

# **Impact assessment modelling of the matter-less stressors in the context of Life Cycle Assessment** Cucurachi, S.

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# A review of the ecological effects of radio-frequency electromagnetic fields (RF-EMF)

# **A review of the ecological effects of radio-frequency electromagnetic fields (RF-EMF)**

#### **Based on:**

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#### **Abstract**

*Objective: This article presents a systematic review of published scientific studies on the potential ecological effects of radiofrequency electromagnetic fields (RF-EMF) in the range of 10 MHz to 3.6 GHz (from amplitude modulation, AM, to lower band microwave, MW, EMF).*

*Methods: Publications in English were searched in ISI Web of Knowledge and Scholar Google with no restriction on publication date. Five species groups were identified: birds, insects, other vertebrates, other organisms, and plants. Not only clear ecological articles, such as field studies, were taken into consideration, but also biological articles on laboratory studies investigating the effects of RF-EMF with biological endpoints such as fertility, reproduction, behaviour and development, which have a clear ecological significance, were also included.* 

*Results: Information was collected from 113 studies from original peer-reviewed publications or from relevant existing reviews. A limited amount of ecological field studies was identified. The majority of the studies were conducted in a laboratory setting on birds (embryos or eggs), small rodents and plants. In 65% of the studies, ecological effects of RF-EMF (50% of the animal studies and about 75% of the plant studies) were found both at high as well as at low dosages. No clear dose–effect relationship could be discerned. Studies finding an effect applied higher durations of exposure and focused more on the GSM frequency ranges.*

*Conclusions: In about two third of the reviewed studies ecological effects of RF-EMF was reported at high as well as at low dosages. The very low dosages are compatible with real field situations, and could be found under environmental conditions. However, a lack of standardisation and a limited number of observations limit the possibility of generalising results from an organism to an ecosystem level.We propose in future studies to conduct more repetitions of observations and explicitly use the available standards for reporting RF-EMF relevant physical parameters in both laboratory and field studies.*

#### **Keywords**

Radiofrequencies, EMF, biodiversity, electromagnetic field exposure, ecological effect, mobile telecommunication\*\*

*\*\*Abbreviations. ELF-EMF = extremely low field electromagnetic field; CW = continuous wave; MW = microwave; PW = pulsed wave* ; *GSM* = global system for mobile communications; UHF = ultra-high frequency; VHF = very-high frequency; DECT = digital *enhanced cordless telecommunications; UWB = ultra wide band; AM = amplitude modulation; FM = frequency modulation; GTEM = gigahertz transverse electromagnetic cell; UMTS = universal mobile telecommunications system; CDMA = code division multiple access; TDMA = time division multiple access; WCDMA = wideband code division multiple access; Wi-Fi = Wireless Fidelity; WLAN = wireless local area network; WiMAX = worldwide interoperability for microwave access.*

# 1 Introduction 1.1

#### Scope

Anthropocene is a term which has been proposed for the current epoch, due to the global environmental effects of increased human population, the economic and industrial development and to the deep overall domination and contamination of the human over the environment (Crutzen and Stoermer 2000; Zalasiewicz et al. 2010). Amongst the many changes, a radical modification has taken place also in the exposure of beings to man-made electromagnetic fields. A continuous, chronic, exposure to a wide range of modulated radiofrequency electromagnetic fields (RF-EMF) burdens all species and groups across the globe.

In terms of mechanisms, the WHO confirms that to date the accepted health effects ascribable to RF-EMF are caused by temperature elevation (van Deventer et al. 2011). Though, several studies have identified possible effects of RF-EMF on organisms, no alternative effect mechanisms have been confirmed to date. Most of the literature has focused on human and occupational health, largely based on animal model studies under laboratory conditions and test subjects exposed to lower frequencies of the spectrum (i.e. extremely low field, ELF -EMF). From the available studies, it became clear that, especially under higher dosages, effects of RF-EMF may be observed. As a response, occupational and human health threshold values and guidelines, proposed by international organisations (ICNIRP 2010), have been increasingly incorporated into national regulations of states (EU 2011). However, results are still not conclusive and there is still some uncertainty about the low dosages and non-thermal effects applied in some studies which did find an effect, and the overall quality of the setup of research in the field. The ever increasing use of RF-EMF in the cellular phone ranges (e.g. GSM, UMTS) and the newer forms of wireless communication (e.g. WiFi, WLAN, WiMAX), which are rarely present in the available studies, require new investigations which will look at possible short and longterm effects.

Over time several monographs and reviews have been compiled as to the biological effects of RF-EMF on humans, and on animals (see among others: Michaelson and Dodge 1971; NCRP 1986; Bryan and Gildersleeve 1987Adair 1990; Verschaeve and Maes 1998; Juutilainen 2005; Balmori 2009; Pourlis 2009; ICNIRP 2010). While of great relevance for the understanding of the phenomenon, these studies lack in the consideration of potential effects which may directly affect other organisms or ecosystems, because of the very limited attention which is usually received by the adverse ecological effects of RF-EMF.

# 1.2

#### Problem definition

Limited research and reviews have focused on investigating the possible ecological effects of RF-EMF. It can be argued that many human-related biological studies using animal models (e.g. rats, rabbits, etc.) may provide also relevant information about potential ecological effects. Many ecological endpoints (e.g. fertility, reproduction, growth) studied at the level of the individual animal, are also crucial from an ecological point of view. Ecology is, one of the sub-disciplines of biology, which studies all living organisms (including human beings), at all organisational levels (i.e. from the smallest molecular system to the largest ecosystem levels). Ecology is the scientific study of the distribution and abundance of organisms and the interactions that determine distribution and abundance (Begon et al. 2005). Those interactions refer to the abiotic and the biotic environment. By definition ecology focuses on the higher organisational levels of populations, communities and ecosystems. Despite the lack of information of the ecological effects of RF-EMF, following this definition, it is then plausible to link biological studies with ecological endpoints at the individual animal level to ecological interpretations at a higher organisation level.

This field of research is of crucial importance for the understanding of mechanisms of interaction between complex ecosystems and the environment. Animal studies have still been identified as a major research agenda point by the WHO (Van Deventer et al. 2011). The WHO stated that high priority in the field should be given to research on the effects of RF-EMF on development and behaviour, on aging and reproduction of animal subjects. The result of these studies might be ecologically interpreted, because they include ecologically relevant endpoints.

As far as strictly ecological research has been conducted, it was mostly presented in the form of non-peer-reviewed grey literature. A review of Balmori (2009) is the only oriented at the effects of RF-EMF on wildlife. However, the contribution by Balmori (2009) has some methodological issues. The criteria for the screening of the literature or the rationale for the inclusion or exclusion of relevant articles have, in fact, not been presented. The review is also missing a detailed analysis of the selected papers (e.g. of the duration of exposure, of the physical parameters, etc.) and it includes only studies finding a significant effect of RF-EMF.

# 1.3

# Research focus

Evidence suggests that there is a large lacuna in research regarding the ecological effects of RF-EMF. The aim of this contribution is to conduct a scientifically sound review of potential ecological effects of RF-EMF. Using the definition and guidelines provided in the clinical sector by Higgins and Green (2006), a systematic review on potential ecological effects of RF-EMF was performed.

The study focuses on the range from 10 MHz to 3.6 GHz (i.e. from AM to the lower band MW EMF), using a transparent, comprehensive and objective substantive review approach and analysis of the available scientific literature on the ecological effects of RF-EMF. The literature search was based on a clear and objective research strategy (see section 2) performed using two databases: ISI Web of Knowledge and Google Scholar. The experimental, physical and biological parameters, which were provided by the selected papers were classified and analysed to look for trends and possible links between dosages and effects.

Papers evaluating ecological endpoints as part of biological investigations were selected with a focus on higher organisational biological levels: ecosystem, community, and species. As much as possible also biological studies, present in biological reviews or in relevant papers, if relevant from an ecological point of view, were included in this review and analysed.

A complete review of the biological literature was beyond the scope of this paper. However, laboratory studies on animals and plants that investigated biological endpoints can still provide information relevant for the ecological level.

First in Section 2 the methods are described, in Section 3 the general results are presented, and in Section 4 the specific results are given for each of the analysed groups (i.e. birds, insects, other vertebrates, other organisms, and plants). The final sections (Section 5 and Section 6) provide a synthesis, with possible links between dose-response relationship, the setup and dosage of the studies, together with general conclusions and recommendations.

# 2

Review method

#### 2.1

#### Criteria of literature search

The literature research was conducted, in the second half of 2011, using ISI Web of Knowledge and Google Scholar databases. Publications on ecological effects of RF-EMF on all relevant endpoints on non-human organisms and parts of organisms (e.g. tissues, cells) were taken in consideration. Additional scientific articles published after December 2011 were added upon indication and suggestion of experts.

