

# Considerations for Developing an RF Exposure System: A Review for *in vitro* Biological Experiments

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**Abstract**—This paper provides a detailed review and classification of exposure systems used in RF *in vitro* research from 1999 up to 2009. Since different endpoints and protocols are used in bioelectromagnetics studies, exposure systems cannot be standardized. However, a standardized procedure to achieve the optimum design of the exposure system is suggested. Following this procedure will lead to a known dose distribution within the biological sample and allow a better comparison with other *in vitro* studies. In addition, the quality of the study will be such that it will be more likely to be included in assessment procedures such as health-risk assessments.

**Index Terms**—Exposure systems, *in vitro* biological experiments, review, RF.

## I. INTRODUCTION

THE SCIENTIFIC literature on electromagnetic (EM) fields contains a large number of conflicting results, especially among those studies evaluating whether exposure to RF fields causes biological effects. Much of this conflict can be attributed to inaccurate dosimetry and to a lack of well-characterized exposure conditions. In many cases, especially in studies published prior to the 1990s, insufficient effort was made by investigators to ensure that their biological samples were exposed to a known “dose,” e.g., the specific absorption rate (SAR) or SAR distribution, to within, say, 3 dB. Only information about the incident field was provided and little or no attention given to field strengths induced within tissue samples or cells. In terms of dosimetry, this is considered insufficient since the ratio between induced fields and incident fields can be highly variable depending on the exposure conditions [1]. In particular, in [2], it was explained how numerical calculations can give proper information on distribution of the induced fields

within the sample showing that variation can be enormous. For example, in experiments with poor dosimetry, having *in vitro* samples exposed to highly nonuniform RF fields, but having low average intensities, may produce positive results. However, if researchers claim that low nonthermal RF fields produced the effect, one cannot rule out that it was due to highly localized peak fields.

Since the International EM Field Project was established at the World Health Organization (WHO) in 1996, WHO’s EM field research agendas have emphasized the importance of accurate dosimetry in all scientific studies. Well-defined and characterized exposure conditions are necessary for health-risk assessments [3]. The reason for this is clear, unless the “dose” is accurately known, the results of EM field studies will have little value for determining exposure thresholds for health risks or for development of exposure limits in standards.

Some authors have tried to provide the necessary characteristics for RF exposure systems to increase the accuracy of the dose induced. For example, [4] and [5] provided information concerning optimization of exposure and how to evaluate the fundamental designs of *in vitro* exposure setups including their advantages and disadvantages.

During the past ten years, several cooperative European and national RF research programs have been carried out. At the commencement of the first European Commission project on EM field studies, discussion on quality assurance led to shared objectives. In particular, a number of European Cooperation in Science and Technology (COST) workshops have been devoted to defining exposure conditions that lead to reproducible and scientifically meaningful results. For example, the COST workshop “Exposure Systems and their Dosimetry,” held in Zurich, Switzerland, in February 1999 [6], [7], and the workshop on “Forum on Future European Research on Mobile Communications and Health,” held in Bordeaux, France, in April 1999 [8].

Following these discussions, the exposure systems adopted in the European projects (see [9, Table I]) were: wire patch cells, TEM cells, and coplanar waveguides used in RAMP2001 (Risk Assessment for Exposure of Nervous System Cell to Mobile Telephone EMF: from *in vitro* to *in vivo* Studies); short-circuited waveguides in REFLEX (Risk Evaluation of Potential Environmental Hazards from Low Energy EM Fields Exposure Using Sensitive *in vitro* Methods); rectangular waveguides in CEMFEC (Combined Effects of EM Fields with Environmental Carcinogens); short-circuited waveguides, TEM cells, and wire patch cells in Perform B (*In vitro* and *in vivo* Replication Studies Related to Mobile Telephones and Base Stations); and wire patch cells in the CRADA-CTIA

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The necessity of conducting coordinated research activities in laboratories of different countries has raised the question of whether standardized exposure systems and protocols should be used. This was discussed at the EMF-NET (Effects of the Exposure to Electromagnetic Fields: from Science to Public Health and Safer Workplace) workshop “EM Field Health Risk Research Lessons Learned and Recommendations for the Future” held in Monte Verita, Switzerland, in 2005 [10]. It was concluded that, because of the different endpoints and protocols used in bioelectromagnetics studies, exposure setups could not be standardized. However, the workshop did conclude that strong quality control on dosimetry is mandatory to assure repeatability and reproducibility of results, even when different exposure systems are used [10]. Some of the authors contributing to these discussions provided specifications that have to be met when designing an *in vitro* exposure system [9].

The issues to be discussed for exposure system design include the biological parameters (biological target, statistical power, exposure environment, end-point(s) to be studied, and the effect of the sample holder) and exposure characteristics (signal, dose, control, and monitoring of dose, sham versus blind conditions, and EM compatibility) [9].

To address these issues, we carried out a detailed review of exposure systems used in RF *in vitro* research from 1999 up to 2009, providing a classification and evolving a standardized procedure for optimal exposure design. More than 100 papers from 28 journals have been reviewed, resulting in the assessment of 51 exposure systems.

The purpose of our review is to discuss the strengths and weaknesses of the various systems used, the frequency ranges over which they are applicable, the type and number of sample holders that can be contemporaneously exposed, the exposure features, and their usefulness for exposing the target tissue or cell sample. Current exposure systems have been designed for frequencies up to about 5 GHz, but systems are now required for investigations at higher frequencies, maybe as high as 10 GHz. This paper will also identify features of exposure systems that lead to optimal conditions needing to be incorporated for these higher frequency studies.

## II. CLASSIFICATION

### A. Classification Criteria

From the more than 100 papers, some focused on the design and characterization of *in vitro* exposure systems, whereas others reported biological experiments involving exposure of cell cultures and tissues to RF fields. Among them, only those reporting a new system or ones adapted to new experimental conditions have been considered. Moreover, biological papers where the exposure system is not described or the dose delivered in terms of the SAR is not given have not been included. The list of the 51 exposure systems with their reference is given in Table I.

