# Review of exposure setups for biological experiments in the radiofrequency range: specifications and emerging trends

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## Abstract

Concern on possible health hazard due to the exposure to radiofrequency electromagnetic fields, as those emitted by mobile telecommunication equipments, has led to the development of biological investigations, both *in vitro* and *in vivo*, to examine possible effects of such fields on biological systems. In the past years, criteria for improving the quality of the exposure and the repeatability of experimental studies were pointed out.

In this work, most of the available exposure systems have been reviewed and classified on the basis of the reference electromagnetic structure and the kind of investigation they have been designed for. General features of more common structures have been discussed. Moreover, besides basic requirements for the exposure systems, specifications rising from emerging trends of *in vivo* and *in vitro* biological investigations have been identified.

## 1. Introduction

Since the early development of mobile telephony, concern aroused on the possibility of the exposure to electromagnetic (EM) signals in the radiofrequency (RF) range having hazardous effects on population health. Consequently, a number of experiments, both *in vivo* and *in vitro*, were carried out [1], [2], aiming at the evaluation of possible biological effects of RF EM fields. The obtained results were contradictory and often difficult to replicate, mostly due to the inadequate knowledge and reproducibility of the exposure conditions.

Since the 90s, the need of a common approach to the bioelectromagnetic research became evident, as pointed out by a number of workshops and publications yielded by the scientific community. In 1994, the Wireless Technology Research (WTR) held a workshop to highlight the appropriate directions for development of *in vitro* and *in vivo* exposure systems [3]. In 1996, the EMF Project of the World Health Organization (WHO) fixed and emphasised these concepts in specific recommendations [4]. Such items, together with a deep discussion on quality assurance, were the main arguments of two COST 281 workshops: *"Exposure systems and their dosimetry"*, in Zurich, in February 1999 and *"Forum on Future European Research on Mobile Communications and Health"*, in Bordeaux, in April 1999. Recommended minimal requirements for exposure systems, in order to obtain reproducible and scientifically valuable results, were synthesized in [5]. Basically, two classes of requirements can be identified: biological and electromagnetic ones. Biological requirements are dictated by the experimental equipment and protocol; the other ones define the exposure parameters on the biological target.

During the last ten years, several cooperative research programmes, e.g. the European projects PERFORM A, PERFORM B and RAMP2001, have been carried out. The necessity of conducting a coordinate research activity in laboratories of different countries has arisen the issue of whether standardized exposure systems and protocols should be used. This was one of the topic of the Workshop: *"EMF health risk research lessons learned and recommendations for the future"* held in Monte Verita in November 2005. The outcome of the work stated that, due to different endpoints and protocols used in bioelectromagnetic investigations, exposure setups cannot be standardized. In the same time, strong quality control on dosimetry is mandatory to assure the repeatability and reproducibility of results even when different exposure systems are used [6].

Recently, the introduction of new communications standards has widened the investigations on possible adverse effects of a continuous exposure to low-level RF fields. According to that, *in vivo* investigation is currently moving towards large scale and long term exposure for ultimately assessing the EM field carcinogenicity; on the other hand, real-time electrophysiological recording from *in vitro* neuronal preparations is revealing a promising technique for the study of possible EM effects on cognitive functions. Emerging experimental protocols have led to new specific requirements for the design of the exposure systems which, moving from standard EM structures, such as TEM cells,

rectangular waveguides etc., have to be adapted to the particular kind of experimental protocol and exposure parameters in order to minimize any possible confounding factor [5]. As examples, in real-time setups, special care must be devoted to the problems of interference and EM compatibility while, in large scale and long term studies, the exposure setup must guarantee a high statistical power and conditions of minimum stress for the animals.

Aim of this work is to give a brief survey of the most used RF exposure systems, both *in vivo* and *in vitro*, published in international journals from 1999 up to now. Special discussion will be reserved to those setups designed *ad hoc* to meet specifications rising from new emerging bioelectromagnetic investigations.

