QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES
(1998 to 2002)

POTENTIAL ADVERSE EFFECTS OF GSM CELLULAR PHONES ON HEARING

GUARD

Key Action No: QOL-2001-4.2; QOL-2001-4.2.1

Environment and Health
Development of new methods of diagnosis, risk assessment and process to reduce causes and harmful environmental health effects
Development of methods to assess environmental hazards including mixed exposures, cumulative and low dose effects

Contract Number: QLK4-CT-2001-00150

FINAL REPORT

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TABLE OF CONTENTS

1. INTRODUCTION

Over a period of just a few years, mobile (cellular) phones have produced a revolution, involving not only communication systems and the technological sphere, but also the whole social and environmental domains. Their widespread use has produced a huge and irreversible modification in the everyday life of all Europeans, irrespective of whether they are among the high percentage of mobile phone users, quickly becoming irreplaceable as communication tools. Due to their widespread use, the environment is subjected to irradiation by their electromagnetic fields (EMF) at GSM radiofrequencies, and in that sense the microwaves represent, now and even more in the near future, an ever-present potential environmental hazard. Moreover, the number of new applications of wireless communications is increasing. Hence, use of wireless communications, and the consequent biological effects and/or risks, cannot be restricted to the domain of personal lifestyle but involves the whole population, and should be considered as a high-priority environmentally related health issue.

So far, concerns have been raised that exposure to these radiofrequency fields may be associated with an increased risk of cancer or some other detrimental health effects. However, to date, there is only limited knowledge on what are the biological systems most influenced by the use of this kind of device. Among them, simple geometrical considerations arising from the typical positioning of the device suggest that the produced EMF could affect, earlier than any other system, the hearing system, and in particular its peripheral part: mainly the inner ear (cochlear cellular) system. As additional fundamental consideration, one should also bear in mind that cochlear hair cells are known to be highly sensitive to a very great variety of exogenous and endogenous agents. These cells are continually lost or damaged throughout life as a result of genetic syndromes, infections, ototoxic drugs, ageing and traumas of various origins, like noise and radiation. Moreover, the outer hair cells of the cochlea show modification in response to externally applied electric and magnetic fields. Furthermore, research in hearing has advanced dramatically over recent years, allowing early identification of even extremely mild hearing losses.

For all the above reasons, the study of the effects of mobile phones on hearing should be considered as one of the major priorities in research into the possible adverse effects of mobile telephony.

State of the art

In the last decade, the health effects of high-level radiofrequencies and microwaves were addressed by many studies and research projects. By contrast, published literature on the effects of low-level microwave exposure, particularly at frequencies and modulation typical for mobile telephony, is not as substantial. Of the relevant reports that do exist, a variety of detrimental effects, independent of the question of cancer or cancer-related outcomes, have been postulated to occur as a result of exposure from the radiation associated with mobile phone handsets and base stations. Due to the close proximity of the head and a mobile phone handset when in use, the tissues and structures within the head may absorb relatively greater amounts of energy than elsewhere in the body, and concern has been expressed about possible adverse effects on the eyes and on the brain and cognitive functions. These conclusions are often inconsistent as disease types and effects. On the other hand, however, some other studies show no evidence of correlation between exposure to microwaves and disease. As a consequence of this lack of agreement among researchers, only a limited scientific database could be developed, increasing the difficulty for health authorities and agencies in improving scientific and public knowledge on the links between exposure and risks. This simply gave rise to the generic consensus opinion (based particularly on animal studies) that no robust biological effects have been conclusively identified with exposure to RF or microwave radiation at levels commonly encountered in the public environment and that no adverse health effects exist when mobile phones are designed, manufactured and operated in agreement with internationally-recognized exposure limits.

Despite all these studies, scientific and politic statements, and the fact that the increase in personal mobile communications has heightened public awareness and concern about the possible biological and health effects of mobile phones, so far only two studies directly addressed the investigation of the effects of GSM phones on any indices of the status of the hearing system. The first study (Marino et al., 2000) discussed some preliminary results on the effect of 900 MHz microwaves Distortion Product Otoacoustic Emissions (DPOAE) recorded from 10 Sprague-Dawley rats, showing the absence of statistically significant functional effects. A second study (Kellényi et al., 1999) presented the preliminary results of a study of the effect of GSM phones on auditory brainstem responses (ABR) recorded from humans (10 subjects), showing a significant increase of the latency of peak V, that could correspond to a 15-18 dB hearing deficiency above 2 kHz, although these results did not match with the status of the auditory system assessed by audiometry measurements in three subjects.

Besides these studies, only microwave hearing (i.e. the perception of microwaves and the consequent production of auditory responses and sensations) was investigated thoroughly in the in the last three decades (see Chou et al., 1982; Frey 1962; Ingalls 1967; Lebovitz et al., 1977).

The lack of studies addressing the potential effects of mobile phones on hearing is difficult to understand, taking into account that simple geometrical considerations about the typical positioning of mobile phones suggest that the produced electromagnetic fields could affect, in preference to any other, the peripheral hearing system (outer, middle and, in particular, inner ear; i.e. the cochlear cellular system). Moreover, it is well known that the outer hair cells of the cochlea show elongation and contraction in response to externally applied electric fields. Moreover, magnetic fields are considered to influence the mechanics of the vestibular outer hair cells in animals.

As an additional but fundamental consideration, one should bear in mind that the majority of the hearing-impaired people suffer from sensorineural hearing loss. Despite the fact that the common name for this condition is "nerve deafness", sensorineural hearing loss most often results from damage to the sensory hair cells of the inner ear, rather than to the associated nerve fibres. Each cochlea normally contains about 16,000 of these transducer cells. Cochlear hair cells are known to be highly sensitive to a huge variety of exogenous and endogenous agents and because they are not replaced when damaged, they are continually lost throughout life as a result of genetic syndromes, infections, ototoxic drugs, ageing and last but not least, acoustic trauma of various origins. As examples one can consider as exogenous agents: ototoxic drugs (a simple aspirin is known to produce a temporary reduction of hair cell motility that can be observed by otoacoustic emissions), high levels of noise, and alcohol. As endogenous agents, simple modification of blood pressure, hormonal balance or high levels of bilirubin can affect cochlear hair cells and produce temporary or permanent hearing loss.

Research in hearing has advanced dramatically over recent years. In the last two decades, advances in the knowledge of cochlear mechanisms, study of the influence of exogenous environmental factors in the onset of deafness, investigations on the relationships between higher cerebral functions, auditory periphery, behaviour and cognition have produced a revolution in this field. At the same time, new methods, instruments and approaches to assess the status of the hearing system as a whole quantitatively have been proposed, thus allowing early identification and treatment of moderate and profound hearing losses. One should also note that in this field European research leads the world. This state of affairs results not only from very good individual laboratories and clinics throughout Europe, but also from the fact that the major advances of the last two decades in this field are due to European discoveries, such as the discovery of active mechanisms in the cochlea, which produced a revolution in the understanding of the whole peripheral auditory system. This led to the development of devices for recording otoacoustic emissions (OAE), which are acoustic signals produced by the activity of the outer hair cells. OAEs are considered at present the most sensitive and reliable method to assess the functionality of the cochlea.

The study of potential effects of GSM microwaves on the hearing system could also include possible effects on the efferent and medial olivocochlear system. In the last decade, evidence was found that study of the amplitude of otoacoustic emissions recorded during contralateral acoustic stimulation (CAS) (i.e., the recording of evoked otoacoustic emissions from one ear during contralateral acoustic stimulation with controlled sound or noise) is a non-invasive technique for functional exploration of the medial olivocochlear efferent system (Berlin et al., 1994; Collet et al., 1992; Collet et al., 1990; Veuillet al., 1991). The status of this system has also been considered as an objective index linked to hearing-related cognitive functions in humans (Collet et al., 1993; Michie et al., 2000; Timpe-Syverson et al., 1999). Based on these considerations and findings, the study of the responses to CAS before and after exposure to GSM exposure can be considered as a non-invasive tool to assess the potential effects of GSM fields on the central nervous system.

Objectives

Based on the above considerations, the originators of the GUARD project felt that a joint effort aimed to explore the potential adverse effects of GSM on hearing should not be delayed.

The GUARD project aimed to eliminate this gap in knowledge, developing new knowledge in the area of potential adverse biological effects of the GSM microwaves produced by mobile phones, in relation to the hearing system. The study of humans and two different species of animals (rats and guinea pigs) allowed investigations in intra-species and inter-species variability in order to quantify uncertainties and to establish a more reliable data set and to improve the scientific basis for extrapolation from animal data to humans, contributing to an European database for risk analysis. This is contributing at the European level to the process of reduction of causes and harmful environmental health effects of this type of EMF.

The results of this research represent the first international and European statement about GSM mobile phone hazards for hearing and hearing-related cognitive functions.

