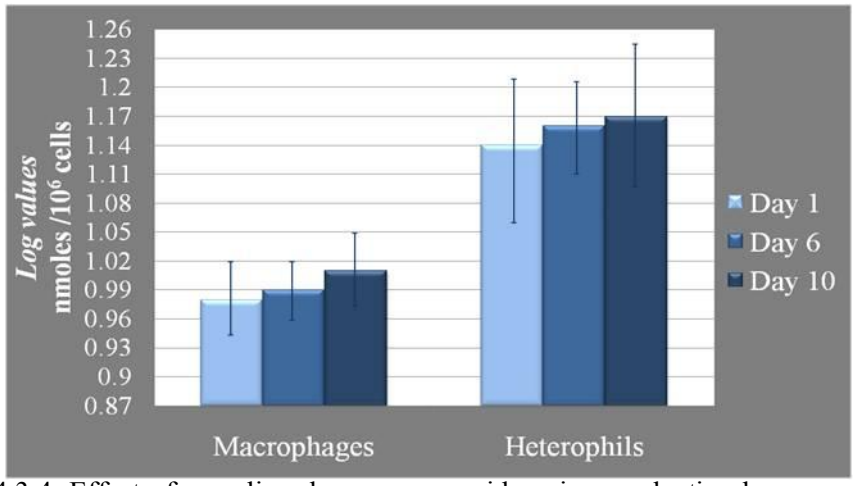


Figure 4.3.4. Effect of sampling day on superoxide anion production by macrophages and heterophils (Least squares means ±SE, log transformed values)

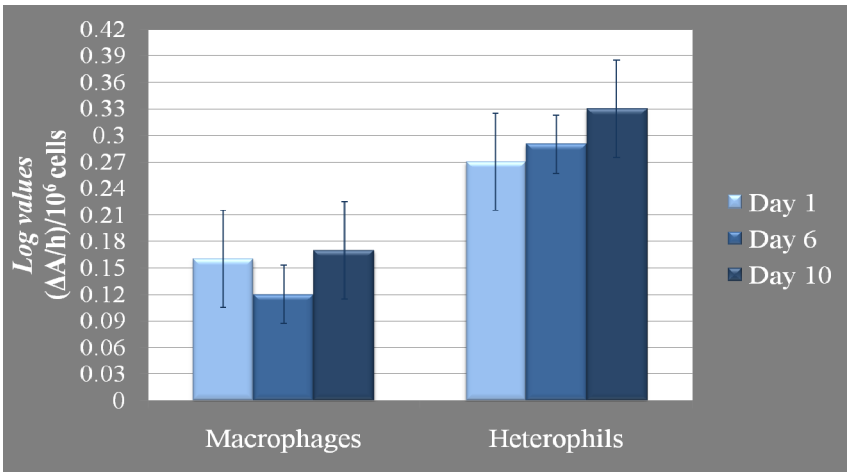


Figure 4.3.5. Effect of sampling day on membrane-bound urokinase plasminogen activator (u-PA) of macrophages and heterophils (least squares means ±SE, log transformed values)

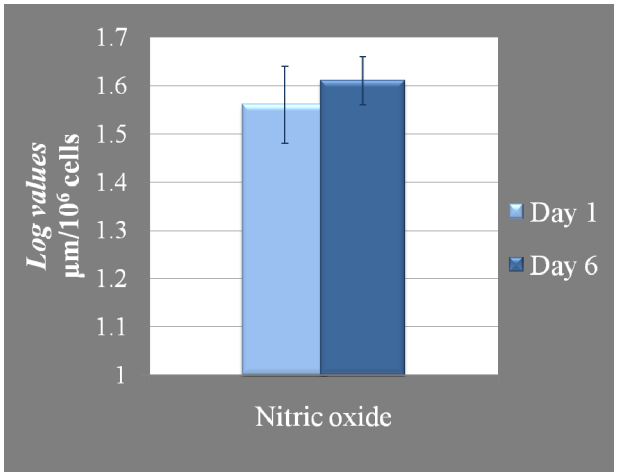


Figure 4.3.6. Effect of sampling day on nitric oxide production by macrophages (least squares means ±SE, log transformed values)