## 2. Review of the exposure systems

The development of exposure systems for biological experiments in the RF range can be represented by a sequence of steps, according to the approach proposed in [4]. Moving from biological protocol and requirements on exposure parameters, the most suitable EM basic structure is chosen and designed on theoretical bases. Generally, the basic structure must be modified in order to meet biological and EM requirements. Thus, a process of adaptation and optimization of the system takes place using numerical tools. To know the EM dose induced in the biological system (animal or cell culture), numerical dosimetry is carried out. As a last step, after the setup fabrication, numerical results must be experimentally validated [5], [6]. Biological requirements represent a crucial point for the design of an exposure system since they could be the most limiting ones, especially when a particular equipment and protocol procedures are needed. Due to the great variety of biological targets and protocols, a number of different exposure systems have been employed in the experimental investigation, both *in vivo* and *in vitro*.

In this review, only considering papers published in international journals over the last nine years, 42 *in vitro* and 33 *in vivo* different exposure systems have been identified. Despite their peculiarities, they can be classified, from an EM point of view, into three main groups: radiating, propagating and resonant structures. In the following two sections, the *in vitro* and *in vivo* setups will be separately examined.

### 2.2 *In vitro* setups

With respect to the experimental protocol, *in vitro* exposure systems have been classified in two different groups: off-line and real-time, depending on the kind of analysis they have been designed for. Proposed classification is reported in Table I

Table I: Classification of the reviewed *in vitro* systems

| | | | | |
|---|---|---|---|---|
| OFF-LINE | RADIATING | Horn antenna | 2 | Peinnequin et al., *Bioelectrochem.*, 2000; Zhao, *Phys. Med. Biol.*, 2005 |
| | | Dielectric lens | 1 | Iyama et al., *Bioelectromagnetics*, 2004 |
| | | Microstrip ant. | 1 | Araneo & Celozzi, *IEEE Trans. Electromagn. Compat.*, 2006 |
| | PROPAGATING | TEM, GTEM cells | 7 | Guy et al., *Bioelectromagnetics*, 1999; Lim et al., *Int. J. Num. Mod. Elec. Net. Dev. Fields,* 2002; Desta et al. *Radiat. Res*., 2003; Zmyslony et al. *Bioelectromagnetics*, 2004; Sarimov et al., *IEEE Trans. Plasma Science*, 2005; Nikoloski et al. *Bioelectromagnetics*, 2005; Zeni et al., *Bioelectromagnetics.*, 2007 |
| | | Rect. waveguide | 7 | Sajin et al., *Trans. Microw. Theory Tech*., 2000; Schirmacher et al., *Bioelectromagnetics*, 2000; Bismuto et al., *Eur. Biophys. J.,* 2003; Markkanen et al. *Bioelectromagnetics*, 2004; Zeni et al. *Bioelectromagnetics*, 2005; Gerber et al., *IEEE Trans. Plasma Science,* 2006; Hoyto et al., *Int. J. Radat. Biol.*, 2006 |
| | | Cyl. waveguide | 1 | Gajda et al., *Bioelectromagnetics*, 2002 |
| | | Radial waveguide | 3 | Moros et al. *Bioelectromagnetics*, 1999; Pickard et al., *IEEE Trans. Biomed. Eng.*, 2000; Franke et al., *Radiat. Res*., 2005 |
| | | Other | 2 | Gaber et al., *Bioelectromagnetics*, 2005; Belloni & Nassisi, *Rev. Sci. Instr.*, 2005 |
| | RESONANT | Short-circuited rect. waveguide | 3 | Shonborn et al., *Bioelectromagnetics*, 2000; Schuderer et al. *IEEE Trans. Microw. Theory Tech*., 2004; Calabrese et al., *IEEE Trans. Microw. Theory Tech*., 2006 |
| | | Parallel plates res. | 1 | Mashevich et al., *Bioelectromagnetics*, 2003 |
| | | Wire patch cell | 3 | Laval et al., *Bioelectromagnetics*, 2000; Ardoino et al., *Radiat. Protec. Dosimetry,* 2004; Joubert et al., *Int. J. Radat. Biol.*, 2006 |
| REAL-TIME | RADIATING | Horn antenna | 1 | Yoon et al., *IEEE Trans. Plasma Science*, 2006 |
| | PROPAGATING | Modified rect. waveguide | 5 | Linz et al., *Bioelectromagnetics*, 1999; Pakhomov et al., *Bioelectromagnetics*, 2000; Hagan et al., *IEEE Trans. Plasma Science,* 2004; Lambrecht et al., *IEEE Trans. Plasma Science,* 2006; Koester et al., *Bioelectrochem*., 2007 |
| | | Modif. TEM cell | 1 | Linz et al., *Bioelectromagnetics*, 1999 |
| | | Modif. stripline | 1 | Ramundo-Orlando et al., *Bioelectromagnetics*, 2004 |
| | | Parallel plates | 1 | Tattersall et al., *Brain Res.*, 2001 |
| | | Coplanar waveguide | 2 | Liberti et al., *IEEE Trans. Microw. Theory Tech*., 2004; Paffi et al., *IEEE Trans. Microw. Theory Tech*., 2007 |