In stating the detailed objectives for this work, primary and secondary aims were proposed. Primary aims are those for which clear importance and more direct future implications were identified. These can be summarized as:

1. Study the potential effects on hearing system structure and function in animals and humans of GSM radiofrequencies, in both 900 and 1800 MHz bands. The investigation addressed the following items:
   1.1. Assess potential changes to hearing possibly occurring after exposure to microwaves at the two frequencies. Quantitative measurements of the effects were obtained for humans by means of subjective and objective audiological protocols, based on typical audiological tests, such as pure tone audiometry, auditory evoked potentials and otoacoustic emissions recorded pre- and post-exposure. For animal investigations, the functional status of the hearing system was assessed by otoacoustic emissions and auditory evoked potentials. In vitro analysis was also performed to look for organic modifications to the cochlear epithelium of exposed animal tissue in comparison with those of control material.
   1.2. Study of the effects of GSM radiofrequencies on the medial olivocochlear system in humans by means of the analysis of OAE contralateral acoustic stimulation (CAS).
   1.3. Cross-sectional investigation of possible effects related to the style of use of the phone (*heavy users* versus *light users*), termed the *between-subject* study.
   1.4. Study of the combined effects of GSM exposure and ototoxic drug administration (gentamicin GM), in animals (both Sprague-Dawley rats and Guinea Pigs).
2. Feedback to producers of GSM mobile phones in order to provide information for health risk assessment.

2. MATERIAL AND METHODS

Animal experimentation

Introduction

The aim of GUARD is to assess potential changes of the hearing function of animals and humans after exposure to low-intensity electromagnetic fields produced by mobile phones at frequencies of 900 and 1800 MHz. The potential effects are investigated by studying the changes in hearing function of laboratory animals monitored by otoacoustic emissions and auditory evoked potentials before, during and after exposure to GSM fields. This chapter includes the reports about animal experiments, relating to the systems for GSM exposure and positioning, the protocols for animal exposure and the protocols for measuring the effects in animals.

The animal experiments are performed by the ENEA Section of Toxicology and Biomedical Sciences in Rome (in the rest of this document ENEA) and by the Biologie cellulaire et moléculaire de l'audition: Université Victor Ségalen Bordeaux 2 and INSERM in Bordeaux (INSERM). The signal analysis and processing and the statistical analysis of the data are performed by the CNR Institute of Biomedical Engineering in Milan (CNR.ISIB). The experimental details and protocols described here are partially based on a previous preliminary study (Marino et al., 2000) and on the research performed in the course of two national projects ("Project COMOBIO COmmunications MObiles et BIOlogie" in France and "Salvaguardia dell'uomo e dell'ambiente dalle emissioni elettromagnetiche" in Italy).

In the remainder of this chapter the material and methods will be separately described for Sprague-Dawley rats and guinea pigs.

Sprague-Dawley rats - ENEA Laboratories

Animals

Male Sprague-Dawley rats (SD), 250-300 g at the beginning of the experiments.

Exposure Parameters

- Exposure timing of 2 hours/day, 5 days/week, for 4 weeks;
- Microwaves at frequencies of 900 and 1800 MHz, with a GSM modulation;
- Level of local exposure (SAR) of 2 W/kg (additional experiments at 4 W/Kg);
- Monaural exposure
- Equal number of exposed and sham animals
- Exposure timing and SAR level of the following experiments defined on the basis of the first results of the exposures

Audiological Assessment

The hearing function was evaluated by recording distortion product otoacoustic emissions (DPOAE), before, during and after exposure (see also the "Experimental protocol" Section). The animal weight is measured in each exposure and measurement day.

DPOAEs tests: DP-gram and DP-Growth Rate (I/O function)

- The induction of gas anaesthesia (induction chamber of IMPAC[6] anaesthesia system[I]) was performed by a mixture of Isoflurane 1-2% in O_2 flow, requiring about 3-5 minutes.
- The maintenance of anaesthesia outside the chamber was performed by a nosecone at one end of a tubing circuit delivering gas mixture from anaesthesia system.
- The recording of DPOAE was performed in one ear of the rat by an ILO92 system by Otodynamics, until November 2002 and by a SmartOAE system by Intelligent Hearing Systems from December 2002. A plastic tip was used to improve the fitting between the acoustic probe and the ear canal.
- The DP-grams parameters were set as follows: F2/F1 ratio equals to 1.22, F2 from 1 to 8 kHz, level of the primaries L1 and L2 of 70 dB SPL (Sound Pressure Level) and 65 dB SPL, respectively, until November 2002. From December 2002, the F2 frequency range was extended from 3 to 14 kHz. .
- The DP-Growth Rates parameters were set as follows: measurements at frequencies F2 of 4.75, 5.5 and 6.25 kHz, with a F2/F1 ratio of 1.22, equal primary level L1=L2, ranging from 70 to 35 dB SPL, in 5 dB steps.
- The test time lasted approximately about 8-10 min for each rat
- The average anaesthesia recovery time was of 1-2 minutes

Exposure set-up

- During the exposure periods, the rats were restrained in single plastic jigs with minimal stress (Fig 2.1). The temperature was controlled by an alcohol or mercury thermometer.

[I] IMPAC[6] system, Vetequip, Inc., Pleasanton, California (USA).

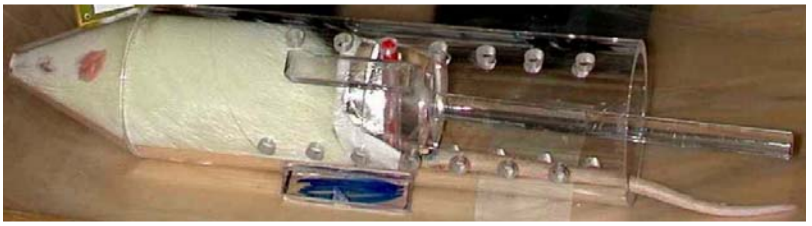

Fig. 2.1 The rat in the plastic jigs

- The loop antennas operating at 900 MHz were developed by the Laboratoire de Physique des Interactions Ondes-Matière (PIOM), Bordeaux, and tested in PIOM and ENEA Casaccia, Rome, whereas the loop antennas operating at 1800 MHz were developed by ENEA on the basis of the antennas at 900 MHz. Both final versions of the antennas were realized by SAMA Sistemi S.r.l. in Rome.
- Two different antennas operating respectively at 900 and 1800 MHz were built using the coplanar line technique on dielectric substrate; the line was terminated with a rectangular loop printed on the substrate (Fig 2.2).
- Electromagnetic matching with the power supply was done using an electric contact sliding on the substrate. The near field of such a loop simulates the localized exposure of the human head exposed to a mobile phone.

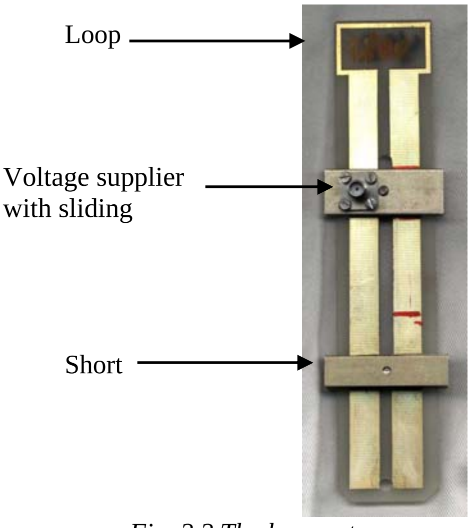

Fig. 2.2 The loop antenna operating at 1800 MHz

- Four loop antennas were set up in an array to expose four rats simultaneously.
- The exposure setup was assembled in a wooden rack with three distinct arrays of four loop antennas positioned at different levels (see Fig. 2.3). RF absorbing panels were inserted between the levels (and on three sides of the wooden rack) to avoid interference signals involving the animals. The arrays were able to work simultaneously: two were supplied for real exposure (at 900 or 1800 MHz) and the third one for sham exposure, so that 8 exposed and 4 sham rats could be tested at the same time.
- The radiofrequencies generator and the amplifiers (HP 100 kHz-1 GHz; HP 100 kHz – 2 GHz; GSM up-link dual band 890–915 MHz and 1710-1785 MHz; CW or GSM modulation signals) were connected to three arrays of four antennas. A four-channel divider fed the loop antennas.

- Each antenna was held perpendicular to the head surface over the ear of rats in contact with the external side of the plastic jig, with a total distance from the cochlea system of about 8-10 mm (about 5 mm due to the plastic jig and about 5 mm due to the position of the cochlea with respect to the head surface).
- Blind operating mode was applied for all operators involved in rats handling procedures (exposure and DPOAE tests).