Our classification of exposure systems was based on those of the authors in [9]. They are based on the experimental protocol

and distinction between offline or real-time systems (Table I). Offline exposure systems, most used by research groups, presuppose experimental data are collected at the end of the exposure, while for real-time setups, data are collected during the RF exposure.

This basic subdivision is further classified according to their reference RF structure and divided in three main families: radiating, propagating, and resonant.

Radiating systems usually consist of commercial or *ad hoc* antennas, such as horn and microstrip antennas, generally exposing samples in the far-field region. They allow simultaneous exposure of many samples, but generally have low uniformity of dose among samples and reduced efficiency in terms of SAR per unit of input power.

Propagating systems are used in many bioelectromagnetics investigations and include different RF structures such as TEM cells and various waveguides (rectangular, circular, radial, coplanar). Their main advantages are versatility and RF field uniformity.

Resonant systems, such as short-circuited waveguides, are closed and compact structures, which can be easily placed inside an incubator when strict environmental control is needed. They are characterized by high efficiency, but the positioning of the sample is critical due to the extremely localized region of field uniformity.

A special type of resonant system is the wire patch cell, based on a wire patch antenna, with the samples positioned inside the structure between the two patches that are short-circuited by metallic rods [11].

In Table I, each system is described by the number and type of sample holders that can be exposed, the operating frequency or the frequency range for wideband systems, the efficiency, and the SAR homogeneity in the sample that is expressed in terms of the coefficient of variation (CV: equal to standard deviation divided by mean value). The information is omitted if it is not provided or cannot be easily determined from the paper. Some of the papers included in the table do not report efficiency or homogeneity. However, all of them give a dosimetric evaluation based on numerical simulations and/or experimental measurements.

These parameters allow an initial comparison among different systems and are described in detail in Sections II-B and C.

### B. Offline Systems

Among offline exposure systems, the most commonly used RF structures are propagating ones, but resonant and radiating structures are also employed according to the experimental requirements.

1) *Propagating Structures*: Propagating structures are mostly closed and confine the RF field inside, and provide good versatility to different situations, usually guaranteeing a uniform field in the biological sample. Based on their propagating structures, 24 different exposure systems were collected. Most of them are based on TEM cells (ten) [12] and rectangular waveguides (six) because these are the best-established structures. However, cylindrical and radial waveguides (three) have also been used.

TABLE I  
CLASSIFICATION OF EXPOSURE SYSTEMS WITH THEIR REFERENCE, NUMBER, AND TYPE OF SAMPLE HOLDERS,  
OPERATING FREQUENCY, SAR EFFICIENCY, SAR HOMOGENEITY, AND NOTES ON TYPE OF DOSIMETRY CONDUCTED

EXPOSURE SYSTEM		REF	SAMPLE HOLDER		FREQ. (MHz)	EFFICIENCY (W/kg)/W	HOMOG. (%)	NOTES		
			#	type						
OFF-LINE	PROPAGATING	TEM cell	[2]	6; 2	Tube; 60 mm Petri dish	837	> 0.10; ≈ 0.10	≈ 80; ≈ 140	Numerical and experimental dosimetry	
			[13]	4	T25 flask	835	6	< 52	Numerical and experimental dosimetry	
			[14]	4	25 mL flask	835	—	—	Experimental dosimetry (temperature measurement)	
			[15]	6	4-well plate	900	0.5	50	Numerical and experimental dosimetry	
			[16]	1	5 mL tube	895+915	0.0215	53	Numerical and experimental dosimetry (power balance)	
			[17]	1	35 mm Petri dish	900	0.144	43	Numerical dosimetry	
			[18]	2	25 mL flask	900	—	—	Numerical dosimetry	
			[19]	2	96 and 24-well plate	900	—	—	Numerical dosimetry	
			[20]	4	25 mL flask	1950	—	—	Numerical dosimetry	
			[21]	2	Petri dish	835	—	—	Experimental dosimetry (temperature measurement)	
		GTEM cell	[22]	1	15 mm tube	930	0.2	—	E field measured in the empty structure. SAR theoretically calculated	
			[23]	1	25 cm <sup>2</sup> flasks	935	—	—	SAR theoretically calculated	
		Rect. waveguide	[24]	1	3 mL cuvette	1950	—	—	Experimental dosimetry (power balance)	
			[25]	10	5 mm well	2450	—	—	Only the incident power measured	
			[26]	4	T25 flasks	900	—	16	Numerical and experimental dosimetry (power balance)	
			[27]	4; 2	T25 flask; T75 flask	900	0.40; 0.19	39; 15	Numerical and experimental dosimetry	
			[28]	8	Flasks	1800	—	—	Numerical dosimetry	
			[29]	1	T25 flask	2450	—	—	Experimental dosimetry (power balance)	
		Radial waveguide	[30]	16	T75 flasks	800+2500	0.016 @ 835; 0.245 @ 2450	15.6; 20.4	Experimental dosimetry (temperature measurement)	
			[31]	24	Cylindrical receptacles	383; 900; 1800	0.34 @ 900	—	Numerical dosimetry	
			[32]	16	T75 flasks	835.62; 915; 2450	<0.1	—	Numerical and experimental dosimetry of [30] with different dielectric loads	
		Cyl. waveguide	[33]	1	Petri dish	1900	8.6	24	Experimental dosimetry	
		Other	[34]	1	35 mm cell plates	900	—	—	SAR theoretically calculated	
			[35]	1	13 mm tube	900	120	—	Experimental dosimetry	
		RESONANT	Short-circuited rect. waveguide	[36]	6	35 mm Petri dish	1800	>10 (susp.); >50 (mon.)	40 (susp.); <30 (mon.)	Numerical and experimental dosimetry
				[37]	2	Tubes	800	1.61; 2.28	6.8; 12.1	Numerical and experimental dosimetry. Efficiency and homogeneity for two different tubes exposed simultaneously
				[38]	1	100 mm Petri dish	1763	5+10	—	Numerical and experimental dosimetry
				[39]	8	35 mm Petri dish	900	0.49 (susp.); 1.3 (mon.)	54 (susp.); 20 (mon.)	Numerical and experimental dosimetry
				[40]	8	60 mm Petri dish	1710	1.5+2	25+35	Numerical and experimental dosimetry