In order to maintain a high scientific standard for this review paper, only publications which were peer reviewed were considered. As criterion for peer review, the presence of the publication in the ISI Web of Knowledge was used. As for papers present only on Google Scholar an expert selection was made based on the ecological relevance and quality of the studies. The criteria used were based on quality criteria defined by relevant methodological reviews (Repacholi and Cardis 1997; Stam 2010). Repacholi and Cardis (1997) suggest that reviews should take into consideration only literature published in scientific peer-reviewed journals to guarantee a selection of articles free from methodological deficiencies and with rigorous analysis and conclusions. They also suggest care when dealing with peer-reviewed reports not published in scientific journals as well as conference abstracts, which are usually not peer-reviewed. In this review, only peerreviewed papers have been selected. In a limited number of cases peer revision could not be guaranteed: the case of a study conducted by Harst et al. (2006) on honey bee (Apis mellifera), in which no sufficient information could be found on the review procedure of the relative journal, and the studies by Van Ummersen (1961; 1963), Carpenter et al. (1960), and Clarke (1978) which were reported by the (peer-reviewed) review by Bryan and Gildersleeve (1988).

The literature search was limited to the range of frequencies from 10 MHz to 3.6 GHz. Papers on the biological and ecological effects of ELF- EMF in the range of  $1$  Hz – 100 KHz (e.g. power line fields) were not considered. Date of publication was not used as a restriction and all publications falling within the above selection criteria were analysed, including those which did not find significant effects.

The keywords used in the literature research process are reported in the appendix to this review. Two main categories were defined: RF-EMF specific keywords (e.g. GSM, DECT, 1800 MHz) and ecological keywords (e.g. growth, population, eco\*).

#### 2.2

#### Description of the literature search

#### *Main search strategy:*

A step-wise search strategy was conducted to find the most relevant articles in the RF-EMF range selected.

As a first step, the literature research was conducted, following the criteria previously

described, on the ISI Web of Knowledge website, which provided 451,031 hits. Since this number of articles was too large to handle, a selection process was started. The collection was further refined by selecting only articles, reviews and proceeding papers as document types (440,528 hits). Then, specific categories were selected: applied physics, cell biology, plant sciences, environmental sciences, biophysics, zoology, ecology, biology, microbiology. The number of hits was so reduced to 98,620.

In order to reduce the number of hits, all the results clearly outside the RF-EMF field of research, or beyond the scope of this review, were excluded. This process reduced the number of hits further to 90,408 hits. A further screening was conducted selecting keywords from the RF-EMF specific and from the ecological defined groups, using one or two of RF-EMF keywords singularly or in combination with a single keyword from the ecological group. The obtained results ranged from 10 hits to 600. Titles were then screened one by one to select papers that could be of interest.

An analogous pattern of searches was performed on Google Scholar and only articles that had not yet been found on ISI Web of Knowledge were added. The number of hits for the initial combination of keywords was 3,600,000, and then reduced with an analogous procedure as described in ISI, but with a more attentive look at the content and the source of the selected papers.

After this first step of the searching process, 709 presumably relevant articles were identified. A one-by-one screening of titles and abstract was performed to investigate which papers would meet the defined criteria (e.g. frequency range, biodiversity exposure to RF-EMF). This second screening led to a new selection of 307 papers.

A closer analysis of the content of these 307 selected papers revealed that most of them regarded highly specific and technical strictly biological studies (e.g. rat tissues, cell-line studies, neuronal studies, calcium signalling, etc.), which were difficult to link directly to ecological effects, and, therefore, discarded. The final selection was reduced to 55 clearly relevant papers.

#### *Related-references search*

As a second step, it was decided to proceed by using a selected number of the 57 available articles to create a search based on "related references" to the ones used by their authors. The first articles used were those that clearly met the scope of the review in terms of focus and content: e.g. Balmori (2005), Panagopoulos et al. (2010) and five others. The screening of a total of 4,000 hits provided 32 additional relevant hits.

Also a selection of the relevant references was conducted from the four relevant reviews (Bryan and Gildersleeve 1987; Verschaeve and Maes 1998; Juutilainen 2005; Pourlis 2009) and this resulted in 15 additional articles.

Regular updates were conducted until October 2012 to include also the most recently published relevant literature. After a careful analysis of all gathered information a total of 113 articles was selected and described in detail in the following sections. The total number of experiments carried out in these articles was 152.

# 3

#### General overview of results

The biggest share of the articles (c. 90 %) involves laboratory studies with biological endpoints with a clear ecological relevance. The remaining part regarded ecological field studies (Table 1).

Most of the laboratory studies included had growth, development, behaviour and reproduction/fertility as biological endpoints. The endpoints analysed in field studies were behaviour, shift in populations and fertility. In circa 65% of the studies a statistical significant effect of RF-EMF on ecological relevant endpoints has been found (Table 1). There were no clear differences in percentage effects between articles included in reviews or not included in reviews. Development seemed to be less significantly affected in percentage than growth and fertility.

Table 1. General overview of effects and no-effects studies across articles types, endpoints and species groups.







The most represented groups include vertebrates, other than birds (i.e. predominantly rats, mice, rabbits), then birds and plants. Articles which found significant effects of RF-EMF were found more frequently in the case of birds, insects (i.e. mostly honey bees and fruit flies) and plants. The group of other vertebrates (Table 1) was equally distributed among significant and non-significant effects. Effects were significant in all the articles on other organisms.

The type of endpoints studied differed across groups. Fertility was the mostly analysed endpoint for the birds. Growth was affected in all the experiments conducted on plants and other organisms, while it was affected In 25% of the studies on other vertebrates and ca. 40% on the birds. The effects of RF-EMF on behaviour were found in thirteen of the twenty of the studies on other vertebrates and in 85% ca. of the studies on insects.

#### 4

# Ecological effects of RF-EMF

#### 4.1

#### **Birds**

Birds have been widely used to analyse the environmental significance of exposure to nonionizing radiation. The ability of birds to detect magnetic stimuli has been documented by several studies (see Keeton 1971; Wiltschko and Wiltschko 1996; Thalau et al. 2005; Wiltschko et al. 2011). A total of 26 articles was selected from the screened literature with 38 relevant endpoints. With the exception of five field studies, all studies were conducted in a laboratory setting.

Of the 26 studies, 70% have been significantly related to the effect of RF-EMF (Table 1). In most cases the effects studied were growth and fertility and were conducted, until the early nineties, under a continuous microwave system of exposure (i.e. 2450 MHz). The physical parameters usually reported regarded the measured level of power flux density

and specific absorption rate (SAR). These parameters were either measured using probes or specific detectors or were based on the information of the manufacturers of the exposure devices.

Chicken (Gallus domesticus) and Japanese quail (Coturnix coturnix subsp. japonica) represented the most studied experimental system in laboratory studies on birds. Approximately 60% of the laboratory studies considered a system at the embryo or egg stages of development.

#### 4.1.1

#### Laboratory studies

#### *Embryo and egg*

In the eighties and early nineties researchers focused on the effects of MW EMF. There was a high level of interest especially in the ranges that would be relevant, at that time, for the possible implementation of new source of renewable power based on the collection of solar energy in space by means of solar power satellites (SPS add to abbreviation list) and its transmission to earth via MW EMF (Glaser 1968; Wasserman et al. 1984). The three more recent studies (Table 2) investigated the typical cellular phones range of frequencies.

All the measured physical parameters varied greatly across studies. The estimated SARs ranged between 0.001W/kg and 140 W/kg (Kleinhaus et al. 1995; Van Ummersen 1961), while the duration of the exposure was as little as 9 seconds (McRee and Hamrick 1977) with peak values of 45 days (Grigoryev 2003). The variation which was found for the power density ranged from 4.4  $\times$  10<sup>-6</sup> mW/cm<sup>2</sup> as in Reijt et al (2007) to the 400 mW/ cm2 measured in Van Ummersen (1961).

The endpoints included growth, hatchability, development based on evidence of abnormal weight of hatchlings, incidences of abnormalities and mortality. Nine of the 15 experiments showed significant differences between RF-EMF and controlled/sham-exposed eggs.

It is a common opinion among experts (Baranski and Czerski 1976; Bryan and Gildersleeve 1987) that the results obtained in most of the studies until the 1980s (i.e. until Inouye et al. 1982 in this selection) relate to increases in the temperature of the egg due to the consequences of hyperthermia a few degrees above normal incubation temperature. An abnormal increase in temperature gradient of 3.5 degrees Celsius had been already observed in the early study by Van Ummersen (1961, 1963), reported in the review conducted by Bryan and Gildersleeve (1987). In a later study, Byman et al. (1985) found no effect on the growth and normal development of born chicks of birds nesting in proximity to antennas. Temperature rise was controlled and the measured power density was 25 mW/cm<sup>2</sup>.Analogous results were obtained by Gildersleeve et al. (1987) who kept the internal temperature of irradiated and sham-exposed eggs to a mean of 37.5 degrees Celsius without detecting any deficiency in the reproductive performance of males and females allowed to hatch.

Among the three more recent studies, Bastide et al. (2001) and Grigoriev (2003) found a significant increase in mortality due to RF-EMF on chicken (Gallus gallus subsp. domesticus) embryos exposed to RF-EMF emitted by a GSM device during the duration of the incubation period.