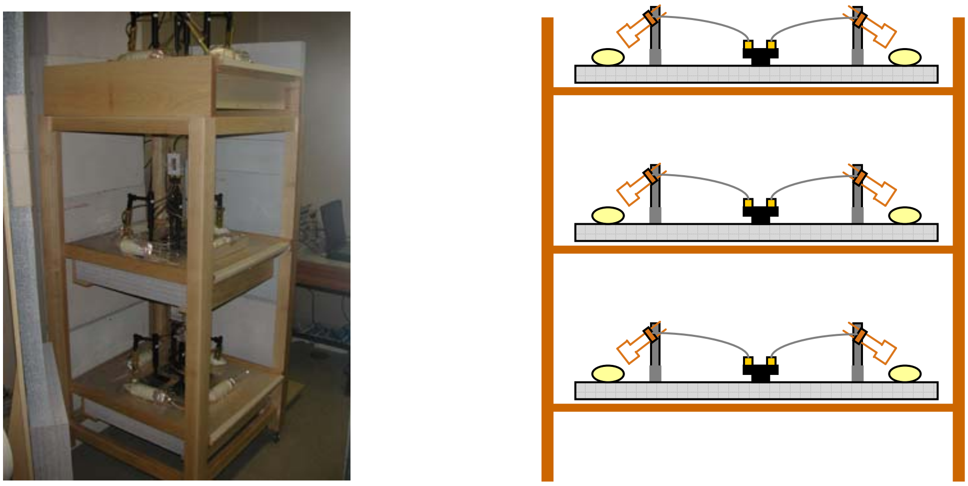

Fig. 2.3 – On the left: The wooden rack, foam panels and one loop array. On the right: the scheme of the three arrays.

Dosimetric assessment

- The experimental dosimetry was performed on a brain-tissue-equivalent homogeneous phantom by the power-pulse method measuring the increase of temperature after a 30-s RF pulse utilizing a Luxtron fibre-optic probe. These data were then verified in the inner ears of sacrificed rats.
- Tests to verified the localized exposure were performed by measuring SAR in different part of phantom (P_{in}=1.7 W) (Fig 2.4)

Location	SAR (W/kg)
Head	1.8
Body	0.5

Fig. 2.4 SAR values measured in different parts of the rat phantom. P_{in}=1.7 W

- Numerical dosimetry was performed by an XFDTD code using a model of rat head with loop antenna positioned at 5 mm from the head surface (Dulou et al., 1998). The rat head was constituted by fundamental tissues (skin, fat, muscle, brain, bone). The loop (900 MHz) has been modelled by a simple wire supplied with a voltage signal corresponding to 1 W (Fig 2.5).

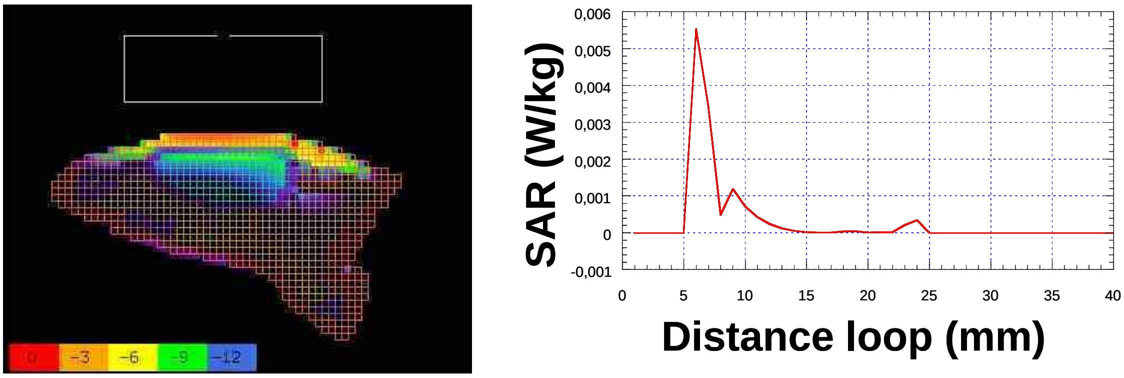

Fig. 2.5 Evaluation of SAR distribution in rat's head (XFDTD)

- Good agreement was obtained between the experimental and numerical data.
- The real efficiency of the antenna (4±1 W/kg/W_{in}) was determined in the phantom at 5 mm depth and in rat cadaver into the cochlea and compared with numerical results.

<u>*Experimental Protocol*</u>

Phase 0: Preliminary studies

Normative studies were performed to define the best DPOAE recording protocol of DP-grams for Sprague-Dawley rats, in terms of reproducibility across time. On the basis of these studies preliminary experiments (*June 2002*) were performed (Galloni et al., 2005; Parazzini et al., 2002), recording both DP-grams and I/O functions, using as exposure set-up a single four-antenna array with RF absorbing panels on three sides.

Phase 1: Exposure protocols at 900 MHz

- Sprague-Dawley rats were divided into two groups: sham and exposed. Exposure condition: 900 MHz GSM radiofrequency, 2 W/kg, 2 h/day, 5 d/week, 4 weeks; DPOAE tests during (at the end of the first, second, and third week) and after (the last day of the fourth week, one day after, one week after) exposure.
- Analysis and processing of the results

Phase 1: Exposure protocols at 1800 MHz

- Sprague-Dawley rats were divided into two groups: sham and exposed. Exposure condition: 1800 MHz GSM radiofrequency, 2 W/kg, 2 h/day, 5 d/week, 4 weeks; DPOAE tests during (at the end of the first, second, and third week) and after (the last day of the fourth week, one day after, one week after) exposure.
- Analysis and processing of the results *(see below)*

Phase 2: Reworking of phase 1

- *Phase 2a*: the SAR level is decreased from 2 W/kg, at 900 and/or 1800 MHz, wherever changes in DPOAE parameters can be related to the exposure performed in *Phase 1*
- *Phase 2b*: the SAR level is increased from 2 W/Kg, at 900 or 1800 MHz, wherever no changes in DPOAE parameters can be related to the exposure performed in *Phase 1*.

- Analysis and processing of the results *(see below)*

Phase 3: Combined effects of ototoxic drug (gentamicin GM) and microwave exposure

- Study 1: Sprague-Dawley rats were divided into three groups; group 1 was treated with daily intramuscular injections of GM of 100 mg/kg bw, for 14 days; group 2 was treated with daily intramuscular injections of GM of 150 mg/kg bw, for 14 days and group 3 was treated with daily intramuscular injection of saline solution (control) for 14 days. All the animals were anesthetized during DPOAE measurements using general gas anaesthesia (isoflurane in O_2 1.5-1.8%). DPOAE measurements were performed with L1/L2 = 70/65 and 65/55 dB SPL, F2/F1 ratio = 1.22. 11 DP frequencies were analyzed from 3 to 14 kHz and the DPOAE tests timing was before treatment and at 1, 2, 3, 4 and 5 weeks from the first injection.
- The 150 mg/kg bw dose caused a marked decrease of DPOAE intensities at the higher frequencies. Therefore, GM treatment can be used as positive control in the study of effects of electromagnetic fields on DPOAEs in this strain of rat.
- Study 2: The Sprague-Dawley rats were divided into four groups; group 1 was treated with daily intramuscular injections of GM of 150 mg/kg bw, for 14 days; group 2 was treated with daily intramuscular injections of GM of 150 mg/kg bw, for 14 days and exposed to EMF; group 3 was only exposed to EMF and group 4 was sham exposed. The exposure was 900 MHz, CW at 4 W/kg of SAR. All the animals were anesthetized during DPOAE measurements using general gas anaesthesia (isoflurane in O_2 1.5-1.8%). DPOAE measurements were performed with L1/L2 = 70/65 and 65/55 dB SPL, F2/F1 ratio = 1.22. 11 DP frequencies were analyzed from 3 to 14 kHz and the DPOAE tests timing was before exposure, at the end of first, second, third and fourth week of exposure, one day after and one week after.

In-vitro and ex-vivo studies

In-vitro experiments aimed to investigate the effect of EMF exposure (1 W/kg, 1800 MHz, 12 hours) on the cochlea of Sprague-Dawley or Wistar (for preliminary tests both were used) newborn rats (3-4 days postnatal). One cochlea for each animal was exposed to RF field in a 37°C incubator, using a WPC (Wire Patch Cell) exposure systems, whereas the other cochlea was used as sham.

For ex vivo experiments, 4 newborn animals were exposed (and 4 sham exposed) for 30 min in a TEM (Transversal ElectroMagnetic) cell, and the cochlea was explanted after exposure for examination (a positive control group was obtained by treating with gentamicin; 2 cochlear cultures from an additional rat are included)

The endpoints for the analysis are:

1. Evaluation of potential morphological alterations of OHCs following RF-exposure, by fluorescence microscopy analysis of specific hair cell marker expression;
2. Evaluation of apoptosis activation associated to RF-exposure, by TUNEL labelling and detection of caspase activation;
3. Evaluation of potential changes in the intercellular calcium fluxes related to exposure by confocal analysis of calcium mobilization in cochlear hair cells.