The TEM cell provides exposure conditions similar to those of free-space and presents great versatility for adaptation to

different experimental requirements [2], [13]. As an example, TEM cells have been used in the European Union (EU) projects

TABLE I  
(Continued.) CLASSIFICATION OF EXPOSURE SYSTEMS WITH THEIR REFERENCE, NUMBER, AND TYPE OF SAMPLE HOLDERS, OPERATING FREQUENCY, SAR EFFICIENCY, SAR HOMOGENEITY, AND NOTES ON TYPE OF DOSIMETRY CONDUCTED

REAL-TIME			[41]	2	Petri dish	872; 900	2.5	30	Numerical dosimetry	
			[42]	up to 4	35 mm Petri dish	1950	>22	<33	Numerical and experimental dosimetry	
			[43]	1	Rect. culture dish	2450	<73	—	Numerical and experimental dosimetry	
		<b>Parallel plates resonator</b>	[44]	1	25 cm <sup>2</sup> flask	830	9.4	—	Numerical and experimental dosimetry	
		<b>Wire patch cell</b>	[11]	8	35 mm Petri dish	900	≈ 0.5	30	Numerical and experimental dosimetry	
			[45]	4	35 mm Petri dish	1800	1.25	25	Numerical and experimental dosimetry	
		<b>RADIATING</b>	<b>Horn antenna</b>	[46]	1	96-well plate	2450	—	—	Experimental dosimetry (calorimetrically evaluated)
				[47]	1; 2; 4	35 mm Petri dish	50000	—	< 20	Numerical dosimetry
				[48]	1	T25 flasks	2450; 8200	0.10; 0.34	—	Numerical dosimetry
	<b>Dielectric lens</b>		[49]	up to 49	35 mm Petri dish	2142.5	0.175 (49 Petri); 0.138 (25 Petri)	59 (49 Petri); 47 (25 Petri)	Numerical and experimental dosimetry	
	<b>Microstrip antenna</b>		[50]	1	35 mm Petri dish	2100	—	—	Numerical dosimetry	
	<b>PROPAGATING</b>		<b>Modif. TEM cell</b>	[53]	1	Round chamber	180; 900	—	—	See [54]
		<b>Modified rect. waveguide</b>	[53]	1	Round chamber	900; 1800	1.66; 3.16	—	Numerical dosimetry	
			[55]	1	Special organ bath	750+1120	>3 @ 1000	78.9	Numerical dosimetry. SAR homogeneity in: (SAR <sub>max</sub> -SAR <sub>min</sub> )/SAR <sub>max</sub>	
			[56]	1	Multi electrode array	1900+2200	—	—	Numerical dosimetry	
			[57]	1	Exposure cell	9200	3300	20 in 2 mm of radius	Experimental dosimetry	
		<b>Parallel plate</b>	[59]	1	Perfusion chamber	700	0.01+0.03	—	Theoretical and numerical dosimetry	
		<b>Coplanar waveguide</b>	[61]	1	35 mm Petri dish	800+2000	17.3 @ 905; 40.9 @ 1750	—	Numerical and experimental dosimetry	
			[62]	2	35 mm Petri dish	800+2000	>27	<7	Numerical and experimental dosimetry	
<b>Modif. stripline</b>	[63]	1	Cuvette	2450	—	—	Experimental dosimetry (temperature measurement)			
<b>RESONANT</b>	<b>Modified rect. waveguide</b>	[51]	1	Cell-perfusion apparatus	750+1120	≈2 @ 1000	30 over the 83% of the area	Numerical dosimetry		
<b>RADIATING</b>	<b>Horn antenna</b>	[52]	1	Cell-perfusion apparatus	1000+6000	0.07 @ 6000	66.5	Numerical dosimetry		

cited above, with different sample holders, such as flasks [14] and multiwells (see Fig. 1) [15]. They are preferred for *in vitro* studies since they can be easily placed in a standard incubator. The typical efficiency values are around 1 (W/kg)/W although there is great variability. For example, the efficiency changes from 0.02 (W/kg)/W for 5-mL round-bottom tubes [16] to 0.144 (W/kg)/W for 35-mm Petri dishes [17] up to 6 (W/kg)/W for exposure of four T25 flasks filled with 5 mL of culture medium [13]. Moreover, Guy *et al.* [2] showed, through a numerical study, the differences in SAR values and distributions in various *in vitro* preparations within commonly used

sample holders, such as tubes and Petri dishes. They noted that uniformity of SAR distribution strongly depends on the vessel used and the field polarization. The most uniform SAR for a layer of cells occurred in Petri dishes with the bottom parallel to the *H*-field. For cell suspensions inside standard vessels, it was not possible to achieve satisfactory uniformity of the SAR >70% [2].

All the TEM cells reviewed operated at frequencies from 835 to 915 MHz in the uplink bands of the GSM850 and GSM900 standards. Some authors used commercial TEM cells [13], [15], [18]–[21] with a SAR evaluation, either numerical

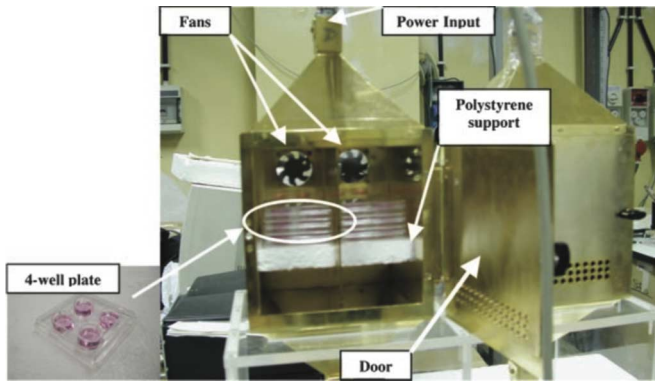


Fig. 1. Two TEM cells for multiwells, as reported in [15, Fig. 1].