For what concerns off-line setups, the most used among different research groups are TEM cells, rectangular waveguides and short-circuited waveguides. The first two are propagating structures, whose main advantages are EM field uniformity and versatility. With a proper dosimetric characterization, TEM cells can be used with several kinds of sample holders (multiwells, Petri dishes, flasks etc.). Resonating systems, such as short-circuited waveguides, are closed and compact structures, which can be easily placed inside an incubator, when a strict environmental control is needed for the well being of the cells during the experiment. They are characterized by high efficiency, in term of absorbed dose per unitary input power, but the positioning of the sample is critical, due to the extremely localized region of field uniformity. On the contrary, radiating systems, usually consisting of commercial antennas, present reduced efficiency and low uniformity of dose, but generally allow the simultaneous exposure of a lot of samples.

Special attention has been recently deserved to real time acquisitions during RF exposure, in order to identify possible cumulative or reversible effects. In particular, electrophysiological techniques are widely spreading in the study of the interaction between nervous system and EM fields. This particular kind of analysis imposes additional constrains which can be summarized in the easy access to the biological sample and the minimal coupling with the acquisition setup. To meet such requirements, two main solutions was presented: closed structures (TEM cell, rectangular waveguide) with holes for sample observation and electrode insertion, and open systems (coplanar waveguide) specially designed to have the field confined in a small volume around the surface. This latter solution also implies high efficiency values [7].

## 2.1 *In vivo* setups

For what concerns the experimental protocol, *in vivo* setups have been classified in two main classes, depending on whether the exposure involves the whole body or is focalized into a specific organ. An additional criterion of organization is the possibility for the animals to move (freely moving) during the exposure. Proposed classification is reported in Table II.

Table II: Classification of the reviewed *in vivo* systems

| | | | | | |
|---|---|---|---|---|---|
| LOCAL EXPOSURE | RESTRAINED | RADIATING | Loop antenna | 5 | Chou et al., *Bioelectromagnetics*, 1999; Dubreuil et al., *Behav. Brain Res.*, 2002; Leveque et al., *IEEE Trans. Microw. Theory Tech*., 2004; Jia et al., *Bioelectromagnetics*, 2007; Lopresto et al., *Radiat. Protec. Dosimetry*, 2006 |
| | | | Wav. antenna | 1 | Wake et al., *IEEE Trans. Microw. Theory Tech*., Mar. 2007 |
| | | | Sleeve dipole ant. (*CAROUSEL*) | 3 | Moros et al. *Bioelectromagnetics*, 1999; Swicord et al., *Bioelectromagnetics*, 1999; Schonborn et al., *Bioelectromagnetics*, 2004 |
| | | | Monopole ant. (*CAROUSEL*) | 3 | Shirai et al., *Bioelectromagnetics*, 2005; Wang et al., *IEEE Trans. Electromagn. Compat.,*2006; Wake et al., *IEEE Trans. Microw. Theory Tech*., Feb. 2007 |
| | FREELY MOVING | RADIATING | Loop antenna | 1 | Bahr et al., *Radiat. Protec. Dosimetry*, 2007 |
| WHOLE BODY | RESTRAINED | PROPAG. | TEM cell | 1 | Ardoino et al., *Phys. Med. Biol.*, 2005 |
| | | | Rect. waveguide | 1 | Eikkinen et al., *Radiat. Res.*, 2001 |
| | | RESONANT | Resonant cavity | 5 | Balzano et al., *IEEE Trans. Microw. Theory Tech*., 2000; Ebert et al., *Phys. Med. Biol.*, 2005; Kainz et al., *Phys. Med. Biol.*, 2006; Tillmann et al., *Bioelectromagnetics*, 2007; Oberto et al., *Radiat. Res.*, 2007 |
| | FREELY MOVING | RADIATING | Horn antenna | 2 | Chagnaud & Veyret, *Int. J. Radiat. Biol.,*1999; Adey et al., *Radiat. Res.*, 2000 |
| | | | Microstrip ant. | 1 | Araneo & Celozzi, *IEEE Trans. Electromagn. Compat.*, 2006 |
| | | | Parabolic reflect. | 1 | Schelkshorn et al., *Radiat. Protec. Dosimetry*, 2007 |
| | | | Flat spiral ant. | 1 | Bartsch et al., *Radiat. Res*, 2002 |
| | | | Base station ant. | 1 | Anane et al. *Radiat. Res*, 2003 |
| | | PROPAG. | GTEM cell | 1 | Bakos et al., *Bioelectromagnetics*, 2003 |
| | | | Rect. waveguide | 1 | Aitken et al., *Int. J. of Andr.*, 2005 |
| | | | Radial waveguide | 4 | Hansen et al. *IEEE Trans. Electromagn. Compat.*, 1999; Reinhardt et al., *Radiat. Protec. Dosimetry*, 2007; Lerchl et al., *J. Pineal Res.,* 2007; Kumlin et al., *Radiat. Res*, 2007 |
| | | | Flared par. plate | 1 | Wilson et al., *Bioelectromagnetics*, 2002 |