In-vitro radiating system - Wire Patch Cell

The radiating structure (Wire Patch Cell) was constituted from two square metal plates, short circuited at the edges by four pillars (Fig 2.6). The RF signal was supplied by means of a coaxial cable with the external conductor fixed on the upper plate and the inner one on the lower one. Their size allowed the simultaneous exposure of four Petri dishes (diameter of 3.5 cm) under the same exposure conditions. A turntable allowed the simple positioning of the dishes. They can be easily placed inside a standard incubator.

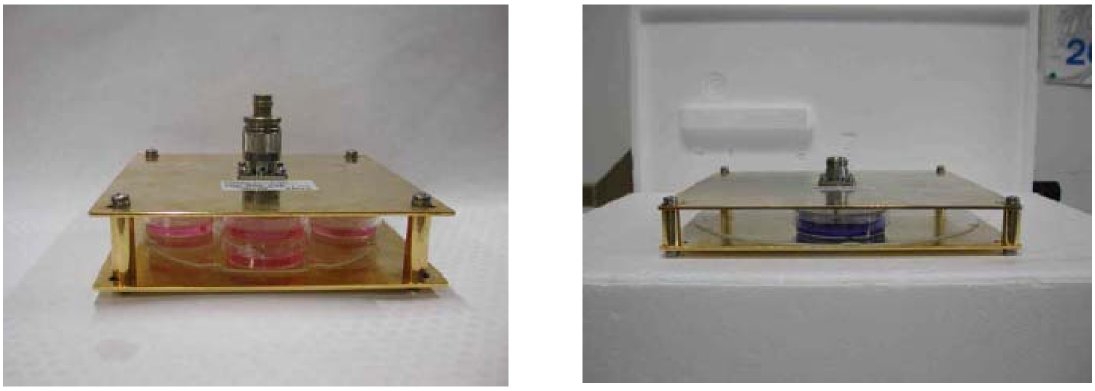

Fig. 2.6 Wire patch cell (WPC) at 900 MHz (left) and 1800 MHz (right).

__Dosimetric assessment - Wire Patch Cell__

- The experimental dosimetry was performed on four Petri dishes filled with 3 mm culture medium and positioned as in the simulations and exposed to high RF power pulse. Thermal increase has been measured at five points (1-5) inside each dish, and average SAR for each dish has been evaluated. Averaged efficiency of WPC is 0.41 W/kg/W with a standard deviation among different measurements of 28%.
- Numerical dosimetry performed by CST code by the evaluation of power loss in four Petri dishes. (Fig 2.7).

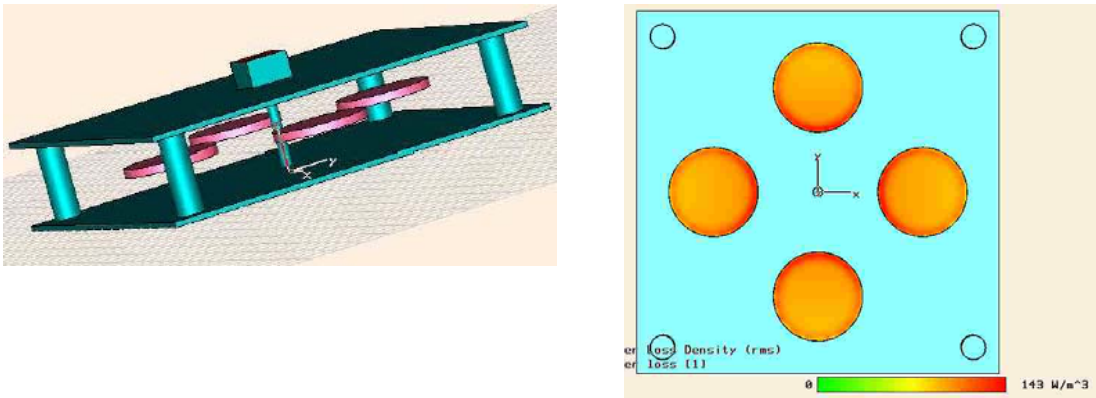

Fig. 2.7 Numerical model (left) and example of evaluation of power loss in four Petri dishes

__Wire Patch Cell Shielding__

The shielding of WPC (necessary for compatibility) was performed by a metal grid box (40×40×20 cm^3) and 4 blocks of foam RF-absorbent material (15 dB).

The environmental conditions inside the incubator (temperature, CO_2) were measured and verified.

Wire Patch Cell Thermal Control

A SAR value of 1.25 W/kg GSM modulated (average, corresponding to 10 W/kg peak) induces a temperature increase of 1-2°C (from 37°C to 38-39°C) during a 24 h exposure. Two water spiral coils were put on external faces of both plates of WPC: the circulating water was maintained at about 36.8°C. In this way the temperature inside the Petri dishes was maintained at about 36.8-37°C even when the electromagnetic power was on. It was verified that in all Petri dishes (sham and exposed) the temperature was 36.8°C $\pm$0.2°C.

Wire Patch Cell In-vitro Exposure Set-up

The in-vitro exposure set-up consists of four independent levels of exposure, simultaneously available (three for real exposure and one for sham exposure), operating in a blind procedure, PC controlled (Fig 2.8a).

Fig. 2.8a In-vitro exposure set-u: WPC

Exposure system – TEM cell

A non standard TEM cell (12 x 12 x 120 cm) operating at 900 MHz has been realized to investigate the biological effects after 900 MHz exposure at different levels of power density. The structure has been designed according to the electromagnetic restraints about cut-off frequency (higher order modes), minimization of VSWR and optimization of the uniform field volume (Fig. 2.8b).

Transversal dimensions has been defined to ensure a pure TEM mode propagation up to 1.25 GHz, and the length has been fixed to 4λ in order to obtain a good TEM mode reconstruction when small objects are placed inside. Typically simultaneous exposure of more biological samples in the same conditions is allowed with this type of exposure system. The EM field distribution, with and without objects inside, has been carefully assessed by numerical code HFSS and by measurements with Network Analyser and calibrated E-field probes.

The system has been realized with a removable wall on one side of the outer conductor in order to easily access and manage the objects to put inside. A grid-wall has been realized for the air grating when animals are exposed.

On the transversal plane, the electric field distribution analysis has shown two regions of uniform electric field big enough for positioning a couple of newborn rats on each side (two over and two below the septum). They were placed with the caudal axis parallels to the propagation direction and radiated in the bottom.

A similar TEM cell, placed in the same room, was used for sham exposure in order to maintain the different groups in the same environmental conditions. To avoid temperature increase in the exposed mice a water-cooling system has been set up with two external metallic jacket filled by circulating water fed through a thermostatic bath, and placed in contact with the bottom and up walls. The temperature in the cavity was monitored during the experiments.

Dosimetry – TEM cell

Exposure conditions were precisely determined by an accurate dosimetry, in order to define experimental standards allowing repeatability of results and their comparison among other laboratories. Numerical dosimetry was performed by two codes (XFDTD and CST Microwave Studio) simulating the exposure system. The dose difference among the animals resulted of 0.11dB, whereas the dose inhomogeneity in single phantom was of about 2.8 dB.

Experimental dosimetry was performed on mouse by power balance method:

$$P_{abs} = 1/4 \, [\, P_{in} - P_{out} - (P_{refl} + P_{loss})]$$

where P_{abs} is the RF power absorbed in one mouse, P_{in} is the power input, P_{out} is the power absorbed in a load, P_{refl} is the power reflected and P_{loss} is the power dissipated in the TEM cell structure. Both homogeneous phantoms and living mice have been used for the evaluation of the absorbed power. For the living mice the average efficiency resulted 0.39 ± 0.08 W/kg/W_{in}.

Fig. 2.8b TEM cell for actual exposure and sham experiments

Guinea Pigs – INSERM Laboratories

Animals

Male pigmented guinea pigs (GP), 250-300 g at the beginning of the experiments.

Exposure Parameters

- Exposure timing of 1 hour/day, 5 day/week, for 4 weeks, unless specified differently.
- Microwaves at frequencies of 900 and 1800 MHz, with a GSM modulation;
- Levels of exposure SAR of 1 and 4 W/kg
- Monaural exposure

Audiological Assessment

All the animals in the experiments were evaluated, separately for each ear, starting systematically with the left ear, before, and twice after the end of the exposures, by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Considering that testing lasted about 1 hour for ABR, and between 1 and 2 hours for DPOAE, a maximum of 4-5 GPs can be tested daily.

ABR recordings: I/O functions and ABR-audiograms

- The animal was weighed.
- The sedation was performed by an IM injection of Domitor 10% (0.1 ml/100g).
- The animal was placed in a restraining box with the head fixed by a nose ring.
- A sub-cutaneous injection of Xylocaïne was performed at the site of the electrodes to reduce the myogenic noise.