Also Batellier et al. (2008) studied the effect of exposure to GSM and UMTS frequencies on chicken eggs over the entire period of incubation. Four replicates with a total 240 eggs each were used in the experiment to assess mortality rates. Results showed an increased mortality of 42.2% for embryos under a regime of controlled temperature, humidity and external EMF. However, it was not possible to establish a proportional relationship between the intensity of the electric field and embryo mortality.

#### *Juvenile and adult*

Five studies focused on the impact of RF-EMF at a later phase of development of chickens: four studies on juvenile and only one on adult subjects (Table 2). The endpoints studied were growth, fertility, rate of egg production, hatchability and mortality.

The only study which found a significant difference between exposed and control/sham groups is the study by Giarola and Krueger (1974) on juvenile chickens. The authors examined exposure to very-high frequency (VHF) and ultra- high frequency (UHF), together with investigation of MW EMF. Exposure determined reduced growth of chicks and consumption. In a follow-up study Krueger et al. (1975), did not find effects either on fertility or hatchability with a continuous exposure period of 12 weeks at a power density (calculated) of 1 mW/cm<sup>2</sup>. Experts from the U.S. department of energy (NASA 1978) attributed the difference in results to the cage used in the first study which may have determined a higher dose of energy absorbed by the target subjects.

# 4.1.2

# Field studies

There were five field studies on the impact of RF-EMF exposure at various frequencies

and physical conditions on populations of birds living in areas in the vicinity of cellular phone masts or base-stations. Anomalies and deviations from normality in the behaviour of exposed subjects and in the level of productivity were found in all these studies.

The values of power density provided by studies ranged from  $4.4 \times 10^{-6}$  mW/cm<sup>2</sup> in the study on sparrows by Everaert and Bauwens (2007) to the highest measured value of 155 mW/cm<sup>2</sup> in Wasserman et al. (1984). In this last case, exposure caused a steady temperature raise which determined a continuous gaping for the total duration of exposure of the exposed population of sparrows (Zonotrichia albicollis) and juncos (Junco hyemalis). Values for the SARs were provided only by the study of Wasserman et al. (1984) and ranged from 0.85 to 0.92 W/kg. The endpoints studied were density, reproduction, behaviour and community composition. In all the studies and experiments conducted, effects of the RF-EMF were found from a variation of 10% to a maximum of 70% compared to control.

Balmori (2005) monitored the variation of a population of white storks (Ciconia ciconia) in the vicinity of a GSM base station.(I.e. 900-1800 MHz with 217 Hz modulation) in search of possible effects from the exposure. Total productivity within 200 m was on average 46% less than that found at a distance greater than 300 m from the emitting station. An analogous significant difference was found in the breeding success: in 40% more of the cases no new-born chicks were found in the nest.

In another study, Reijt et al. (2007) studied the influence of long term exposure to RF-EMF from radar (200-1300 MHz) on a population of Great tits (Parus major) and Blue tits (Cyanistes caeruleus) living around a military radar station. Possible other sources of co-variance (e.g. from human interactions with the location of birds, other pollutants, etc.) were not considered in the study. Unlike in the case of Balmori (2005), the exposure seemed not to have affected the number of nesting tits, but the distribution of the different species. The authors state that the results contradict with the study of Balmori (2005), probably because of the exposure of targets to radar MW (i.e. 1200-3000 MHz), instead of mobile phone exposure (i.e. 900-1800 MHz with 217 Hz modulation).

Additionally, Reijt et al. (2007) found that exposed nests were occupied, compared to control, by the less dominant species of tits (Blue tit), which would suggest that birds can perceive high frequency RF-EMF as a stressful factor and, thus, would try to avoid nesting in those areas. An average of 50% of the great tits moved from a more exposed section of the study area to a less exposed one: in the interaction with the great tit, the blue tit is usually less dominant according to behavioural studies by Tanner (1966) and Tanner and

Romero-Sierra (1974). Therefore, the Great tit would move to areas where the power density is lower, and therefore the Blue tit would have to nest elsewhere.

Figure 1 presents a plot of the effect with the relative measured power density, from studies with a significant effect (see Table 2 for details on the studies). It is not possible to define a clear dose-effect relationship, but also at low values of power density strong effects of RF-EMF are found.



Figure 1. Size of the ecological effects of RF-EMF on birds related to the power density of exposure. Articles reported in graph: (1) - Hills et al. (1974); (2) - Inouye et al. (1982); (3) - McRee et al. (1975); (4) - Wasserman et al. (1984); (5) - Balmori (2005); (6) - Balmori and Hallberg (2007); (7) - Everaert and Bauwens (2007); (8) – Reijt et al. (2007); (9) - Batellier et al. (2008); (11) – McRee et al. (1975); (12) - Krueger et al. (1975); (13) - Davidson et al. (1976); (14) - McRee and Hamrick (1977); (15) - Byman et al. (1985); (16) - Gildersleeve et al. (1987). See Table 2 for a complete description of studies. Data is reported for studies from which information could be extracted. The equation of the regression line is  $y = -0.0078x + 0.2908$ .

#### Summary

Most studies on birds were laboratory investigations. The target subjects were in the majority of laboratory studies chicken and Japanese quail. Older laboratory studies exposed targets to high level of MW EMF which probably determined an uncontrolled raise in temperature which affected the exposed systems. Amongst the more recent laboratory studies, evidence of an effect of RF-EMF on mortality and development of embryos was in all cases found at both high and low dosages. In all the five field studies found a significant effect of RF-EMF on breeding density, reproduction or species composition. Field observations give a closer representation of real-life exposure, thus RF-EMF, especially in the 900 MHz GSM band could be a certain factor influencing the ecology of birds.



Table 2. Summary of articles reporting ecological effects of RF-EMF on birds. Table 2. Summary of articles reporting ecological effects of RF-EMF on birds.









<sup>d</sup> Wave/Modulation indicates the type of RF-EMF applied/measured in the study. CW = continuous wave, MW = microwave, PW d Wave/Modulation indicates the type of RF-EMF applied/measured in the study. **CW** = continuous wave, **MW** = microwave, **PW** = pulsed wave **GSM** = Global System for Mobile Communications, **UHF** = Ultra-High Frequency, **VHF** = Very high frequency. = pulsed wave GSM = Global System for Mobile Communications, UHF = Ultra-High Frequency, VHF = Very high frequency. e Values of power density are reported as provided by authors or recalculated by conversion of electric field values e Values of power density are reported as provided by authors or recalculated by conversion of electric field values

(PD=EF2/3770) and expressed in mW/cm<sup>2</sup>. (PD=EF2/3770) and expressed in mW/cm2.

' Values of SAR are reported as provided by authors and expressed in W/kg. f Values of SAR are reported as provided by authors and expressed in W/kg.

<sup>8</sup> Biological or ecologically relevant endpoints studied. <sup>8</sup> Biological or ecologically relevant endpoints studied.

h Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to h Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect, a – sign indicates that no significant effect was found. control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found.

n/a indicates that data was not provided by authors. i n/a indicates that data was not provided by authors.

# 4.2

#### Insects

Insects are a useful target system for the investigation of RF-EMF because of the limited size, the short life cycle and the possibility of easily detecting developmental defects (Schwartz 1985). It has been demonstrated that insects can sense magnetic fields as a means for navigation and orientation (Kirschvink et al. 2001; Abraçado et al. 2005; Liedvogel and Mouritsen 2010; Wajnberg et al. 2010; Winklhofer 2010). Magneto-reception has been associated with the use of ferromagnetic iron oxide particles embedded in tissue or through pairs of molecules with unpaired electrons (known as radical pairs) that are associated with a light sensitive photoreceptor (Ritz 2000; Knight 2009; Vácha et al. 2009). The exposure to RF-EMF might disrupt this magneto-reception mechanism, which could in turn affect the survival of insects. The most commonly studied species are the honey bee (Apis mellifera) and the fruit fly (Drosophila melanogaster).

# 4.2.1

# Honey bees (*Apis mellifera*)

Over the past few years, a phenomenon known as Colony Collapse Disorder (CCD) has increased the attention of experts on the survival of colonies of honey bees (Schacker 2008; Balmori 2009). The reduction in population of bees worldwide could have serious ecological, economic and, thus, political implications given their role as pollinators. It has been estimated that 15% of wild plant species in Europe (Kwak et al. 1998) and 35% of the global crops produced (Klein et al. 2007) are visited by honey bees. Bees are interesting for this reason from an economic perspective: their economic role has been estimated to be around 153 billion euros in the year 2005 (Gallai et al. 2009). RF-EMF has been classified as one of the possible causes of honey bee colonies collapse (Ratnieks and Carreck 2010). Even though the interest of media and the public in the effects of exposure of honey bees to mobile communication RF-EMF has drastically increased, there does not seem to be a thorough body of research into their effects in the scientific literature. As a result, the screening conducted in this contribution identified only eight studies which matched the defined criteria (Table 3), for a total of 12 experiments. Six of the studies focused on the frequency ranges specific to mobile communication and in all cases found a significant relationship between the exposure to the field and the effects studied. Only two of the studies found were not produced in the last decade (Westerdahl and Gary 1981a, b). These studies were the only ones which did not find any significant effect on flight, orientation of behaviour of bees exposed to CW microwaves (i.e. 2450 MHz) at power densities from 3 to 50 mW/cm<sup>2</sup>.