4.3.4. Discussion

This is the first study reporting effects of low dosing 900 MHz continuous wave exposure during early embryonic development on post-hatching immunosuppression of young chicken. The findings of the present study are that macrophages and heterophils from EMF exposed chicks produced significantly lower amounts of nitric oxide, superoxide and u-PA on their plasma membrane than the corresponding cells isolated from controls. The nitric oxide (NO) reduction may be of particular importance since it is a mediator of the antimicrobial and tumoricidal activities of macrophages. Moreover, NO is an important intra- and intercellular signaling molecule that acts in many tissues to regulate a diverse range of physiological and cellular processes including immune system and cardiovascular system functions (ICNIRP, 2009).

It should be noted that the present EMF related findings cannot be directly compared with others since there no relevant studies in the literature reported. However, in a study using the same EMF frequency, Irmak et al. (2002) studied the influence of the EMF of a digital GSM mobile telephone 900 MHz on oxidant and antioxidant levels in rabbits (2Wpeak power, average power density 0.02 mW/cm^2, for 30 min day, for 7 days). They found a significant decrease in serum NO levels after the exposure. The authors suggested that, EMF may destroy NO by generation of superoxide anion and/or EMF causes a reduction in production of NO. However, the NO is a protective molecule in part because of its ability to react with and inactivate superoxide anion in EMF-exposed rabbits. Moreover, nitric oxide reacts with superoxide anion at a rate that is three times faster than the dismutation of superoxide anion by superoxide dismutase SOD (Irmak et al., 2002).

Moreover, the effects of lower EMF exposure, than used here, on processes involving NO have been investigated by two studies. Vasodilatation of arterioles in the webbing of the feet of anaesthetized South African clawed toads was found to be increased under exposure to pulse modulated 10 MHz EMF (Miura and Okada, 1991). This effect was abolished by the addition of an NO synthase inhibitor (Miura et al., 1993). These authors also reported that the exposure of a supernatant fraction of homogenized rat cerebellum to a similar pulsed 10 MHz EMF resulted in an increase in concentration of NO and cyclic guanosine monophosphate (cyclic GMP). Additionally, Morimoto et al (2005) used a similar exposure setup (10 MHz, 50% duty factor, up to 8 mW/kg) and found that exposure caused a decrease in the thrombin-induced production of endothelin-1 (ET-1), a potent vasoconstrictor, and ET-1 mRNA by cultured endothelial cells. The effect on ET-1 production was abolished by addition of a nitric oxide synthase inhibitor, which was interpreted by the authors as evidence that the inhibitory effect of RF exposure is mediated, at least partly, via an NO related pathway.

There are some studies dealing with the effects of 900 EMF at different intensities on immunity systems using several other parameters. Radon et al. (2001) found that mobile phone EMF radiation had no effect on immune function in on eight healthy young men.

The men were exposed to 900 MHz fields, pulsed with 217 Hz, pulse width 217 μs. An antenna was positioned 10 cm behind the subject's head. The power-flux density was approximately 1 W/m^2, and the maximum local SAR in the head (averaged over 10g tissue) was 0.025 W/kg. The study was designed to assess the effects of the RF fields on salivary levels of melatonin, cortisol, neopterin, and IgA. Neopterin and IgA are substances that are part of the immune system. Moreover, Gatta et al. (2003) found that 900 MHz GSM-modulated radiation for 1, 2, and 4 weeks (2 h/day) in a TEM cell to a SAR of 1 or 2 W/kg had no substantial effects on immune function in mice. Moreover, two studies evaluated the effects of EMF radiation on mouse peripheral lymphocytes and on B-cell peripheral differentiation and antibody response in mice (Gatta et al., 2003; Nasta et al., 2006). Mice were exposed or sham-exposed to GSM900 at whole body SARs of 1 or 2 W/kg for up to 4 weeks. The authors concluded that T- and B-lymphocytes were not substantially affected by exposure to EMF.