- Placement of the needle electrodes (4): 1 at the vertex (active electrode), 2 at mastoid level respectively on each side and connected together (reference electrode), one at the nose (ground electrode).
- An earphone (Sennheiser) was placed against the left ear.
- Amplification: 100,000 times with Tucker-Davis Technologies (TDT) Equipment.
- Stimulation and recording: PC-controlled TDT hardware and SigGen and BioSig software.
- The ABR input-output recordings were performed at 6 frequencies (1, 2, 4, 8, 16 and 24 kHz) from –20 to +40 dB nRL (normal Response Level). Recordings start only when residual background noise (with no stimulation) is below 200 nV.
- Automatic amplitude and threshold determination (sound level corresponding to a 200 nV response amplitude) was performed by processing the TDT files, using custom made software developed with LabView. All results were filed and printed.
- Same recordings and procedures for the right ear, by changing only the placement of the earphone (same electrodes).

DPOAEs: DP-grams and Fast Adaptation

- The animal was weighed.
- The sedation was performed by an IM injection of Domitor 10% (0.1 ml/100g).
- The animal was placed in a restraining box with the head fixed by a nose ring.
- Two Etymotic probes (2 ER1 insert earphones, 1 low-noise microphone ER10B+ in each probe) were connected to each ear respectively through a short (5 mm) silastic tube sealed with a surgical glue (Histoacryl) into each ear canal.
- Sound production and analysis, monaural or binaural, was performed using PC-controlled TDT hardware and software developed by David Smith (Duke University) with MathLab.
- The recording of the DP-grams was performed simultaneously in both ears, with F1 from 1 to 12 kHz, F2/F1 = 1.21, L1 equal to 70 dB SPL and L2 set to 65 dB SPL.
- The recording of DPOAE fast adaptation (over a 3-s window) was performed with F1=2.5 kHz, L1=70 dB SPL, F2 = 3.025 kHz, L2=65 dB SPL, during binaural and monaural (left ear) stimulation.

<u>*Exposure set-up*</u>

- The loop antennas for 900 MHz have been developed by the PIOM Laboratory. The antennas for 1800 MHz are provided by SAMA Sistemi S.r.l., Rome (see above the Exposure set-up section).
- Each antenna, held by a PVC mount, is placed perpendicular to the ear.
- The GSM microwave generators (RFS900-60, 900 MHz; RSTA 1800 MHz, CW or with GSM modulation) are connected to an 8-channel divider so that 8 GPs can be exposed simultaneously.
- Exposure can be realised for 8 GPs at a time (see Fig. 2.9)

Fig. 2.9 – On the left: Exposure system: the 8-channel divider, the generator and the antenna. On the right: Exposure systems for 8 GPs at a time.

<u>*Dosimetric assessment*</u>

- Numerical dosimetry was already performed for 900 MHz. Additional measurements were performed both for 900 and 1800 MHz on the GP inner ear, using a thermal probe place against the round window membrane.
- Numerical models were also developed (FDTD method) to evaluate the microwaves absorption by biological tissues.

<u>*Experimental Protocol*</u>

Phase 1: Normative study

GPs were tested three times, both ears, for ABR I/O and ABR-audiograms and for DP-grams and fast adaptation as defined above.

Phase 2: Pilot study with positive controls - October-November 2002

At the end of phase 1, GPs were divided into 4 groups

- Group 1: sham exposed
- Group 2: exposed (one ear) to GSM at 900 MHz, 4 W/kg
- Group 3: sham-exposed and simultaneously treated with gentamicin (GM) (60 mg/kg, 10 days, beginning at the start of exposure of Groups 2 and 4)
- Group 4: GM treatment as Group 3 and GSM exposed as Group 2

Phase 3: Exposure at 900 MHz

GPs were divided into 5 groups

- Group 5 sham exposed
- Group 6 and Group 7 exposed to 4 W/kg
- Group 8 and Group 9 exposed to 1 W/kg

Phase 4: Exposure to 1800 MHz

Groups 10 to 13 as in Phase 3 but with 1800 MHz instead of 900 MHz

Phase 5: Results

Analysis and processing of the results (see below)

Phases 6-8:

Duplication or modification of protocols of phases 2-4

Phase 9: Combined GSM microwaves exposure and gentamicin ototoxicity

Although former experiments (French COMOBIO project) have shown no toxic effects of daily exposure of GP ears to 900 MHz microwaves at SAR of 1, 2 and 4 W/kg (1 hour/day, 5 days/week for 2 months), there is still the possibility that GSM electromagnetic fields could interfere with other pathological conditions, e.g. by modifying the access and uptake of toxic agents to and by the ear structures. In this respect, aminoglycoside antibiotics are well known for producing irreversible damage after the molecule has penetrated in the sensory hair cells of the inner ear. Thus we investigated the putative potentiating effect of GSM microwaves on the ototoxicity of gentamicin (GM) in “normal” GPs. “Normal” GPs were treated with daily intramuscular injections of GM at 60 mg/kg (a dose close to ototoxic threshold) one hour prior exposure of the left ear to 900 MHz GSM microwaves at SAR 2W/kg for 2 hours. They were so treated 5 days/week for 4 weeks. Another group of GPs were submitted to the same GM treatment but sham-exposed to the GSM microwaves. A final group of GPs were kept in the animal quarters as controls. All GPs were tested before, at the end and one month after the end of the treatment to measure DPOAEs and ABR thresholds.

A second experimental series consisted in the exposure of albino GPs (more prone to develop ototoxic damage) treated with a higher daily dose of GM (75 mg/kg/day), 5 days/week during 2 weeks. A sub-group of these GP had their left ear simultaneously exposed to the GSM microwaves at SAR of 4 W/kg (thus 2 hours/day, 5 days/week for 2 weeks). A final group of GPs were sham-exposed. DPOAEs and ABR thresholds were measured for each ear before, at the end and 1 month after the end of the treatment/exposure period.

In-vitro experiments: Combined effects of gentamicin (GM) ototoxicity and GSM exposure in vitro

GSM exposure and hair cell counts

The technique was similar to that used in former experiments and reported elsewhere (Aran et al., 2004):

- Two wire-patch cell (WPC) sets of apparatus are used to expose the organs of Corti in Petri dishes at well characterized SAR levels. The WPC has been described elsewhere (Laval et al., 2000). Briefly, it consists in two parallel square metallic plates, the bottom one being powered with RFR through a coaxial line going through the top plate. Four pillars at the four corners between the plates allow for optimal tuning of the device, which in these experiments was set at 900 MHz. Extensive dosimetric measurements showed that the uniformity of the SAR in the culture medium was very good (<15%) and that the efficiency was 0.6 W/kg per incident watt. Each WPC was placed in its own incubator for true blind operation.
- Organs of Corti of newborn rats were dissected out at post-natal day 3-5.
- The OCs were isolated under sterile conditions and the stria vascularis and spiral ganglion removed. Dissection of the OC was carried out in Hanks balanced salt solution (Sigma, France) (mM: $CaCl_2$ 1.3, $MgSO_4$ 1, KCl 5.4, NaCl 137, KH_2PO_4 0.4, NaH_2PO_4 3.4, D-glucose 5.5, pH 7.4, 290 mOsm/kg H_2O). The OCs were then placed in a culture medium composed of Dulbecco’s modified Eagle’s medium

(DMEM) (Sigma, France) containing 10% foetal bovine serum (Sigma, France), 25 mM HEPES buffer and penicillin (2500 IU). Cultures were grown at 37°C in 5% CO_2 for 2-3days.

- After exposure or sham-exposure to GM or GSM alone or to both GM and GSM, the OCs were fixed with 4% paraformaldehyde (1 h) and permeabilized using a 0.5% solution of triton X-100 (15 min). They were rinsed three times in PBS and incubated in 1-µg/ml phalloidine-rhodamine for 45 minutes at room temperature in darkness. They were rinsed again three times in PBS (2×6 min and 1×4 min). Finally the OCs were dissected in different fragments which were mounted on glass slides, labelled with phalloidine-FITC which reveals actin in the hair and allows the visualization of hair cells. Present and missing hair cells (inner hair cells - IHCs and outer hair cells - OHCs) were counted on each OC fragment.

Experimental phases

Two sets of experiments have been realized:

1. Experiment 1 (no post-culture)

This consisted of defining the dose-response curve for the effects of 24 h GM exposure, the specimens being fixed just after the GM exposure, and then choosing the dose on the lower edge of the slope of the curve; then study of OCs in the same conditions with/without exposure to that concentration of GM and with/without exposure to GSM (1W/kg) during the same period (24 h), with no delay between the end of the GM/GSM exposure and the fixation and fluorescent observation

2. Experiment 2 (48 h post-culture)

This consisted of the same protocol, but after the 24 h GM/GSM exposure, the OCs were rinsed and maintained in culture (thus without GM and GSM exposure) for 48 additional hours before fixation and fluorescent observation. Then dose-response relations were established for different doses of GM exposure, with or without GSM exposure (1W/kg, 24 h).

Analysis of the Results

The main aim of the experimental design was to evaluate the effect of the SAR level and of the exposure time on the variations of the DPOAE (both DP-gram and DP growth rate) and ABR, measured at different time (i.e. before, during and after exposure).