Among the studies that did find an effect, Sharma and Kumar (2010), Kumar et al. (2011)

and Sahib (2011) found a critical reduction of all studied parameters of the exposed colonies of bees as a response to RF-EMF. In all cases, an acute decrease in the breeding performance or even a collapse of the entire colony resulted as a consequence of exposure to RF-EMF. However, the studies provide limited statistical information on the scale of the effect found and did not take into account other confounding parameters (e.g. the placement of the emitting device inside the hive).

The work by Harst et al. (2006) and Kimmel et al. (2007) from a German research group seems to support the previously described findings, but do not provide any statistical measure of the effects found and did not report any system of control or sham-exposure.

Clearer conclusions can be drawn from the study by Favre (2011), which seems to be the most complete and qualitatively interesting contribution. Using sound-analysis techniques, the author investigated the changes that were triggered in the behaviour of a population of honey bees of the carnica group (Apis mellifera subsp. carnica). The sounds produced by the bees from five healthy and unexposed hives were used as a negative control and compared with recordings made when the same hives were exposed to a mobile phone handset in a calling position. Another inactive mobile phone was placed, at an earlier stage, to investigate the possible disturbing influence of the sheer presence of the tool in the hives. The analysis of the recorded sounds revealed that the bees produced sounds at higher frequency and amplitude after about 25 to 40 minutes after the communication had started and became quiet when the handset was switched off.

No particular difference in behaviour and sounds was found for exposure to the inactive handsets. The analysis of the sound data revealed that the bees were, in fact, producing the so-called "worker piping", which usually serves as a signal for swarm exodus as a response to danger or stress, thus RF-EMF directly affected the community of bees under exposure.

#### 4.2.2

#### Fruit flies (*Drosophila melanogaster)*

The screening of the literature identified five studies on the fruit fly (*Drosophila melanogaster*) for a total of nine experiments (see Table 3). All the available studies found a significant effect. The RF-EMF applied focused on the GSM 900 MHz and GSM 1800 MHz (named also DCS –Digital Cellular System) systems.

RF-EMF power density was measured in the range of 0.0002 to 0.0407 mW/cm<sup>2</sup>, several order of magnitudes lower than those measured in the previously analysed laboratory

studies on birds and bees. All the values can be considered typical for digital mobile telephony handsets and in most cases fall within the current exposure criteria (ICNIRP 1998). Unlike the previous cases, in most studies it was possible to collect information about the magnetic flux density, which ranged from to the time-averaged 0.003  $\mu$ T of Panagopoulos et al. (2004) measured for a DCS frequency to 0.09  $\mu$ T in the study by Panagopoulos et al. (2010). SAR levels were, when provided, obtained by elaboration of data provided by the manufacturer (i.e. for the human head) of the system used for exposure and not directly measured.

The ecologically relevant endpoints analysed in the studies were growth and reproduction. All of the analysed studies found a significant effect compared to the control. With the exception of a study by Weisbrot et al. (2003), all studies were conducted by a research group from the University of Athens, Greece. In the study of Weisbrot et al. (2003) the irradiation determined a beneficial effect on the reproductive success of the exposed system. The number of off-springs even increased of up to 50% compared to control. All the other studies found a significant depression of growth and reproduction as a response to exposure. Several studies performed by Panagopoulos and co-authors (see Table 4) found a maximum decrease in the endpoints of at least 40% compared to control. Exposure duration lasted for six minutes per day or increased over time up to a maximum of twenty-one minutes over a period of six or five days. The reproduction of experiments performed at several distances from the emitting system (i.e. a telephone device) suggested in all cases a quasi-linear decrease at increasing durations of exposure (Panagopoulos and Margaritis 2010) and increase in proximity to the source of the emission (Panagopoulos et al. 2010). In this last study a window-effect was found at distances of 20-30 cm from the device, which resulted in the highest decrease of the measured values.

#### 4.2.3

#### Effect on other insects

The remaining studies in this section focus on the Tobacco Hornworm (*Manduca sexta*), on the American cockroach (Periplaneta americana) and on a species of ant (*Myrmica sabuleti;* Table 3).

The study by Schwartz et al. (1985) analysed differences in development, reproduction and mortality in Tobacco Hornworms exposed during their larval stage to PW RF-EMF at a frequency of 2695 MHz and a power density of 4 MW/cm<sup>2</sup>.All the measured parameters were affected and effect size was as high as 50% lower compared to control.

The studies on the American cockroach (Vácha et al. 2009) and the ant (Cammaerts et al. 2012) focused on the effects of RF-EMF on the magneto-reception of the insects. In the study by Vácha et al. (2009), it was found that, during and after the rotation of the natural geomagnetic field, the insects turned around, as a response of the detection of the field. However, their ability to detect the geomagnetic field was disrupted after exposure to a field at 1.2 MHz with a magnetic flux density between 12 and 18 nT.

Cammaerts et al. (2012) investigated the impact of RF-EMF on the acquisition and loss of olfactory and visual cues of six experimental colonies of the ant Myrmica sabuleti. The exposure to a GSM- generated signal determined a loss in the acquired association between food and a visual cue (40% worse than control), a decreased retention of acquired knowledge, and a total loss of visual memory.

The representation of the size of the effect compared to the power density (Figure 2) shows that significant effects are found at both at high and low dosages, revealing no clear dose-response relationship. In one of the analysed studies, no effects were found at high levels of power density.



Figure 2. Size of the effects of RF-EMF on insects compared to the power density of exposure. Articles reported in graph: (1) - Westerdahl and Gary (1981a); (2) Westerdahl and Gary (1981b); (3) - Sharma and Kumar (2010); (4) - Panagoupolos et al. (2004); (5) - Panagoupoulos et al. (2007); (6) - Panagoupoulos et al. 2010; (7) - Panagoupoulos and Margaritis 2010; (8) – Panagoupoulos (2012); (9) - Schwatz et al. (1985); (10) - Cammaerts et al. (2012). (See Table 3 for a complete description of studies.

#### Summary

A limited set of articles regards the possible impact of RF-EMF on honey bees is available in literature. Most of the analysed studies found an effect on the target colonies. The most affected endpoints seemed to be behaviour and orientation of exposed bees, which lead to disruptive consequences in the colonies. The majority of the studies did not provide statistical analysis and did not use clear control measures to analyse results. One exception is the study conducted by Favre (2011), in which the behaviour of the bees seems to be comparable to that experienced by colonies exposed to extreme danger and stress.

The studies analysing the effects of RF-EMF on fruit flies found in all cases a significant effect. One study results show an increased reproductive success after exposure. The remaining studies, which were conducted by the same research institute in Greece, found in all cases a significant depression of growth and reproduction at both 900 and 1800 MHz. Two studies on the American cockroach and a species of ant analysed the effects of exposure to RF-EMF on the magneto-reception and orientation of the insects. The behaviour of target systems was disrupted by the exposure to RF-EMF.











a Life stage refers to the age of the tested subject at the moment of the performance of the experiment. b Studies divided in laboratory and field studies. **Lab** = laboratory study, **Field** = field study. Number of subjects involved in the experiment or field study where reported in the study. In brackets information about number of control subjects. Further specifications of type of subjects involved in the studies are reported if provided by authors. In the case of studies regarding number of control subjects. Further specifications of type of subjects involved in the studies are reported if provided by authors. In the case of studies regarding the fruit fly the distances applied are reported. d Wave/Modulation indicates the type of RF-EMF applied/measured in the study. **CW** = continuous wave, **MW** = microwave, **GSM** = Global System for Mobile Communications, **DECT** = Digital Enhanced Cordless Telecommunications. e Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm2. f Values of SAR are reported as provided by authors and expressed in W/kg. <sup>g</sup> Biological or ecologically relevant endpoints studied. <sup>h</sup> Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found. In/a Life stage refers to the age of the tested subject at the moment of the performance of the experiment.  $b$  Studies divided in laboratory and field studies. Lab = aboratory study, Field = field study. Number of subjects involved in the experiment or field study where reported in the study. In brackets information about the fruit fly the distances applied are reported."Wave/Modulation indicates the type of RF-EMF applied/measured in the study.  $\mathsf{CW}$  = continuous wave,  $\mathsf{MW}$  = microwave, GSM = Global System for Mobile Communications, DECT = Digital Enhanced Cordless Telecommunications. "Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm<sup>2</sup>. 'Values of SAR are reported as provided by authors and expressed in W/kg. 8 Biological or ecologically relevant endpoints studied.<sup>h</sup> Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found. In/a ndicates that data was not provided by authors. indicates that data was not provided by authors.

# 4.3

#### Other vertebrates

The impossibility of conducting laboratory experiments into the effects of RF-EMF on humans steadily increased the number of scientific studies on laboratory vertebrate models. As suggested by the WHO (2006), studies conducted on immature animals can, for instance, provide a useful indicator of possible cognitive and behavioural effects on children. The vast majority of studies focused on the analysis of intracellular pathways, for instance through changes in calcium permeability across membranes (e.g. Maskey et al. 2010); or on gene expression, namely on the neurons of rats exposed to RF-EMF (e.g. Salford et al. 2003; Zhao et al. 2007); or on possible chromosomal damage in mice cells (e.g. Nikolova et al. 2005).