[17]–[20] or experimental [14], [21], for the frequency and sample holders employed. For example, Capri *et al.* [19] used the same system as [18] at the same frequency, but with well microplates instead of flasks. In [20], the same TEM cell used in [18] was employed, but at a higher frequency (1950 MHz). Under each condition, a new numerical dosimetry was conducted, even though neither the efficiency, nor the dose uniformity were quantified.

Two commercial GTEM cells were used in [22] and [23] to expose tubes of lymphocytes at 930 MHz and flasks of fibroblasts at 935 MHz, respectively. In both cases the average SAR in the sample was only theoretically estimated. The GTEM cell has broad bandwidth (up to several gigahertz) application and potentially large capacity, but is characterized by a very low volume efficiency (ratio between the target volume and the space of the entire exposure unit). Due to its quite large dimension, it does not fit inside an incubator so *ad hoc* systems to maintain environmental control have to be adopted.

Even the rectangular waveguide is quite a versatile structure, allowing, with satisfactory efficiency values, exposure from 900 MHz up to 2.45 GHz of different kinds of sample holders: cuvettes (1950 MHz [24]), multiwells (2.45 GHz [25]), and flasks (900 MHz [26], [27]; 1800 MHz [28]; 2.45 GHz [29]).

A common characteristic of both TEM cells and rectangular waveguides is the small volume of sample (up to eight flasks) they can expose under similar conditions. A radial transmission line can overcome such limitations as presented in [30]–[32]. It can also be used over a wide frequency band (up to 3 GHz) to simultaneously expose 16 T75 flasks [30], [32] or 24 pineal glands located in cylindrical receptacles [31]. Nevertheless, the efficiency is significantly lower: 0.016 (W/kg)/W at 835 MHz [30], 0.34 (W/kg)/W at 900 MHz [31], and 0.245 (W/kg)/W at 2.45 GHz [30].

The exposure system used in [33] was a truncated cylindrical waveguide based on a totally different principle. The efficiency is high (8.6 (W/kg)/W) and the dose homogeneity good, but due to the fact that only one Petri dish can be exposed at a time, the whole system was made up of six waveguides to have enough statistical power. Moreover, this system allows only the exposure of cells not needing CO<sub>2</sub> since it does not fit into an incubator so an arrangement has to be set up to control the environment of the sample.

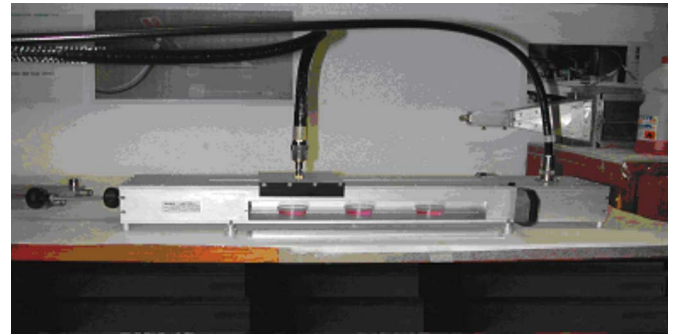


Fig. 2. Short-circuited rectangular waveguide with Petri dish samples inside as used in the laboratories of the Department of Biotechnology, Health and Ecosystems Protection, ENEA.

Other systems supporting a traveling wave are the two described in [34] and [35]. The first is based on two parallel conductors with bent lateral edges to limit the RF radiation and supports plane wave transmission. In [35], a modified coaxial cable was developed that includes a special glass tube used as the sample holder, reaching a very high efficiency (120 (W/kg)/W), as determined by experimental measurements.

2) *Resonant Structures*: There were 11 systems classified as resonant, in particular, eight short-circuited rectangular waveguides.

Resonant systems are generally closed structures that allow standing waves inside due to total reflection. They have high volume efficiency and are usually compact systems enabling the placement of both active and sham systems in the same incubator. As they are based on resonance, they are strongly affected by the position and size of the biological samples, and have a narrow operating frequency band. In spite of this, they guarantee high SAR efficiency for cells in monolayers or suspensions since samples can be located at the *E*- or *H*-field maxima. The temperature is usually controlled by forced airflow through the guides [36].

Most of the resonant structures reviewed are based on shortening the rectangular waveguide at one end, as reported in Fig. 2. These structures permit the simultaneous exposure of different sample holders: tubes [37] and Petri dishes (from one 100 mm [38] up to eight 35 mm [39] or 60 mm [40] dishes). They are used at most frequencies typical of mobile communications: 800 MHz [37], 900 MHz [39], [41], 1710 MHz [40], 1800 MHz [36], and 1950 MHz [42]. The efficiency and SAR homogeneity vary strongly with the frequency. For 900-MHz exposures of cell monolayers [39], the efficiency is about 1.3 (W/kg)/W and homogeneity 20%; for 1800 MHz [36], the homogeneity is 30%–40% for both monolayers and suspensions, while the efficiency is much better than 10 (W/kg)/W for suspensions and 50 for monolayers. Such differences depend on the dominant coupling mechanism (inductive or capacitive). For example, Schuderer *et al.* [36] exposed monolayers to the *H*-field and cell suspensions to *E*-field maxima to improve both the SAR value and homogeneity.

The custom-made resonant structure used in [37] allowed the simultaneous exposure of eight tubes. Numerical and experimental dosimetry was performed for all tubes, but only two



Fig. 3. Wire patch cell operating at 1800 MHz with four Petri dishes and a thermostated water jacket as reported in [45, Fig. 11].

of them were chosen to expose the biological samples to two different doses at the same time. Efficiencies were 1.61 and 2.28 (W/kg)/W for the two tubes and good homogeneities of 6.8% and 12.1%, respectively.

A short-circuited modified rectangular waveguide, operating at 2.45 GHz, was used in [43], and an *ad hoc* rectangular culture dish placed outside the structure over two slits on the top wall of the waveguide. With this arrangement the maximum SAR was around 70 W/kg, but the SAR distribution varied strongly along the length of the waveguide.