A statistical analysis on the biomedical signals recorded as described before was carried out by means of two-way analysis of variance (ANOVA) for repeated measures with *time* as within-subject factor and *sham vs. exposure* as between subject factor.

After two-way ANOVA, where a statistically significant difference among the means of the groups (group of shams and group of exposed) was found, multiple comparison post hoc test, was used for calculating the comparison between pairs of groups, in order to determine which groups were statistically different from which other groups.

HUMAN EXPERIMENTATION

Introduction

The studies were performed after the initial animal experiments, the results of which informed the detailed design of the human studies. The rationale of the human protocol was to maintain as much similarity as possible with the animal experiments so results may be compared. However, some differences were necessary in view of physiological differences between species.

The animal experiments and the initial GUARD proposal entail a longitudinal (within subject) design where participants are assessed before and after exposure to electromagnetic fields. This approach maximises sensitivity to change because between subject variation in the results is minimised by calculating the difference between before and after measurements. However, for human studies, practical and ethical considerations impose severe restraints on the intensity and duration of exposure that can be allowed. Therefore, exposure dose was necessarily low and such studies may miss important chronic effects. For this reason, the human studies differed from the animal experiments by including two parallel strands of investigation: longitudinal assessment of acute effects of short-term exposure and cross-sectional comparison of groups of mobile telephone users. The latter strand utilised two groups: heavy users and light users of mobile telephones. The differences between groups were assessed to determine if there may be any chronic effects of mobile telephone use. In the following, the two strands will be termed the *within subject* and *between subject* studies. While there were similarities in the measurement protocols, there were also some important differences that reflect the different aims and constraints. The exposure protocols were obviously different.

The human studies were carried out in several laboratories. Each laboratory had different interests, expertise and equipment. The protocols defined a common core of measurements to be carried out in all laboratories, using equipment that was similar in principle but may differ in some respects. Additional protocols defined further measurements that were carried out in only a limited set of laboratories. For example, measurement of the effects of contralateral stimulation via the efferent nervous system was spearheaded by NSS Laboratories in Lyon and carried out also by AHEPA in Thessaloniki and RCA in Moscow.

Participants and screening

Participants were healthy young adults without any evidence of hearing or ear disorder, corresponding to the ISO definition of *otologically normal*. Similar numbers of males and females were included. The rationale is to test a group that is representative of the population of young otologically normal people. Absence of pre-existing hearing or ear disorder maximised the sensitivity of the study to detect small changes that may occur. Specifically, participants satisfied the following criteria.

- Age between 18 and 30 years.
- In a good state of general health.
- Hearing threshold levels (HTL) in both ears no worse than 20 dB at any of the standard audiometric frequencies between 0.5 and 8 kHz.
- No evidence of conductive hearing loss based on air-conduction and bone-conduction audiograms.

- Normal tympanograms and acoustic reflexes present in both ears for stimulation using a 1-kHz tone at 100 dB HL.
- Normal appearance of the tympanic membrane on otoscopy.
- No history of otological disorder.
- No history of familial hearing disorder.
- Noise exposure infrequent (e.g. night clubs) and without persistent effects.
- No self-reported hearing difficulty or persistent tinnitus.
- No exposure to ototoxic drugs by injection or topical spray (e.g. for severe burns).
- No excess consumption of alcohol or drugs 24 hours prior to testing.
- Presence of clear recordable TEOAE (*within-subject* study only), defined as signal-to-noise ratio (SNR) greater than 6 dB in two or more half octave bands centred at 1.5, 2, 3 and 4 kHz

Acceptance as participants was based on otoscopy, audiometry by air conduction (0.5, 1, 2, 3, 4, 6, 8 kHz) and bone conduction (0.5, 1, 2 kHz), tympanometry and acoustic reflex testing, and a simple screening questionnaire concerning medical and otological history (see Appendix I).

Additionally, for the *between-subject* study, participants were either *heavy* or *light* users of mobile telephones, defined as follows.

- *Heavy users*: typically speaking for at least 30 minutes per day using a mobile phone held to the ear (i.e. disregarding text messaging and hands-free use).
- *Light users*: typically speaking for less than 5 minutes per day using a mobile phone held to the ear, including non-users.

Membership of these two groups was established by a simple questionnaire and verified during the test session where possible by reference to recent itemised bills.

Methods for assessment of auditory function

The following tests formed the core methods for assessment of auditory function. For the *within-subject* study, they were performed immediately before and after exposure to electromagnetic fields (see below for exposure protocol). Only the exposed ear was tested. For the *between-subject* study, they were performed only once and both ears were tested. All testing was carried out in a sound-treated room or booth satisfying criteria in ISO 8253-1 for air conduction audiometry using earphones down to 0 dB HL.

- Transient otoacoustic emissions (TEOAE) using click stimuli.
- Distortion product otoacoustic emissions (DPOAE): DP-gram and I/O function.
- Auditory brainstem response (ABR) using clicks at medium and high rates.

Optionally the following tests were performed.

- Contralateral suppression of TEOAEs.
- DPOAE microstructure sweep.

Further details of the protocols are given below. The equipment used varied amongst laboratories; the apparatus specified here is described as an example. As a consequence, there were minor variations in the implementation and parameters of the test protocols amongst laboratories.

TEOAE measurement

The Otodynamics ILO-88 or ILO-288 system was used to record TEOAEs according to a "linear" protocol, using clicks at 60, 70 and 80 dB. Each measurement run included a minimum of 500 "sweeps" (i.e. 2000 clicks). The rationale of using the lower click intensities is to maximise sensitivity to change. The range of intensities enabled part of the input-output function to be characterised. [Duration approximately 6 minutes per ear].

DPOAE measurement

Where possible, PC-based apparatus consisting of DSP card, A/D and D/A converters, Etymotic ER-10B probe microphone, Etymotic ER-2 insert earphones and associated software was used to record the 2F1–F2 distortion product. As this equipment was not available at all centres, the equipment configuration used to record DPOAEs varied between test centres, for example using ILO-96 systems.

The frequency ratio F2/F1 was constant at 1.22. Primary tone levels L1 and L2 were 60 and 50 dB respectively. The tones were swept with F2 covering the range 2 to 8 kHz in 1/16-octave steps. For each step, measurement of the DPOAE utilised signal averaging for 6 s, or until SNR was at least 15 dB, whichever occurred first. [Duration 3 minutes per ear].

For the *within-subject* study only, with a frequency ratio F2/F1 of 1.22, an input-output (I/O) function was measured for F2 = 2 and 4 kHz and the following combinations of L1 and L2: 50/35, 55/40, 60/50, 65/60, 70/70. These combinations approximate the "scissor-level" paradigm of Kummer et al. (2000). For each step, measurement of the DPOAE utilised signal averaging for 6 s, or until SNR reached at least 15 dB, whichever occurred first. [Duration 1 minute per ear.]

Additionally, for the *between-subject* study, the F2 sweep measurement was repeated for the following combinations of L1 and L2: 50/35, 55/40, 65/60, 70/70. This also allowed input-output (I/O) functions to be determined for every frequency. [Duration 12 minutes per ear.]

Auditory brainstem response (ABR)

Standard apparatus was used with recording electrodes positioned on the vertex, ipsilateral mastoid and contralateral mastoid. Stimuli were 0.1-ms broadband clicks presented at an intensity level of 93 dB peak-equivalent SPL. Note that 33 dB peak-equivalent SPL corresponds to 0 dB nHL (Lightfoot, 1992). Two click rates were used: 33.1 and 74.1/s with filter settings of 100 Hz (high-pass) and 3000 Hz (low-pass). Recordings entailed averaging the responses from 2000 clicks at each rate. The amplitudes and latencies of waves I and V and the ABR were measured. The rationale was to test the refractory behaviour of the auditory nerve and brainstem under a high rate of stimulation. The difference in magnitude of response (e.g. wave V) at the two rates gives an indication of the ability of the nerve to sustain high rates. [Duration 4 minutes per ear].

Medial efferent system testing using TEOAEs and Contralateral Acoustic Stimulation (CAS effect)

Two TEOAEs were recorded using ILO-88 apparatus (one with and one without contralateral acoustic stimulation), using the linear mode, at five different intensities (3-dB steps between the different intensities), with intra-meatal intensities from 57 to 69 dB pSPL (usually, *gain between -21 and -6*). The click intensities were presented randomly.

The contralateral stimulation consisted of 35 dB SL white noise, (*Menu 3, Noise generation, option 6: white noise*), generated by the ILO-88 system (or, alternatively, by a separate audiometer), by means of ILO alternating protocol (called difference on/off): 6 epochs of 80 clicks (3 with and 3 without CAS) = 480 response averaged (240 with/240 without). If the rejection rate was higher than 15%, the recording was repeated [Duration: 15 – 20 minutes per ear].