A total of 50 scientific articles were selected for a total of 62 relevant ecological experiments (Table 4). The endpoints analysed which were of interest were fertility, growth, behaviour and mortality (Table 1).

With the exception of one study on bats (*Pipistrellus pipistrellus; Pipistrellus pygmaeus; Myotis daubentonii; Myotis nattereri*) breeding nearby a wind turbine and one study on the tadpoles of frogs (*Rana temporaria*), all studies were conducted in a laboratory setting. The animal systems under investigation were rats commonly used in laboratory studies (Wistar albino rat; Sprague Dawley rat), mice (*Balb/c; Balb/c/f*), rabbits (*White New Zealand Rabbit*), rhesus monkeys (*Macaca mulatta*). Of the total of 50 articles, 50% of the studies were conducted on rats. A total of 27 experiments (43%) showed no significant results of an impact of RF-EMF under the physical and experimental settings used. The power density ranged from  $0.6 \times 10$ -6 to 20 mW/cm<sup>2</sup>, which was the maximum value measured for MW CW exposures (Table 4). The SARs values measured ranged from 0.00194 to 44 W/kg, with a peak value measured at 2450 MHz for MW PW exposure. In the studies in which higher level of exposure to RF-EMF were applied and temperature was not controlled, results may be related to an increase in body temperature as a consequence of exposure.

A large share of the studies on vertebrate animal models focused on changes in behaviour as a result of exposure. This choice may be related to investigating of possible influences of RF-EMF on the behaviour and cognitive performance of humans, who use mobile phone devices in close proximity to their heads. Some commonalities between human and rat response to noxious substances have been explored by other fields of science (Hammond et al. 2004). Lai et al. (1994) suggested that rats suffer from a deficit in spatial working memory function when exposed to RF-EMF (50% decreased performance compared to

control). The repetition of the experiment with similar conditions of exposure by Cassel et al. (2004), Cobb et al. (2004), and Cosquer et al. (2005) found no effects on learning abilities of rats in the performance of spatial tasks and no evidence of altered brain development.

Another example in this direction is the work of Daniels et al. (2009), who investigated the effect of RF-EMF in the mobile phone range on the behaviour of the rat with controversial results. Spatial memory was tested using the Morris water maze test (Morris 1981), and mood disturbances and anxiety-like behaviour were tested in an open field test, for twelve radiated and twelve control subjects. Results showed no significant differences between groups in the Morris test, suggesting no significant difference in the behaviour of exposed and control rats. However, male rats performed significantly worse (60%) in the open field test.

The articles by Lee et al. (2009; 2012), Imai et al. (2011) are the only studies focusing on the impact of the frequencies network standards found in 3G mobile communication (Smith and Collins 2000), working with protocols like wideband code division multiple access (W-CDMA) or CDMA. All experiments, on mice and rats, did not have any observable adverse effect on development, reproduction or mutation of tested subjects. No effects on the development of rats were also observed by the study of Poullettier de Gannes et al. (2012), where Wireless Fidelity (Wi-Fi) signal at 2450 MHz was applied on rats, and by the study of Jiang et al. (2012), where mice were exposed to a wireless transmitter at 900 MHz. These studies represent the first attempt to investigate the effects of wireless communication on health.

The field experiment of Balmori (2010) on the behaviour and growth of the tadpoles of frogs (Rana temporaria) placed 140 m from a field station provides evidence of the effect of RF-EMF. The exposed group showed low coordination of movements, an asynchronous growth and a high mortality (90%). The control group was exposed to the same environmental conditions, but placed inside a Faraday cage. As a result, the coordination of movements was normal, the development was synchronous, and the mortality rate was 4.2%. The research goal of the field study by Nicholls and Racey (2009) was to test whether PW RF-EMF emitted by a radar could be used as a method of preventing bats from death caused by collisions with wind turbines. The authors analysed 20 foraging sites. The exposure of bats to a pulsed wave radar system determined a significant reduction in foraging activity of bats.

The plotting of the size of the effect with the relative measured power density (where

the value was provided by authors) of positive studies does not show any detectable trend (see Figure 2). No clear pattern is visible from the analysis of the data and effects were found both at high and low levels of power density.



Figure 2. Size of the effects of RF-EMF compared to the power density of exposed vertebrate animal models. Articles reported in graph: (1) - Berman et al. (1992); (2) - Magras and Xenos (1997); (3) - Khillare and Behari (1998); (4) - Nittby et al. (2008); (5) - Mathur (2008); (6) - Salama et al, (2010a); (7) - Kesari et al. (2011); (8) – Lai et al. (1994); (9) – Daniels et al. (2009); (10) – Nicholls and Racey (2009); (11) – Fragopoulou et al. (2010); (12) – Balmori (2010); (13) – Hao et al. (2012); (14) – Yang et al. (2012); (15) – Jian et al. (2012); –(16) - Chernovetz et al. (1975); (17) - Jensh et al. (1982); (18) - Lary et al. (1983); (19) - D'Andrea et al. (1989); (20) - Sherry et al. (1995); (21) - Klug et al. (1997); (22) - Jensh (1997); (23) - Bornhausen and Scheingraber (2000); (24) - Dasdag et al. (2007); (25) - Lee et al. (2009); (26) - Sommer et al. (2009); (27) - Sienkiewicz et al. (2000). See Table 4 for a complete description of studies

#### Summary

Rats and rabbits exposed to RF-EMF in a laboratory setting represented the most studied animal model. Changes in behaviour as a result of exposure were analysed in most studies and presented contradictory results. As for the other endpoints, significant effects were found under various conditions of exposure and under different laboratory setups. A field study showed a significant effect of exposure on the growth and mortality rates of tadpoles of frogs under field conditions. In another RF-EMF reduced the foraging activity of bats.


















a Life stage refers to the age of the tested subject at the moment of the performance of the experiment. a Life stage refers to the age of the tested subject at the moment of the performance of the experiment.

b Number of subjects involved in the experiment or field study where reported in the study. In brackets information about number of control subjects. Further b Number of subjects involved in the experiment or field study where reported in the study. In brackets information about number of control subjects. Further specifications of type of subjects involved in the studies are reported if provided by authors. specifications of type of subjects involved in the studies are reported if provided by authors.

· Wave/Modulation indicates the type of RF-EMF applied/measured in the study. CW = continuous wave, MW = microwave, GSM = Global System for Mobile c Wave/Modulation indicates the type of RF-EMF applied/measured in the study. **CW** = continuous wave, **MW** = microwave, **GSM** = Global System for Mobile Communications, DECT = Digital Enhanced Cordless Telecommunications, PW = pulsed wave, UWB = ultra wide band, AM = amplitude modulation, FM Communications, **DECT** = Digital Enhanced Cordless Telecommunications, **PW** = pulsed wave, **UWB** = ultra wide band, **AM** = amplitude modulation, **FM**

3

= frequency modulation; UMTS = Universal Mobile Telecommunications System; CDMA = Code division multiple access; TDMA = time division multiple = frequency modulation; **UMTS** = Universal Mobile Telecommunications System; **CDMA** = Code division multiple access; **TDMA** = time division multiple access; WCDMA = Wideband Code Division Multiple Access. access; **WCDMA** = Wideband Code Division Multiple Access. <sup>4</sup>Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mVV/cm<sup>2</sup>. d Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm2. e Values of SAR are reported as provided by authors and expressed in W/kg. e Values of SAR are reported as provided by authors and expressed in W/kg.

f Biological or ecologically relevant endpoints studied. f Biological or ecologically relevant endpoints studied.

a Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a  $^{\mathrm{s}}$  Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found. h n/a indicates that data was not provided by authors. significant effect, a - sign indicates that no significant effect was found. h n/a indicates that data was not provided by authors.

## 4.4 Other organisms

This section includes studies on the effect of RF-EMF on the bacterium (Escherichia coli), the nematode (*Caenorhabditis elegans*), and the land snail (*Helix pomatia*), which constitute the species not yet included in the previous sections.

The screening of the literature identified four studies for a total of eight experiments (Table 5). In all cases effects were significant. The RF-EMF applied were mainly the GSM 900 MHz and GSM 1800 MHz (DCS –Digital Cellular System) systems, with the exception of the study of Grospietsch et al.(1995) and de Pomerai et al. (2002), which studied respectively a pulsed wave modulated frequency at 150 MHz and a microwave continuous wave frequency at 1000 MHz (Table 5).

RF-EMF power density was measured in the range of 0.0005 to 0.679 mW/cm<sup>2</sup>. All the values can be considered typical for digital mobile telephony handsets and in most cases fall within the current exposure criteria (ICNIRP 1998).