A different solution was adopted in [44] where a culture flask was exposed to an 830-MHz field with an efficiency of 9.4 (W/kg)/W. A parallel-plate resonator fed by a coaxial cable through a tapered transition section was used. The entire exposure system (6-cm length, 5-cm width, and 2.4-cm height) was installed within an incubator.

Finally, the wire patch cell is a structure first proposed by [11] and constructed of two squared parallel metallic plates short circuited by special props at the corners (see Fig. 3). One of the two plates is fed from above through a coaxial cable whose inner conductor extends to the plate below. The biological sample is placed in Petri dishes between the two plates. The dimensions of this system depend on the operating frequency. In spite of the need for an EM compatible arrangement for the wire patch cell, its reduced size permits it to fit inside an incubator. This system allows simultaneous exposure of eight Petri dishes filled with cell suspensions or monolayers at 900 MHz [11] and four at 1800 MHz [45] due to its reduced dimensions. The efficiency of the two systems is low compared to resonant structures, but comparable to the propagating ones: 0.5 (W/kg)/W at 900 MHz and 1.25 (W/kg)/W at 1800 MHz. SAR homogeneity for monolayers decreases with frequency and remains below 30%, which is acceptable according to [4] and [5]. Local temperature control of the samples can be maintained using two spiral plate water jackets (Fig. 3) [45].

3) *Radiating Structures*: Radiating systems allow large experiments where many samples can be simultaneously exposed. These are the only systems currently used for frequencies over 2.45 GHz. Nevertheless, they have low efficiency, due to the low incident power densities, and poor homogeneity. They also need

EM compatible arrangements due to the lack of enclosures confining the field. Moreover, if environmental control is needed, the setup may become complex.

Five radiating systems have been reviewed. A horn antenna was used [46] to conduct 2.45-GHz exposures of 96-well culture plates in a Plexiglass incubator. They determined the SAR to be 4 W/kg. Zhao [47] used a horn antenna for millimeter waves (50 GHz) to irradiate one, two, or four Petri dishes with a SAR distribution varying below 20%.

Vijayalaxmi [48] also used two horn antennas operating at 2.45 and 8.2 GHz to expose T25 flasks in an incubator inside an anechoic room. Exposures were conducted at 1.75 m from the opening of the antenna at a frequency of 2.45 GHz and 1.46 m at 8.2 GHz. Numerical dosimetry confirmed low efficiencies (0.1 and 0.34 (W/kg)/W at 2.45 and 8.2 GHz, respectively). SAR homogeneities were given in terms of a dose distribution function.

The system described in [49] allowed exposures of up to 25 or 49 Petri dishes. It was comprised of a horn antenna operating at 2142.5 MHz and a dielectric lens that focused the beam onto the samples. The efficiency was low (0.175 (W/kg)/W), but the SAR variation was high (*CV* of 59%). In this case, the environmental control was very complex with two different forced air sources placed in the culture room and in the anechoic chamber.

The exposure system described in [50] consisted of six microstrip antennas operating at 2.1 GHz and placed on each face of a cubic box. The system attempts to replicate the field distribution of radio base stations in an area of 6 cm × 6 cm at the center of the box. Only the electric field inside the sample (Petri dish filled with Dulbecco solution) was provided using numerical simulations.

### C. Real-Time Systems

Special attention has recently been given to real-time data acquisition during RF exposures to identify possible cumulative or reversible effects. In particular, electrophysiological techniques are now widely used to study interactions between the nervous system and RF fields. Real-time analysis imposes additional requirements of easy access to the biological sample and minimal coupling with the data acquisition setup.

Most real-time systems are propagating structures with the exceptions of one resonant [51] and one radiating [52] system.

1) *Propagating Structures*: Propagating systems for real-time studies are generally closed structures, such as TEM cells or rectangular waveguides, modified with holes for sample observation and perfusion, and for online monitoring of biochemical or biophysical parameters.

In [53], Meyer *et al.* used a TEM cell at 180 and 900 MHz [54] and two rectangular waveguides at 900 and 1800 MHz to expose myocyte cultures during patch-clamp recordings of electrophysiological activity [53]. These systems had two holes in their top and bottom plates: one to insert the recording electrodes and the other to observe the sample with a microscope. To avoid interference between the *E*-field inside the guide and the wire of the patch-clamp electrode, long glass microelectrodes were used between the solution and a wire positioned outside the exposure device. The calculated efficiency was 1.66 (W/kg)/W for the waveguide at 900 MHz and 3.16 (W/kg)/W for 1800 MHz.

A rectangular waveguide operating between 0.75–1.12 GHz was proposed by [55] to evaluate effects on skeletal muscle contraction exposed to continuous wave (CW), amplitude modulated, or pulse modulated fields. The muscle was inserted in a bath placed in the center of the waveguide. A force transducer continuously measured muscle contractions induced by a voltage difference between the two metal electrodes. The waveguide walls contained slots to allow connection with measurement, control, and stimulating devices outside the system. Their detailed numerical dosimetry in the muscle sample accounted for the bath, metal electrodes inside the guide, and openings in the walls. The efficiency was higher than 3 and homogeneity around 79%, calculated as  $(SAR_{MAX}-SAR_{MIN})/SAR_{MAX}$ .

A modified rectangular waveguide giving CW or Universal Mobile Telecommunications System (UMTS) signals was used for electrophysiological recordings of neuronal networks cultivated on microsensor chips [56]. The chip is fitted into a recess in the guide to avoid short circuiting the measuring probes while exposing the neuronal cells.

To expose heart slices [57] and brain slices [58] to high-power microwave pulses (repetition frequency 9.2 GHz), a WR90 waveguide was used terminated with an exposure cell containing the sample and a sapphire matching plate below. An extremely high efficiency of 3.3 kW/kg/W was measured at 0.5 mm above the matching plate, but decreased about twofold per millimeter with distance from it.