The main advantages of the generation of the contralateral stimulus directly by the ILO apparatus were:

- The contralateral stimulation is not long enough to have a residual effect after it has been switched off.
- The likelihood of probe movements between the trace without and with contralateral stimulation is reduced.

On the other hand, the disadvantage is that the white noise stimulus is generated by the ILO-88 and hence is modulated by the generation process.

Variation in testing between centres

The audiological measurements differed across laboratories. The following Table *a* shows the planned allocation of test methods to laboratories. All laboratories carried out audiometry before and after exposure. The principle of sharing test types among laboratories aimed to keep test times as short as possible.

The timing shown in Table *b* illustrates the schedule for testing where TEOAE and DPOAE were included. The schedule was somewhat different for other combinations. Note the reversal of order before and after exposure, with tests most likely to show an effect of GSM closest to exposure period.

Table a – Audiological measurement across GUARD laboratories

Laboratory	TEOAE	DPOAE	CAS	ABR
AHEPA	☑	☑	☑	
ISVR	☑	☑		
KMU	☑			
NIRR				☑
NSS	☑	☑	☑	
RCA	☑	☑	☑	☑

<u>*Methods for exposure (within-subject study)*</u>

The within-subject study consisted of baseline audiological measurements, genuine or sham GSM exposure, followed by repeat audiological measurements. Participants attend for two sessions: genuine and sham exposures. The administration of genuine and sham exposure was double-blind and counterbalanced in order.

Objectives

The exposure consisted of speech at a typical conversational level delivered via an earphone to one ear, plus GSM exposure in either genuine (test) or sham (control) conditions. Genuine and sham exposures were on separate days (at least 24 hours apart) with the test participant and tester both blind to the condition being used. GSM exposure utilised the normal output of a consumer mobile phone (NOKIA 6310) at full power for 10 minutes. Half of the participants received GSM exposure at 900 MHz (full power = 2W) and the other half received GSM exposure at 1800 MHz (full power = 1W).

Equipment requirements

1. NOKIA 6310 mobile phone without SIM card (provided by NOKIA Research Laboratory), including battery charger and spare battery.
2. Phoenix software to control mobile phone (carrier frequency, output level, transmit/receive mode). This should was installed from CD onto a suitable PC or notebook computer.
3. Headband and positioning system (provided by NIRR, Gyorgy Thuroczy) with bracket attached to spare battery for phone.
4. A method of using either a “sham load” or “dummy load” was available. The “sham load” intercepts the RF signal to the internal antenna on the phone and dissipates the RF in the load. The “dummy load” looks identical but does nothing, allowing the RF to reach the antenna.
5. Sound replay system with Etymotic ER-3A insert earphone to replay speech to the ear of the participant. For example, the replay system might consist of a CD player, audiometer and insert earphone.

Table b – Timing to illustrate the schedule for testing where TEOAE and DPOAE are included

Phase	Timing	Action
Pre-exposure	5 minutes	Audiometry (air conduction using 2-dB steps in test ear only)
	1 minute	Prepare for DPOAE
	4 minutes	DPOAE measurements
	1 minute	Prepare for TEOAE
	6 minutes	TEOAE measurements
	5 minutes	Prepare for GSM exposure
Exposure	10 minutes	GSM exposure
Post-exposure	1 minute	Prepare for TEOAE
	6 minutes	TEOAE measurements
	1 minute	Prepare for DPOAE
	4 minutes	DPOAE measurements
	1 minute	Prepare for audiometry
	5 minutes	Audiometry

Operational Procedure

The operational procedure was as follows.

1. Collect the phone to be used for the current test participant (Fig. 2.10). This was fitted with either the genuine or sham dummy load by an independent researcher.
2. Ensure that there are no other mobile phones switched on in the test room.
3. Clean the phone with alcohol wipes before use.
4. Ensure that the battery on the phone is fully charged (if not, change to fully charged spare battery).
5. Select the test ear according to the audiometric and other audiological test data that have already been obtained. The principle is to select the ear with the better results on the main outcome measure(s) for the laboratory (TEOAE, DPOAE, ABR). This approach is designed to give maximum sensitivity to detect any change.

6. Examine the test ear to check for excessive earwax. Ears with more than 50% occlusion will be rejected. If the test ear is occluded with earwax while the opposite ear is not, and the opposite ear is suitable in all other respects, change the selection of the test ear.
7. Ask the participant to remove spectacle frames, earrings or any other metal ornaments on the pinna or close by that might alter the electromagnetic field generated by the phone. (If ABR measurements are being made, disposable electrode pads should be left in place but leads should be detached and moved away from the ear.)
8. Attach the sound tube from the ER-3A so that it lies along the jaw with the end within the tragal notch of the pinna. Fix the tube on place using tape designed for attachment to the skin (e.g. Micropore).
9. Place the headband and positioning system on the head. Adjust the position of the mobile phone as follows, ensuring that it will remain comfortable for the 10-minute exposure period. The longitudinal axis of the phone should follow an imaginary line from the entrance to the ear canal to the corner of the mouth. The centre of the radiated field should be over the entrance to the ear canal. The centre of the field corresponds approximately to the letter K in the word NOKIA printed on the back (battery side) of the phone. The area of the phone around the earphone grille should rest with light pressure on the pinna, causing slight deformation of the pinna in most participants. Tighten the adjustment screws on the headband and check that it is comfortable.
10. Connect the serial data cable from the PC to the phone. Run the Phoenix software to set the exposure parameters to the required frequency (900 or 1800 MHz) maximum power.
11. The subject is asked to perform an attention task so that they attend to the speech stimulus, such as counting the number of times a specific work occurs in the speech material. Start the speech replay system, set the software into Transmit mode, disconnect the serial data cable and start the exposure timer. The exposure has now started.
12. When the timer indicates that the exposure is complete, stop the speech replay system, remove the phone immediately from the positioning system and ask the participant appropriate questions about the speech material. The purpose of asking questions is to ensure that the participant has been actively listening to the speech.
13. Ask the subject if they experience any subjective effect from the exposure.
14. Remove the ER-3A earphone and prepare for audiological testing.
15. After audiological testing, re-connect the phone to the PC and set the phone back to the default mode.

Fig 2.10 Example of exposure system positioning during experiments

Experimental procedures

Ethical Committee approvals were required for all studies involving human participants. Particular emphasis was attached to the deliberate exposure to electromagnetic fields involved in the *within-subject* study. Justification relied on similarity with usual levels of exposure for users of mobile telephones, careful control of exposure levels and absence of effects in animal studies for similar exposures.

Pilot studies

The purpose of the pilot studies was to ensure that the test protocols could be implemented in all laboratories successfully and timings were viable. Each laboratory tested a minimum of three participants according to each protocol (*within-subject* and *between-subject*). Translation of questionnaires was required into the local languages.

Recruitment of participants

Participants were divided across the seven laboratories in approximately equal numbers. Initial recruitment included explanation of the purpose of the study, signature by the participant indicating informed consent, and screening tests against inclusion criteria. Sufficient potential participants were screened to allow for attrition due to exclusion and poor data acquisition.

Within-subject study

Participants who have satisfied the inclusion criteria were scheduled to attend for two test sessions lasting approximately one hour (depending on optional measurements). During one test session the exposure system was active; during the other it was inactive (sham exposure). The participant was unaware of the exposure condition. The order of conditions was counter-balanced across participants. Electrodes were attached for ABR measurement and remained in place for the entire session. Electrode contact impedance was lower than 5 kΩ. Suitable probe tips were selected for TEOAE, DPOAE measurement. Pre-exposure measurements were taken, with order of tests (TEOAE, DPOAE, ABR) counterbalanced across participants. Post-exposure measurements were in the same order as pre-exposure for each participant. The measurements were completed in succession without unnecessary delays. Following pre-exposure measurements, the exposure system was adjusted and electromagnetic fields applied according to the previous “Methods for exposures (within-subject study)” section (see above). Immediately following exposure, the post-exposure measurements were carried out. The time at which each measurement was recorded, relative to the end of exposure, was logged. Finally, the electrodes were removed and participants asked whether they noticed any effects of the exposure.

Between-subject study

The recruitment process involved division of participants into *light user* and *heavy user* groups. Both groups were tested in exactly the same way. The order of testing ears and methods (TEOAE, DPOAE, ABR) was counterbalanced using the same design for each group (participants in the two groups were paired for each order). Where the same testing was used, procedures were identical to the *within-subject* study.

Following testing, a detailed questionnaire on noise exposure history was completed by interview with the participant. The purpose was to assess any history of noise exposure as accurately as possible, for inclusion as a covariate in the statistical analysis.