Systems used for local exposure are small antennas placed in the vicinity of the target organ (brain, ear, eye) to induce significant and localized power absorption. A single antenna can be used for the simultaneous exposure of several animals if they are arranged in a sort of carousel around it. Generally, animals are restrained with plastic holders for a more accurate exposure even though body-mounted antennas become necessary for the well being of animals when the exposure is prolonged.

For whole body exposure setups, one of the most demanding requirements is the uniformity of dose absorbed by animals of the same group and within each animal. This is difficult to achieve especially for large scale experiments. Radiating structures, in spite of providing the exposure of a lot of bodies to a plane-wave equivalent field, present low efficiency. In closed structures, such as rectangular or radial waveguides, the efficiency is higher and a further increase is observed in resonant cavities, obtained by shorting radial waveguides. These structures are largely used since they provide collective illumination of numerous bodies (up to 65 animals in each structure) in a limited space. Nevertheless, they are narrow-band operating and, due to the relevance of body positioning, animals are restrained within plastic cylinders. This makes them not much suitable for chronic long term (days or moths) exposures even if they were used for some two years bioassay experiments limiting the exposure to few hours per day to reduce the stress (PERFORM A). Some protocols require the animals to be exposed during all the period of their life, so they must be allowed to move inside plastic cages provided with food and water. This generally implies a reduction in the uniformity of the absorbed dose which depends on position and orientation of the body with respect to the field; moreover the presence of the water must be taken into account when dosimetry is carried out. Nevertheless, for very long exposures, each animal should reasonably absorb the same mean dose, even though an accurate dosimetry is necessary to assess uncertainty and variation [8]. The evidenced trend towards long term and large scale experiments is confirmed by the study conducted by the IT'IS Foundation, Zurich, Switzerland, for the use of a reverberation chamber in testing a large number of rodents at cellular telephone frequencies [9]. Accurate dosimetric studies are also required for those recent experiments aiming at the evaluation of the effects of the RF exposure on growing up animals, due to the great differences in size and weight as a function of age, sex, and pregnancy.

## 3. Conclusions

From the proposed classification of the exposure systems, both *in vitro* and *in vivo*, it is evident as, moving from a limited number of standard EM structures, a lot of exposure setups have been developed, able to meet specific requirements imposed by the biological protocols. The procedure of adaptation and optimization, during the design of the system, becomes demanding especially when the experiment needs a particular equipment and procedure, as in real-time acquisitions which are assuming growing importance, in particular in neurophysiologic recording and for new emerging communication standards. Due to the great variety of exposure setups which cannot be standardized, the knowledge of the induced EM dose is an essential precondition to compare results obtained in different laboratories. It is worthwhile to highlight this point since, in spite of the efforts of the scientific community, it is still possible to find some papers where the exposure conditions are not controlled. Another unavoidable aspect that must be taken into account when designing an exposure setup is the well being of the exposed target, animal or cell culture, in order to rely on the results achieved from an exposure experiment. The avoidance of the stress for the animals and an accurate dosimetric evaluation have to be carefully taken into account especially in large scale, long term exposure, emerging trend for the *in vivo* biological investigation.

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