To expose brain slices to 700-MHz CW fields while recording electrophysiological activity, Tattersall *et al.* [59] employed a parallel-plate waveguide apparatus. In this case, as in [53], the top and bottom plates of the guide had holes to illuminate the sample and allow insertion of both stimulating and recording electrodes. The electrodes were placed at an angle of about 45° to the *E*-field raising the possibility of artifacts in the recorded traces [60]. The SAR in the slice was estimated to be less than 0.01 W/kg for an input power of 0.126 W, giving an efficiency value lower than 0.03 (W/kg)/W.

An open coplanar waveguide was used for studies involving patch-clamp recordings of neuronal cells [61] and field potential recordings of brain slices [62]. Fig. 4 shows the system of [61] mounted on a microscope. The two systems operate in the 800–2000-MHz band, encompassing all typical frequencies for mobile telephony. They differ from each other by the distance between the central and lateral conductors because of the different size samples to be irradiated.

The open planar geometry allows easy access to the samples and the *E*- and *H*-fields are confined in a small volume around the surface that guarantees the avoidance of interference with the data acquisition setup. Field confinement also provides high-efficiency values, higher than 17 (W/kg)/W, for both systems at all frequencies.

A modified stripline system was used to evaluate effects of a CW 2.45-GHz field on the activity of ascorbate oxidase trapped in liposomes [63]. In this system, both the sample cuvette and the temperature regulating chamber were adjacent parts of the dielectric substrate of the stripline. The whole system was located inside a spectrophotometer monitoring the enzymatic activity during exposure (Fig. 5).



Fig. 4. CPW system for patch-clamp recordings while mounted on a microscope, as used in the laboratory of the Department of Human and General Physiology, University of Bologna.

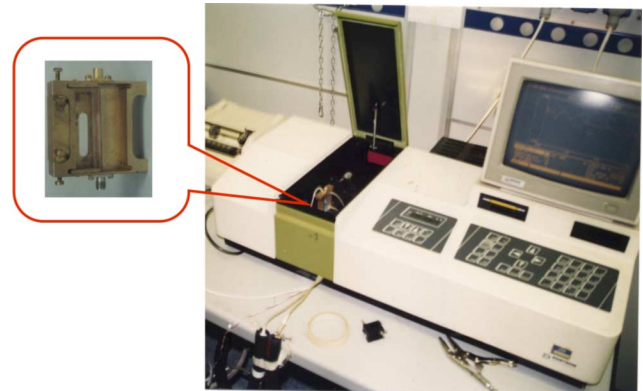


Fig. 5. Modified stripline system reported in [63] installed in a spectrophotometer at the laboratory of the Institute of Neurobiology and Molecular Medicine-CNR, Rome, Italy.

2) *Resonant Structures*: A resonant system employed by Hagan *et al.* [51] was based on a WR-975 rectangular waveguide terminated with a shorting plate. It was designed to expose neural cells in the frequency range of 0.75–1.12 GHz while monitoring catecholamine release online. The cell perfusion apparatus was placed inside the waveguide and communicates with the exterior through slots on the guide plates. The highest calculated SAR was achieved when the cell perfusion chamber was located at the *E*-field maximum.

3) *Radiating Structures*: Yoon *et al.* [52] had the same biological protocol as Hagan *et al.* [51], but exposed at frequencies from 1 to 6 GHz. For this higher frequency range, a standard waveguide is too small to accommodate the sample and the cell-perfusion apparatus. Thus, a radiating system was chosen using a horn antenna with the perfusion chamber placed in the far-field region. This solution required a special arrangement to avoid perturbing the field and interfering with the experimental equipment. All instruments were shielded in a conducting box behind the perfusion chamber and a layer of absorber material used to prevent *E*-field reflection. In addition, the whole system was placed within an anechoic chamber.

While Yoon *et al.* [52] recognized the need for exposure systems that could operate at higher frequencies, in their system

SAR homogeneity became critical due to the higher conductivity of the medium and the dimensions of the sample holder becoming comparable to the wavelength of the incident field.

#### D. Considerations

From Table I, it is evident that the majority of experiments in bioelectromagnetics in the last ten years used offline analysis, but in recent years there has been an increasing trend toward real-time systems.

Most offline exposure systems are based on standard RF structures dimensioned to operate at the frequency of interest and accommodate the biological sample in holders required by the protocol. Real-time systems generally require modifications of standard RF structures and features that allow continuous monitoring of the sample while avoiding RF coupling and interference with the recording apparatus. As is evident from Table I, real-time investigations generally require the use of nonstandard sample holders.

Currently the majority of exposure systems operate at typical mobile communication frequencies. However, some papers report systems designed to expose biological samples at higher frequencies: 2.45 GHz [25], [29], [30], [32], [43], [46], [48], [63], millimeter waves [47], 6.00 GHz [52], 8.20 GHz [48], and 9.20 GHz [57].

Different strategies exist for exposure system development. In some, the same reference structure is maintained for exposures at different frequencies. For example, for wire patch cells, at 1800 [45] and 2450 MHz [64], [65], a resizing and a new dosimetry are necessary [11]. In others, the change of exposure frequency imposes a change of reference structure, such as in the real-time systems used at 1 GHz by Hagan *et al.* [51] and at 6 GHz by Yoon *et al.* [52]. However, systems operating at the same frequency and delivering the same dose can be based on completely different structures, determined by the experimental protocol (e.g., for real-time and offline experiments).

The efficiency (see Table I) depends strongly on the type of RF structure (e.g., open, closed, resonant) and frequency while SAR homogeneity also depends on the sample volume and holder shape.

### III. RESULTS

#### A. From the Classification to a Procedure for Developing an Appropriate Exposure System

From Table I, it is evident that many different exposure systems have been developed and employed for a great variety of experimental protocols in the last ten years. As already noted in [9] and [10], the concept of using a standardized exposure system for all types of studies is not possible. However, the choice, design, and characterization of the system can be standardized to obtain repeatable and reproducible results from biological experiments.