The ecologically relevant endpoints analysed in the studies were growth, reproduction and stress. All of the analysed studies found a significant effect compared to the control. The exposure of the bacteria E. coli and the nematode C. elegans suggests that RF-EMF tend to enhance growth of the organisms. The study on the land snail (Nittby et al. 2012) found a beneficial non-thermal analgesic effect on a group of 29 land snails placed on a hot plate. The response time to heat of GSM-exposed snails was 20% higher than that of the control. The study by Daniells et al. (1998), which exposed a transgenic nematode (C. elegans PC72) to RF-EMF at a frequency of 750 MHz, found a significant drastic effect on the stress levels (i.e. 150% higher than control) of the exposed target system.

## Summary

Studies on the effects of RF-EMF on the bacterium (*Escherichia coli*), the nematode (*Caenorhabditis elegans*) and the land snail (*Helix pomatia*) reported in all cases a significant effect on behaviour and growth of target subjects and under all laboratory setups applied. The study on the E. coli and C. elegans beneficially affected growth. The exposure of the land snail to RF-EMF retarded the response to heat determining a beneficial analgesic effect.



Table 5. Summary of articles on ecological effects of RF-EMF on the bacterium Escherichia coli, the nematode Caenorhabditis elegans, the land Table 5. Summary of articles on ecological effects of RF-EMF on the bacterium Escherichia coli, the nematode Caenorhabditis elegans, the land snail Helix pomatia.

= microwave, **GSM** = Global System for Mobile Communications, **PW** = pulsed wave, **DCS** = digital cellular system. b Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm2. c Values of magnetic flux density if provided by authors. d Values of SAR are reported as provided by authors and expressed in W/kg. e Biological or ecologically relevant endpoints studied. f Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect, a - sign Wave/Modulation indicates the type of RF-EMF applied/measured in the study. Modulation value reported if provided by authors. CW = continuous wave, MW = microwave, GSM = Global System for Mobile Communications, PW = pulsed wave, DCS = digital cellular system. <sup>b</sup> Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm<sup>2</sup>. Values of magnetic flux density if provided by authors. d'Values of SAR are reported as provided by authors and expressed in W/kg.® Biological or ecologically relevant endpoints studied. Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to control. A sign + indicates a significant effect a - sign a Wave/Modulation indicates the type of RF-EMF applied/measured in the study. Modulation value reported if provided by authors. **CW** = continuous wave, **MW** indicates that no significant effect was found.<sup>8</sup> n/a indicates that data was not provided by authors. indicates that no significant effect was found.  $\epsilon$  n/a indicates that data was not provided by authors.

## 4.5

#### Plants and yeasts

The influence of the earth's natural magnetic field or that of superimposed artificial magnetic fields on plants has been known for many years. Static magnetic fields, in fact, have been proven to have a beneficial impact on the stimulation of growth and germination of plants (Savostin 1930; Pittman 1965; Dulbinskaya 1973), or inhibitive impact depending on the species and their physiological state (Krizaj and Valencic 1989; Ruzic et al. 1998). According to Soltani et al. (2006), until now no proper physiological explanation has been provided for the described effects, though the biological effects of weak static MF do not only depend on the physical conditions of the exposure (e.g. power density, frequency), but also on the environmental conditions in place.

The analysed literature considered that plants are continuously exposed to RF-EMF as they cannot avoid them, by moving away from the source of emission. As in the case of the studies explored in earlier sections, little is known about a possible mechanism explaining how exposure to RF-EMF may cause biological/ecological effects, and therefore most of the investigations were aimed at the possible mechanisms underlying the effects in plants.

In total, 16 studies and 29 experiments were selected based on the ecological relevance of the endpoints studied (Table 6). Ten experiments investigated the impact of RF-EMF on the inhibition of the regular growth of plants. Four experiments directly investigated the stress levels of plants exposed to RF-EMF as a variation in specific test methods. The remaining studies focused on abnormalities as a consequence of the exposure, and on the effect on the photosynthesis.

The frequency investigated ranged from as low as 10 MHz from an AM CW system (Haider et al. 1994) to 2450 MHz MW CW EMF (Schmutz et al. 1996). Power density ranged from 0.015 mW/cm<sup>2</sup> to 50 mW/cm<sup>2</sup>, therefore lower than the values measured in the previous section on the fruitfly (*D. melanogaster,* in section 4.4) and in line with the applications measured for birds and bees (section 4.1 and section 4.2). When measured and provided, SAR values were in the range of 0.0-4.7 W/kg (see Table 6).

The experiment by Schmutz et al. (1996) investigated the effects of a long term exposure to 900 MHz MW on the spruce and the beech (*Picea abies; Fagus silvatica*). At a measured power density of 10 mW/cm<sup>2</sup>, growth parameters and photosynthetic activities of the systems were not affected. No evidence was found on the mutation and the stress levels of yeast (*Saccharomyces cerevisiae*) in the laboratory experiment by Gos et al. (2000) and on mutation in the study by Chen et al. (2012). No information was provided on the levels of power density.

Among the studies with a significant effect on plants, three were published in 1996 by a Latvian group of researchers (Balodis et al. 1996; Magone 1996; Selga and Selga 1996). The researchers focused on the area of Skundra, Latvia, where a radio location station had been operating for 20 years. The three studies provide a unique experience of a complete set of experiments and field studies conducted around a radio station in the short as well as in the long term. The area of study also allowed for the investigation of RF-EMF effects at different distances from the station. The effects of other environmental and anthropogenic factors (e.g. pollution levels, population density, etc.) were also evaluated without revealing any significant effect on the parameters studied. As a result, the nonthermal RF-EMF under investigation indicated that the effects of short term exposure (i.e. up to five days) are dependent on the stage of growth of Great duckweed (Spirodela polyrhiza; Magone 1996) at the time of exposure. The vegetative growth of young plants decreased as a consequence of exposure, while it even accelerated in the case of older plants. The exposed population of adult plants was on average growing 150% more than the control unexposed samples. In the other two studies the pine tree (Pinus sylvestris) was under investigation. The effects of RF-EMF emitted by the radio station were analysed using retrospective tree ring data in Balodis et a (1996): a significant negative correlation between the measured electric field at specific sample locations and the mean relative 4additional annual increment of pines has been identified. Selga and Selga (1996) found significant cytological and ultra-structural changes in exposed pine needles and cones.

Duckweed (*Lemna minor*) was used as a model plant for the monitoring of effects on growth and other physiological responses also in two studies by Tkalec et al. (2005; 2007), which confirmed that under most of the investigated conditions of field frequencies, modulation, and exposure time growth was significantly reduced (i.e. 29% on average less) compared to control.

A connection between exposure and very rapid molecular stress responses was made in the studies performed by Roux et al. (2006; 2008) focusing on the molecular responses of tomato plants (*Lycopersicon esculentum Mill* VFN8). The study was based on the use of several stress related transcripts (e.g. energy charge, protease inhibitor). Great differences were found in the exposed population compared to the control (up to 300%). The data supports the evidence that plants respond to exposure as they would respond to any other injurious treatment. Even though the RF-EMF used was non-thermal and the total

power used was low, results, as the authors commented, are strikingly similar to those found when plants are wounded, cut or burned.

Plotting of the size of the effect and the power density measured in studies (i.e. where provided) did not show any identifiable trend (see Figure 4): effects were found at high and low dosages and the size of effects varied greatly across studies.



Figure 4. Size of the effects of RF-EMF compared to the power density of exposed plants. Articles reported in graph: (1) - Tkalec et al. (2005); (2) – Tkalec et al. (2007); (3) - Roux et al. (2008); (4) - Ursache et al. (2009); (5) -Jinapang et al. (2010); (6) - Sharma et al. (2009); (7) - Urech et al. (1996); (8) – Magone (1996); (9) - Haider et al. (1994); (10) - Schmutz et al. (1996). See Table 6 for a complete description of studies

#### Summary

Significant effects of RF-EMF were found mostly on the inhibition of the growth of exposed plants. Oxidative stress (e.g. for tomato plants or duckweed) and continuous abiotic stress have been presented in some studies as possible determinants of the mechanism. Of interest is the case of studies performed for an extensive period of time in an area in Latvia around a radar station and involving both field and laboratory investigations. These studies showed possible effects of RF-EMF on the radial growth of pine trees (*Pinus sylvestris*), and on the growth of duckweed (*Lemna minor*) or great duckweed (*Spirodela polyrhiza*).



Table 6. Summary of articles on the ecological effects of RF-EMF on plants. Table 6. Summary of articles on the ecological effects of RF-EMF on plants.









number of control subjects. Further specifications of type of subjects involved in the studies are reported if provided by authors.<sup>4</sup> number of control subjects. Further specifications of type of subjects involved in the studies are reported if provided by authors. d Wave/Modulation indicates the type of RF-EMF applied/measured in the study. **CW** = continuous wave, MW = microwave, **GSM**  Wave/Modulation indicates the type of RF-EMF applied/measured in the study.  $\text{CW}$  = continuous wave, MW = microwave, GSM = Global System for Mobile Communications, **PW** = pulsed wave, **UWB** = ultra wide band, **AM** = amplitude modulation, **FM** = = Global System for Mobile Communications, PW = pulsed wave, UWB = ultra wide band, AM = amplitude modulation, FM = frequency modulation,  $GTEM = gigahertz$  transverse electromagnetic cell. frequency modulation, **GTEM** = gigahertz transverse electromagnetic cell.

e Values of power density are reported as provided by authors or recalculated by conversion of electric field values e Values of power density are reported as provided by authors or recalculated by conversion of electric field values (PD=EF2/3770) and expressed in mW/cm2. (PD=EF2/3770) and expressed in mW/cm2.