Most studies indicate that the most consistently observed EMF induced changes in immune function and hematology are transient and associated with temperature rise of 1 ºC or more (ICNIRP, 2009). There is no clear evidence that using low intensity radiation, like that of the present study (average SAR values of 0.13 mW/kg), is expected to be associated with any marked rise of embryos' body temperature. This hypothesis was confirmed by dosimetry analysis where maximum temperature rise during radiation was calculated as high as 0.028 ºC. Therefore, the defects detected in this study on the immune parameters are not associated with thermal effects; another mechanism of effect could be involved.

Overall, reduced levels of important macrophages and heterophils parameters studied here (nitric oxide, superoxide anion, u-PA) may suggest a serious adverse effect of the EMF on the immune system. However, no direct extrapolation of the present findings to early human, or even mammal development could be made. Further studies are needed to elucidate the exact mechanism of function of EMF on the immune system.

Part C
General Discussion and Conclusion

Part C: General Discussion and Conclusion(s)

The main objectives of this research was to examine the effects of 900MHz EMF low intensity exposure on developing embryos by assessing the embryonic development, aspects of the post hatching behaviour as well as the levels of some immune parameters.

The teratogenic effect of the low dosing EMFs exposure detected in the present research seems not to be thermal related, since the temperature rise and the variation due to radiation was classified as not significant. Further detailed studies focusing on precisely measuring the embryos' temperature in situ are needed to verify this hypothesis. Whatever the real scenario is, it should always kept in mind that the generally considered very low dosing used in this research may indeed have detrimental effects in small embryos especially in the absence of any maternal protective mechanism. If this is the case, the effect of even lower dosing with more detailed dosimetry analysis needs to be investigated.

Effects of EMFs on post-hatching behavioral aspects were clearly detected in the present study. Although exposure resulted in modifications of the daily behavioural pattern as well as impairment of the memory function, these findings do not suggest particular risks nor do they conclude which parameters should be tested in experimental studies. Behaviour is a complex phenomenon correlated to the electrophysiological and neuroanatomical complexity of the nervous system. Behavioural deviations and/or memory deficits may primarily imply defects in the CNS. In case of behavioural manifestations, there are additional factors e.g. hormones, neurotransmitters as well as genetic and environmental factors that are being involved. Thus, more neurological and behavioral studies are needed to clarify how EMF exposure during early embryogenesis may affect behaviour and the exact mechanism(s) of action. These studies could provide some answers as to whether defects are primarily caused by hormones changes or/and damages in the structure and/or the impaired function of some parts of the CNS (e.g. hypothalamus). Knowledge on the exact mechanism of action is apparently of pronounced importance also for humans and other mammalian species. This is also valid for the memory function. Several well designed studies are being carried out to investigate the way of action of EMFs on spatial learning and memory of animals and this knowledge could be useful for humans who are apparently the heaviest users of mobile phones. Therefore, hypothesis-driven research on plausible mechanisms is imperative for the evaluation of the biological effects of low intensities EMFs.

Another important aspect of consideration raised by the present findings was the reduced levels of the immune parameters studied (immunosuppression), implying reduced immune response ability of the exposed animals. This hypothesis also needs to be further investigated since immunosuppression does not necessarily translate to increased disease susceptibility under exposure to common or specific pathogens.

The present study detected some defects after continuous exposure during the first five days of development and suggested some possible study extensions. Such extensions may include: the use of the EMF pulsed energy, different EMF levels, longer exposure periods and/or exposure in different or the whole developmental stage(s). There seems also to be a large requirement for studies including combinations of different EMFs sources. Since radiation dosimetry in chicken embryos may considerably differ from that in humans and/or other mammals, the present findings cannot directly be extrapolated to humans or other mammals. However, similar studies are useful for investigating basic mechanisms and as screening tests to detect potential risks for other species, humans included. Although findings are species specific, they may ring the alarm towards the heavy use of some human population parts such as, for instance, the pregnant women.

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