The effect of the exposure depends only on the RF dose characteristics, while the reliability of the observed effect depends on avoiding any confounding factor due to RF interference, changes in environmental parameters, or loss in the well being of the cells. In turn, an accurate knowledge of the dose and control of possible confounding factors depend on proper

design and characterization of the system employed, which can be achieved following a standardized procedure, as suggested in Section III-B.

Analysis of the experimental protocol requirements allows one to choose the RF structure with the required features or to design a system through a sequence of standardized steps.

One sees from Table I that some studies do not match all the procedural steps.

#### B. Standardized Procedure

The proposed procedure for reaching the optimum exposure design is shown in the flowchart (Fig. 6) and consists of seven main steps.

First, an experimental hypothesis is formulated (step 1), leading to the choice of an appropriate biological system to be exposed. The experiment to test the hypothesis is then defined (step 2), including biological models, endpoints, techniques, and exposure parameters. The outcome of these analyses determines the requirements of the exposure system. The best RF structure is then chosen (step 3). If one chooses to adopt an existing system, then determine whether it has already been reported in literature, or whether it is necessary to design one (step 4) that leads to a first dimensioning of the structure. The final design parameters (dimensions, materials, sample position, etc.) are obtained through numerical simulations with and without the sample (step 5), using an iterative adjustment procedure to optimize sensitive parameters.

The next two steps are the manufacture (step 6) and experimental validation (step 7) of the exposure system. Measurements should be conducted first with the structure empty and then loaded with the biological sample to validate the behavior of the system and to experimentally evaluate the dosimetry. If acceptable agreement between measurement and simulation is not achieved, one must return to steps 5, 6, or 7 depending on the degree of mismatch.

To explain how this procedure can be applied in actual situations, some practical examples are given in Section III-C.

#### C. Some Examples

The following two examples provide some practical guidance on how to use the standardized procedure.

1) *How to Start to Identify the Exposure System:* Assume we want to test the hypothesis that an RF field typical of wireless technologies (i.e., Wi-Fi) at a frequency of 2.45 GHz can produce genotoxic effects in blood cells. From a review of available publications, an experimental protocol like the one used in [20] for UMTS may be considered. The biological test system is human leucocytes and is used to detect primary DNA damage, i.e., strand breaks using alkaline comet assays. This endpoint suggests the use of an offline system and an incubator with at least six donors to obtain sufficient statistical power. Assume the exposure protocol requires 24 h of intermittent RF exposure at a SAR ranging from 1 to 4 W/kg.

Different vessels could be used; flasks, Petri dishes, or tubes. Referring to Table I, we note that seven exposure systems for 2.45 GHz exist. The first two are radiating structures from [46] and [48], which do not seem adequate due to their low efficiency and poor RF characterization.



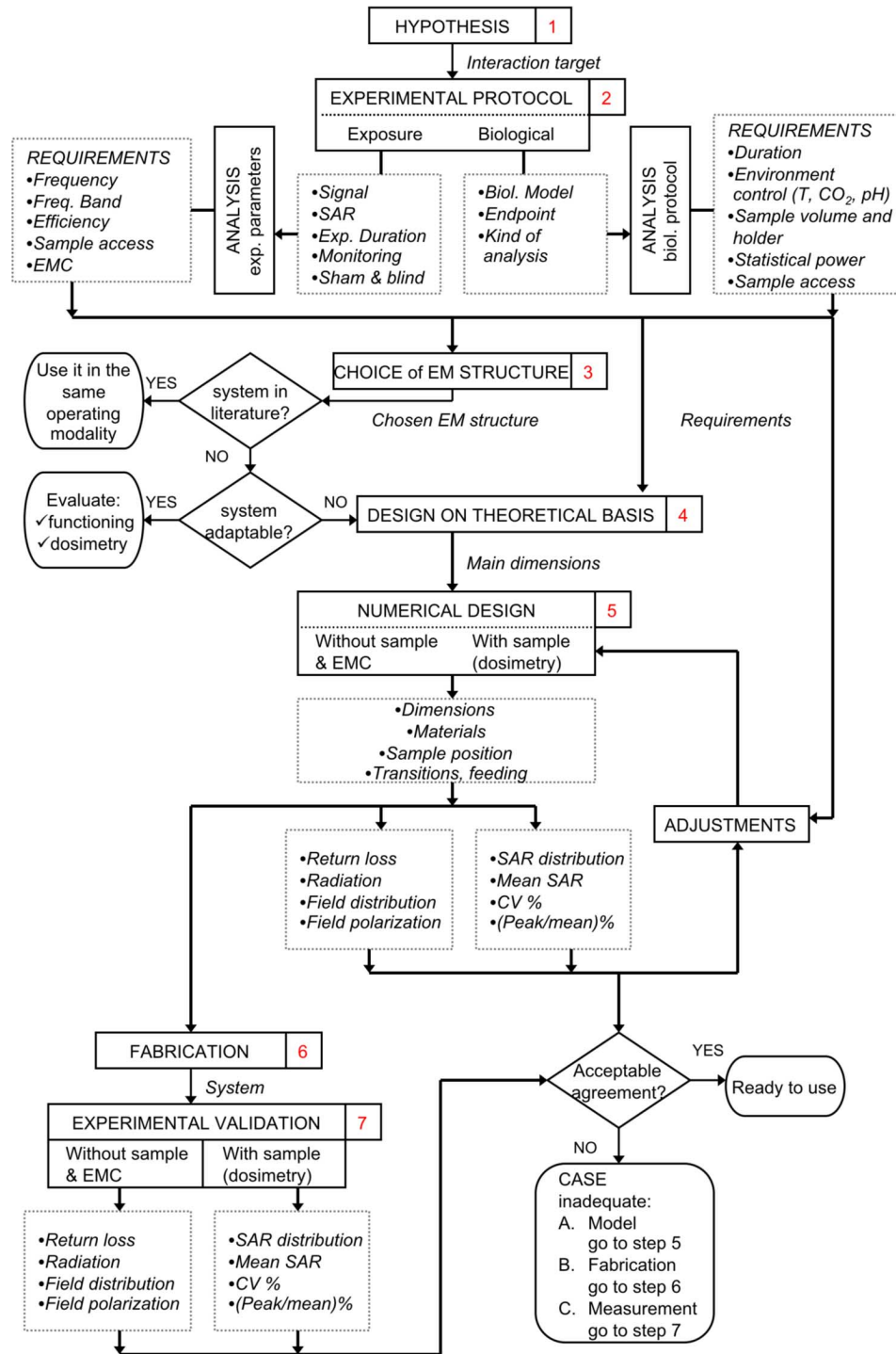


Fig. 6. Flowchart of the proposed standardized procedure for reaching the optimum exposure system design.