'Yalues of SAR are reported as provided by authors and expressed in W/kg. f Values of SAR are reported as provided by authors and expressed in W/kg.

<sup>8</sup> Biological or ecologically relevant endpoints studied. <sup>8</sup> Biological or ecologically relevant endpoints studied.

control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found. j n/a indicates that data was control. A sign + indicates a significant effect, a - sign indicates that no significant effect was found. j n/a indicates that data was Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to h Size of the effect where significant. It indicates the ration between maximum effect and percentual difference compared to not provided by authors. not provided by authors.

## 5 **Synthesis** 5.1

## **General**

The reviewed literature focused on birds, insects, plants and other vertebrates studied as model species. Other important ecological groups such as e.g. bumble bees, were underrepresented. Field studies were limited and mostly focused on the analysis of the response of birds and honey bees to RF-EMF. Irrespective of the studied group, development and reproduction were the most studied ecological endpoints.

The number of studies finding effects was highest for plants (90%) and insects (90%), lower for birds (70%), other vertebrates (56%) and other organisms (50%). In all the available field studies significant effects of RF-EMF were found. In laboratory experiments, birds and vertebrate animal subjects were in most cases tested at higher frequencies than smaller organisms (e.g. fruit flies) and plants. Older experiments on birds were often carried out at relatively high frequency MW (i.e. 2450 MHz and higher) and dosages (power density greater than 100 mW/cm<sup>2</sup>), which possibly determined a thermal increase of body temperature. In later experiments temperature was kept under control.

The quality of the reported RF-EMF characteristics was heterogeneous. Some studies only provided the frequencies of the RF-EMF emitting device and one dosage parameter (e.g. power density in mW/cm2 ). A limited number of studies supplied the full list of physical parameters needed for an adequate description of the exposure (e.g. modulation, spatial connotation of field, polarization, field pattern, modulation, measuring techniques). The reporting of the measured or extrapolated power density values and relative electric field values were discordant and no precise information was given on measurement or calculation procedures. Also relevant biological parameters were often neglected or not described (i.e. size, tissue dielectric properties, size, geometry, relation to polarization; see Michaelson 1991).

The overall quality of the studies varied across and within groups. In the case of the studies regarding bees (with the exception of the study of Favre, 2011) a limited definition of the characterisation parameters of the exposure, and a low number of control/sham measurements limits the possibility of generalising results and for possible ecological effects.

## 5.2 Dose-effect relationships

The studies that did find an effect did not always refer to the existence of a dose-effect relationship. Two studies from a Greek research group (Panagopoulos and Margaritis 2010; Panagopoulos et al. 2010) described a non-linear window-effect of RF-EMF at a specific distance from the emitting source. Despite a high number of studies finding a significant effect, there was no clear relationship between applied dosage and size of effect, at the level of ecological groups. However, the analysis was hampered by the use of different and scarcely comparable physical parameters to characterize dosage and the use of different ways of shielding control groups (e.g. not always a Faraday cage was used). Experimental groups were not always shielded from extraneous sources of RF-EMF and other types of RF-EMF not expressly taken into consideration.

One important conclusion is that even at low dosages, high effect percentages were described in the range of between 10 and 90%. There seem to be no specific physical parameters and experimental conditions that seem to determine an effect. In the field experiment the proximity to the emitting device (i.e. usually a base station) contributes to increase the size of the effect.

## 5.3

## From biological to ecological mechanisms and effects

In studies involving RF-EMF exposure temperature increase is often the only recognised and recognisable agent causing an effect. WHO (2010) considers temperature as the only clear mechanism active, especially in the studies exposing subjects to higher dosages. Most studies only report an effect of RF-EMF, without paying any attention to possible explanations. Stress is often mentioned as a possible influential element. Studies which use a sham-exposed group investigating also the possible influence of the sheer presence of the emitting device in the test area tend to exclude stress as the sole triggering factor for the effect, suggesting that the effect should be ascribed totally to the physical composition of the EMF and to the exposure conditions.

In the case of plants, an used theory is that the effects of RF-EMF- could be described and explained, also at non-thermal exposure dosages, as an ordinary stress factor, like drought or heat. The size of effects mentioned in studies with effects is relatively large in comparison with the control situations, and therefore it may be tentatively concluded from these studies that RF-EMF might have a significant ecological effect.

## 5.4

## Differences between effect and no-effect studies: a possible bias?

The differences in articles between effect and no-effect RF-EMF studies were compared

regarding the country of the origin, the exposure duration, the applied RF-EMF frequencies, and the impact factor of the journal of publication (see Table 7).

Parameter	<b>Effect</b>	No effect
Country (number)†		
<b>USA</b>	18	17
India	8	3
Greece	8	2
France	5	8
Croatia, China, Germany, Latvia, Spain, UK	3	
Canada, Japan, Switzerland	$\mathbf{2}$	
Others	10	$\overline{2}$
<b>Exposure duration [mins]††</b>		
Mean	146960,5	63241,26
Median	1800	1800
Mode	30	300
<b>Standard Deviation</b>	836108,1	232212,2
Sample Variance	$6,99E+11$	$5,39E+10$
Minimum	5	0,0875
Maximum	7257600	1238400
Based on number of articles	79	39
Frequency ranges [MHz](number)‡		
$0 - 30$	3	2
$31 - 200$	7	2
201-900	38	9
901-1200	7	
1201-1800	4	5
1801-2000	3	4
>2000	19	16
Journal Impact Factor +		
Mean	2,079973	2,449725
Median	2,291	2,371
Mode	0,73	2,291
<b>Standard Deviation</b>	1,094949	0,897919
Sample Variance	1,198914	0,806259
Minimum	0, 13	0,246
Maximum	4,411	4,411
Based on number of articles	73	40

Table 7. Analysis of differences in articles between RF-EMF effect and no-effect studies.

a Country: location of the university where main author or research group are based. Data tested by Fisher Exact Test (p-value = 0.1595). <sup>b</sup> Exposure duration [mins]: duration of exposure of target subject in minutes as reported by author. Data tested by Kruskal-Wallis (p-value = 0.9514).  $^{\circ}$  Frequency ranges [MHz]: type of RF-EMF frequency ranges applied in studies. Data tested by Fisher Exact Test (p-value = 0.03531). d Journal Impact Factor: impact factor of journal of publication, if available, of RF-EMF study as reported by Journal of Citation Reports on the Web (JRC WEB). Data tested by Kruskal-Wallis (p-value = 0.3233)

The comparison of the countries of origin of the main authors and research groups showed in both groups a clear prevalence of studies coming from the USA (Table 7). Among the studies that did find a significant effect the most represented countries were India, Greece, France, Croatia, Germany, and Latvia (see Table 7). A lower variation in countries was found in the case of no-effect studies.

The analysis of the duration of the exposure showed that exposure was on average twice as high in the case of positive studies than in studies with no significant effects. Minimum and maximum values were also higher in the first case (see Table 7).

The distribution of studies according to the RF-EMF frequencies applied confirmed a clear prevalence of the range between GSM and MW lower band in the case of studies finding an effect. Most of the studies which did not find an effect applied RF-EMF frequencies higher than 2000 MHz (see Table 7). The analysis of the impact factors (JRC WEB 2012) of the journals where the selected articles were published showed on average a higher score for studies not finding an effect (see Table 7).

In conclusion, possible ecological effects of RF-EMF seem to be found more at higher duration in the GSM bands and in the MW frequency bands (> 2000 MHz).

## 5.5

## Minimum requirements for studies ecological effects RF-EMF

In Michaelson (1991) and Beers (1999) attention is paid to the experimental set up of RF-EMF experiments, and to the criteria to conduct biological (therefore, also ecological) RF-EMF field and laboratory studies. The criteria are in line with the propositions of WHO (van Deventer et al. 2011) and their proposed research agenda. None of the studies analysed in this review reported the use of these standard procedures of exposure and analysis.

According to Michaelson (1991) and Beers (1999), experimental conditions should be meticulously defined, selecting the most appropriate animal species to investigate the

effect of RF-EMF: intrinsic physical and physiological dissimilarities between species could be confounding elements. The experiments/studies should include a total precise duration of exposure, the length of periods of exposure, intervals (if any) between exposures and heating amplitude. Relatively to the SAR levels, the experts warn that they are often predicted using models which fail to characterise specific features of the species exposed (bone, tissue, energy deposition, etc.). All the factors that can influence biological responses at the same SAR level (e.g. sex, age, number of subjects, etc.) need to be reported.

As for the setup of laboratory experiments, standard laboratory stressors should be avoided or at least accounted for (e.g. using sham-exposure). The effects of other intervening factors (e.g. temperature, noise, chemicals) should be considered (or avoided).

Relative to the characteristics of the RF-EMF, some effects might be related to (or influenced by) the local geomagnetic field and, oddly enough, by the variation occurring in RF-EMF because of lunar phases (Beers 1999). Other factors that affect the absorption of the RF-EMF (e.g. frequency, polarisation, modulation, field pattern) have to be considered and reported, together with other possible confounding elements (e.g. RF-EMF alien to the experiment/study under investigation).

In the number of studies analysed in this review, it appears that too little attention is paid to these important recommendations. The majority of the reviewed research has been done using small rodents. Scaling of results to other species is needed to further investigate and extend results to the ecosystem level. Some exposure setups are capable of reflecting or focusing the EMF, inducing the SAR levels to increase more than experimenters may have realised, which may lead to erroneous conclusions. There is a clear need for proper dosimetry in experimental procedures with a detailed description of the methods. A special point of attention is the control: not only a control situation, but also a sham situation should be included. This procedure might introduce some extra difficulties in field situations but might still be possible (e.g. by experimentally shutting down the communication stations for a period of maintenance).

There is a great need for more ecological experiment/studies on the effects of RF-EMF, taking into account the reported guidelines. From this ecological review it became, in fact, clear that the way in which RF-EMF were applied and measured was very heterogeneous, limiting the possible comparison of the effects found.

#### Conclusions and recommendations

The screening of literature in the field ranges that were analysed provided a limited number of strictly ecological studies. The distinction between biological studies and ecological studies as intended in this review has been detailed in section 1 of this contribution. Only endpoints that may provide an ecologically relevant picture were selected, in order to quantify significant biological effects, which may provide valuable hints on the ecological implications of results. The effects of RF-EMF on different biological groups were investigated. With reference to the groups under investigations in the selected studies (i.e. birds, honeybees, mammals, plants, Drosophila and others) there is ecologically relevant evidence that the RF-EMF caused an effect in about 50% of the animal studies and about 90% of the plant studies. No studies, in fact, were found on the impact of RF-EMF at the ecosystem level. The sole study by Reijt et al. (2007) investigated the alteration in the interaction among two species of Tits. Only eight studies were conducted in the field.

Nevertheless, an ecological interpretation of the biological studies under review was necessary. The information and results on effects gathered in laboratory studies may need to be cautiously handled due to the sheer nature of the laboratory solutions adopted. The conditions applied in the laboratory studies, in fact, do not always reflect real conditions of exposure, and at times it is important to carefully evaluate the plausibility that biological systems exposed to RF-EMF could likely translate into ecologically relevant effects.

As suggested by the expert panel to the European Commission SCENIHR (2009) and Foster and Repacholi (2004), while it seems appropriate to perform experimental studies using pure experimental RF fields, it may be necessary to emulate the complex modulation patterns and intensity variations typical to real RF-EMF exposure. Few of the studies found were performed in the field and engaged in real exposure conditions and only few laboratory studies dealt with real-exposure modulation.

The ICNIRP guidelines (1998; 2010) provide limiting values as basic restrictions and reference levels for the exposure of humans to RF-EMF. These guidelines have been adopted by most European countries which have imposed limits (EU Commission Implementation Reports 2008). To our knowledge, there are currently no guidelines for the exposure of biodiversity to RF-EMF. The available data has so far been inadequate to judge whether the ICNIRP guidelines and other environmental standards should be the same or significantly different from those appropriate to protect human health (EU 2011).

However, if we consider that the guidelines might protect biodiversity (i.e. with the

consideration of differences in size and exposure conditions), in some studies analysed we encountered applications of dosages hardly experienced by animals and plants in case of real outdoor conditions. As a general trend, no clear relationship was determined between maximum effects found in different studies and the dosage of the RF-EMF applied. Also at very low dosages significant ecologically relevant effects were found. These values are compatible with real field situations, and could be found under environmental conditions. From the limited number of field studies decreasing effects could be determined at increasing distances from the emitting source, but residual relevant effects were still detected as far as 300 metres away and with an average measured electric field of 0.53 V/m, thus 7,45x10-5 mW/cm2 (ICNIRIP limit for general human population 0,0004 V/m).

As ICNIRP suggests (2010), when reference levels are exceeded restrictions values are not necessarily exceeded. Further investigations, need to be undertaken. For instance, localized fields in excess of the reference levels can be emitted by certain devices (i.e. wireless or remotely-controlled devices) but there might be a weak coupling of the field with the body of the exposed target subject (e.g. due to the size of the exposed subject). Therefore, while it is not possible to rule out the adverse ecological relevance of effects, ICNIRP (2010) and WHO (2010) suggest to extrapolating only cautious indications on the global impact of RF-EMF on ecosystems.

Considering the relevant remark of Beers (1999) "a long list of reports of positive results yielded by inadequate experiments may appear impressive in a review and yet mean little". No clear relationships, in fact, could be found between dosage and effects because of a wide variety of exposure strengths, durations, conditions, frequencies, time between exposures, assessment methods, measurement systems, replications efforts, and adequate dosimetry. In the older laboratory studies the interpretation of results needs to be filtered by the consideration of a lack of control of temperature. In the other studies the balance of experimental evidence points towards a non-thermal effect of RF-EMF exposure. In field studies additional confusion might be caused by the simultaneous exposure to multiple field strengths and frequencies and other environmental confounding variables. A similar conclusion can be drawn for those laboratory studies that did not adequately control the exposure to other sources of electromagnetic fields, in which also the influence of other variables on the result was usually not handled in the design or in the analysis.

The plotting of the size of the ecologically relevant effects in relationship to the dose conditions applied did not seem to define a trend. Thus, the result of the graphical metaanalysis leads to no definitive conclusions about whether the effects are real, not real, or can be found only under certain conditions. The study of the differences between significant and non-significant studies presented in section 5 revealed differences in the duration of the exposure of the target subjects, in the selection of the frequency band of exposure and in the impact score of the journals where articles were published.

Potential further sources of bias should be further examined using tools such as *funnel or forest plots* (Egger et al. 1997; Peters et al. 2006, 2008). These might reveal asymmetries due to: location biases (e.g. language bias, citation bias, multiple publication bias), heterogeneity (e.g. intensity of intervention, differences in odds ratios),data irregularities (e.g. poor or inadequate analysis), poor choice of effect measure, and chance.

At the current state of our knowledge, it is possible to conclude that there is an urgent need for repetitions of experiments and field studies by other research groups and under other (standard) situations and setup in order to confirm the presence/absence of effects. We, once again, refer to the ICNIRP statement of 2010, suggesting that results can only be accepted 'for health risk assessment if a complete description of the experimental technique and dosimetry are provided, all data are fully analysed and completely objective, results show a high level of statistical significance, are quantifiable and susceptible to independent confirmation, and the same effects can be reproduced by independent laboratories (Repacholi and Cardis 1997). If the significant conclusions found by studies are confirmed, they will be important for a mechanistic understanding of the interaction of RF fields with ecosystems.

In the synthesis the requirements to conduct an adequate study of the (ecological) effects of RF-EMF have been described in detail. Advances in dosimetric investigations in terms of precision and resolution were appreciable in some of the more recent studies, while standards seemed to be totally neglected in others. The application of the suggested best practise would allow to handle the information on the reported effect or absence of effect with greater precision.

Our review highlights that there is a clear need for the study of the effects of RF-EMF on more species and organisms and, by means of field studies, on populations and interactions between species. Studies at the ecosystem level should start from the consideration of micro-ecosystems and micro-cosmos, which would allow for laboratory results to be more informative and ecologically-relevant, also at a policy level.

The number of experiments assessing new technologies is limited: only 5 matched the ecological criteria set in this review. Experiments evaluating the impact of newer wireless technologies (e.g. WiMAX, WLAN, WiFi), together with studies analysing new generations of mobile phone technologies (e.g. 3G, 4G) would shade some light on the impact of these technologies for ecosystems. To our knowledge solely the study on mice by Lee et al. (2009) investigated the possible impacts of these technologies. In order to minimize the uncertainties as efficiently as possible a number of situations with limited number of studies should be investigated: the long-term monitoring of selected species and/ or ecosystems, field studies under a controlled system of exposure, laboratory studies following given recommendations, and studies on important ecological groups, other than those here analysed, would be a solid base on which to focus future studies.

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## Appendix

Keywords for literature screening.

Main search strategy

RF-EMF OR SAR OR electromagnetic OR "power density" OR "internal electric field" OR "current density" OR nonionising OR non-ionizing OR RF OR "electric field" OR "magnetic field" OR Wi-Max OR WiMax OR W-LAN OR WiFi OR Wi-Fi OR modulation OR DCS OR GSM OR FM OR UMTS OR AM OR television OR TV or FM or AM or radio OR transmitter OR broadcast OR antenna OR aerial OR "base station" OR phone OR wireless OR DECT OR TETRA OR radar OR phone mast

## AND

reproduction OR fecundity OR mortality OR behaviour OR behavior OR activity OR density OR growth OR navigation OR orientation OR eco\* OR malformation OR insect OR honey bee OR bee OR bat OR fruitfly OR mammal OR plant OR fauna OR biodiversity OR community OR population OR wildlife OR animal OR organism OR tree OR plant OR fish OR invertebrates OR fauna OR flora OR fungi OR birds OR vegetation