The system in [25] is not adaptable because it uses a nonstandard vessel (polystyrene block with ten holes) and has inadequate sample volume exposed (approximately 0.5 mL per each hole). Similarly, in [43], an *ad hoc* vessel is used that is divided into four compartments to simultaneously expose to four different SARs.

Of the two remaining systems, the radial waveguide of [30] and [32] and the rectangular one of [29], the first cannot be used since it is not possible to insert it inside an incubator. Therefore the rectangular waveguide seems to be the appropriate choice

for this study. Nevertheless, to expose samples from at least six donors it would be necessary to perform six separate experiments since the system from [29] can hold only one flask at a time. This disadvantage may be overcome by a wire patch cell system like the one proposed in [64] and [65], which permits the exposure of four Petri dishes at a time. Otherwise one can choose to use a completely new design by going through steps from 4 to 7 (Fig. 6).

2) *Use of Different Systems in Cooperative Studies:* This example addresses the evaluation of RF effects on biological sys-

tems of different levels of complexity, as part of a cooperative study program, such as the RAMP2001 Project (Table I in [9]).

From that table, both real-time and offline protocols were used with many biological endpoints.

The primary hypothesis was that RF fields (900 and 1800 MHz) may affect nerve cells, thus the interaction targets were neuroblastoma cells and hippocampal and cortical neural cultures from a rat brain. The endpoints were proliferation, apoptosis, gene expression, cell differentiation, activation, and inactivation kinetic changes in the ratio of the different calcium channel subtypes and ionic currents. Doses in the range of 1–4 W/kg were chosen for all experiments, requiring a low number of samples. This excludes radiating structures due to their low efficiency.

For neuronal phenotype maturation, neuroblastoma cell lines were used. A reduced number of neurites, possibly related to increased expression of a specific mRNA, was observed [66]. For this study, a multiwell sample holder was used in an incubator. From the literature, the possibility of using a TEM cell has been identified (step 3 of Fig. 6) and its functioning and dosimetry have been evaluated [15].

Cell proliferation and gene expression have been evaluated using standard Petri dishes. For this purpose, the wire patch cell was chosen and used at 900 MHz with the same operating modality as in [11]. For exposures at 1800 MHz, a resizing was carried out. Details on steps from 4 to 7 are given in [45].

Finally, for an ionic currents endpoint, a real-time analysis with a microscope and current recorder is necessary. In this case, the specific requirements are described in [61], which are: 1) a transparent dielectric substrate to achieve visibility of the sample; 2) the exposure region where the external electrode is placed is large enough; 3) a substrate thickness less than the microscope optical length; 4) avoiding RF power losses due to dissipation effects in the substrate; 5) avoiding RF power losses due to RF radiation; and 6) a characteristic impedance of 50  $\Omega$  to achieve good impedance matching when the structure is connected to a standard coaxial cable.

From the literature, no systems for both frequencies of interest are available, therefore a new design is necessary. All steps from 4 to 7 in Fig. 6 are discussed in [61]. This system has been used in [67] to investigate the effects of 900-MHz CW fields on  $Ba^{2+}$  currents through voltage-gated calcium channels in rat cortical neurons.

#### IV. DISCUSSION AND CONCLUSION

The appropriate exposure system for *in vitro* RF research depends on the biological endpoints and the exposure parameters required. The WHO has placed great importance on accurate dosimetry if studies are to be useful for determining any health risks of exposure to RF fields. While standardized exposure systems are not necessary, there is a place for a standardized design procedure to ensure that the appropriate exposure system is identified and used.

When determining the exposure structure, some priorities must be kept in mind. The ability to accurately determine the dose in the exposed sample and the well being of the cells. Furthermore, a sham group and blind modality must be adopted when possible [9].

This paper has provided practical information on the processes necessary to arrive at the best exposure system to properly test the study hypothesis. Our approach has been to review papers in the literature (summarized in Table I) to: 1) divide the exposure systems into categories defined by their RF structure; 2) identify their strengths and weaknesses, efficiencies, and ability to expose the sample volume and numbers necessary to achieve good statistical power; and 3) finally provide a flowchart of information necessary to achieve the best results. Two guidance examples on how to use such a flowchart have been provided. The first one for identifying the best exposure system for a new experiment and the second describes how to manage exposure systems in a large cooperative study. The two examples describe three different ways to use the flowchart. In the first case, the suggestion is the use of an existing system (“use it in the same operating modality,” step 3 in Fig. 6). The second example describes how to develop two new exposure systems, a wire patch cell and a real-time one, following the overall procedure to step 7 of Fig. 6 (“ready to use”). For TEM cells, a reference system was adopted with a proper characterization (“evaluate functioning and dosimetry,” step 3 of Fig. 6).

During the last ten years, the quality of exposure systems has greatly improved and the use of well-grounded experimental protocols (sham exposure, blinding of exposure and biological tests, positive and negative controls) has become a reference for the scientific community. Standardized criteria for the choice of exposure system represent a solid base for conducting high-quality investigations, as required by bodies such as the International Commission on Non-Ionizing Radiation Protection (ICNIRP), IEEE, International Agency for Research on Cancer (IARC), and WHO, to have confidence in the results so they can be included in the process of health-risk assessment. The procedure proposed in this paper can help to provide a quality exposure system.

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