<u>Positioning systems</u>

A positioning system for exposure system fixation was designed and developed according to the following requirements:

- Comfortable for the subjects under investigation
- Repeatable positioning
- Free positioning of the phone in any directions
- Adjustable positioning to the different head sizes of the subjects
- Good fixation after the position
- Easy handling
- No perturbation of the radiofrequency field
- Light weight

The phone

According to the human protocol, the mobile phone was connected via serial data cable from the PC to the phone and running a software to set the exposure parameters to the required frequency (900 or 1800 MHz) and required power (maximum 2 W peak, 250 mW average). Within the GUARD project a NOKIA 6310 phone was chosen for the investigation.

Comfortable system for the subjects

In order to develop a comfortable holder, a system of phone fixation with a possibility of freely moving of the subjects' head was designed. Therefore the positioning holder has three main parts: a headband, an adjustable arm and the phone holder (Fig. 2.11).

Structure

All parts of the positioning system were made by non-metallic plastic materials in order to avoid any perturbation of the electromagnetic fields emitted by the mobile phone. The headband (1) provides free movement of the head without any replacement of the phone from the adjusted position. By using the adjustable arm (2) the phone may be placed into the requested position and may be adjusted according to the size of the subject under investigation. The adjustable arm can be placed on both side of the headband (Fig. 2.11). The phone holder (3) is placed on the battery-side of the phone. In this way the weight of the holder may be kept as light as possible.

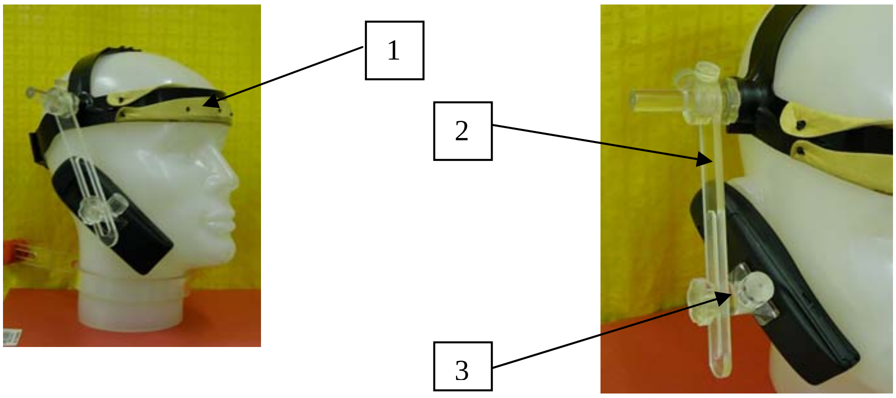

Fig. 2.11 - The positioning system: headband (1), adjustable arm (2) and phone holder (3)

Positioning

According to the GUARD human protocol the researcher doing experiments must place the headband and positioning system on the head then adjust the position of the mobile phone as follows, ensuring that it will remain comfortable for the 10-minute exposure period. The longitudinal axis of the phone should follow an imaginary line from the entrance to the ear canal to the corner of the mouth. The centre of the radiated field should be over the entrance to the ear canal. The centre of the field corresponds approximately to the letter K in the work NOKIA printed on the back (battery side) of the phone. The area of the phone around the earphone grille should rest with light pressure on the pinna, causing slight deformation of the pinna in most participants. Tighten the adjustment screws on the headband and check that it is comfortable. The position of the mobile phone should mimic the normal use of wireless devices (Fig. 2.12). These procedures can be made by the plastic screws of the adjustable arms and phone holder.

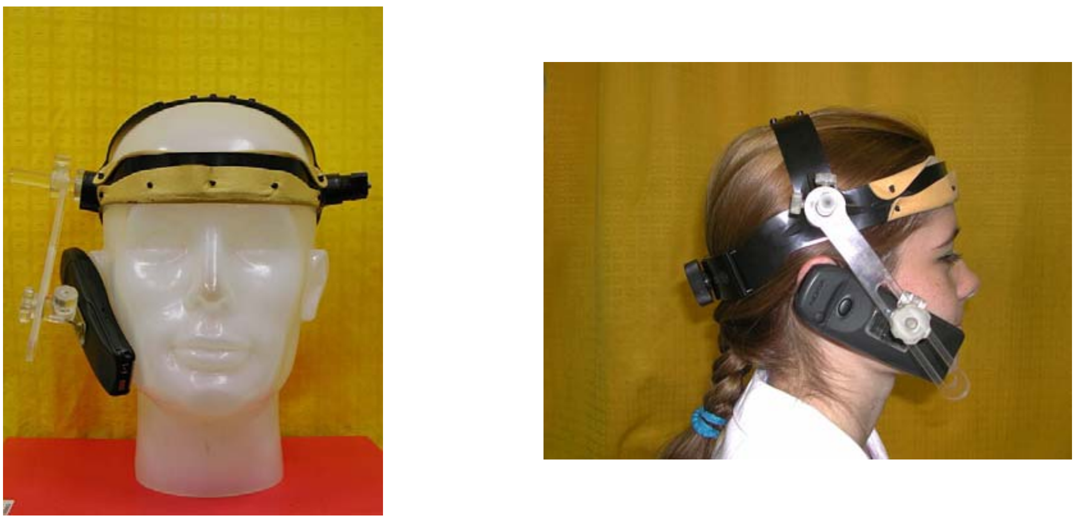

Fig. 2.12 Positioning system for human study within GUARD project.

Exposure system test and dosimetry

Selection of the source

The specific aim was to provide relatively high absorbed power (SAR) within the ear region. The other specific requirement was to use a commercial phone (for ethical reasons). The exposure level must be below the EU 519/99 recommendation in any condition within the study (2 W/kg)

The following requirements were preferred for choosing the commercial phone used in the research:

- The phone must be commercially available in the market
- The phone has to have the EC label according to the CENELEC product standard
- Having two GSM band (900/1800)
- The phone has to have maximum exposure at the ear region of the head symmetrically
- Needs an external antenna connector
- Relatively recent model
- Relatively widely used model
- Low weight
- Available PC connection for external PC control

Following the above requirements, the Nokia 6310 phone was chosen for the human study. The Nokia 6310 mobile phone provides all of above requirements. The phone has an integrated antenna, external antenna connection, is widely used type in Europe, dual band, low weight. Both exposure systems, related control codes and technical assistance and support was provided by the NOKIA Research Laboratory in Helsinki (Dr. Sakari Lang), whose collaboration resulted fundamental in performing these activities.

RF power output measurements

According to the human protocol, the mobile phone was connected via a serial data cable from the PC to the phone and running software in order to set the exposure parameters to the required frequency (900 or 1800 MHz) and required power (maximum 2 W peak, 250 mW average, at 900 MHz; 1 W peak, 125 mW average at 1800 MHz).

Within the project six Nokia 6310 phones were distributed for the human studies. Each human laboratory received a phone, a PC control system and a positioning system. The phones were measured before and after the whole human experimental series. Before the distribution of the phones, the output power of the phones via the external RF output was measured.

The phones have an identity number and we measured the RF output power for each using the external antenna connector (Fig. 2.13). The aim of this measurement was to compare the power output of the selected phones. The results show that the phones had the same output power at each power level provided by the software and the GSM system. Therefore the laboratories used the devices with the same RF powers in the human experimental studies.

Fig. 2.13. Power measurements (left) and the external RF output and cable on the phone (right)

RF power output stability measurement

According to the human experimental protocol, the phones must emit the same RF power during the exposure period. Since the phones used battery power, the measurement of long term RF output stability was validated before the experimental work. The long term measurement was made by PC data acquisition of the output power during the discharge cycle of the battery. The phones were switched to the highest output RF power level (level 5 at 900 MHz, level 0 at 1800 MHz).

The sample rate of PC controlled Voltage multimeter was 20 s. The RF power uncertainty of the phone during the whole lifetime was below 1% using the highest power levels: at 900 MHz, 2W; at 1800 MHz, 1W. In the first 10 minutes the uncertainty was below 0.4%.

The Nokia GSM phone system did not show any decrease of the output RF power during the discharge of the battery. Decreasing the battery voltage below a critical level caused the phones to switch off. Therefore the RF power stability during the experimental exposure period was assured by the selected GSM phone.

Surface scanning of the RF emission

For the evaluation of the exposure characteristic of the phone, the near E-field has been scanned on the surface. The scanning was performed within 6×16 cm area at 1 cm distance from the surface of the phone (Fig. 2.14). The resolution of step was 0.5 cm. An automatic scanning driver was used for moving the near field probe (Kuster-probe)

External load application

For the sham exposure conditions an external power load was applied using the external antenna connector output of the phone. Small and light 50-ohm loads and dummy loads were developed for sham/exposed conditions with the same shape and structure (Fig. 2.15). In order to control the efficiency of the load, surface scanning near field measurements were performed. The external load application provided the double-blind conditions for the human studies. The sham or genuine exposure was performed using a “load” or a “dummy load”. The “load” intercepts the RF signal to the internal antenna on the phone and dissipates the RF in the load, while the “dummy load” looks identical but does nothing, allowing the RF to reach the antenna. No radiated RF fields were measured using the RF load connected to the external antenna output.

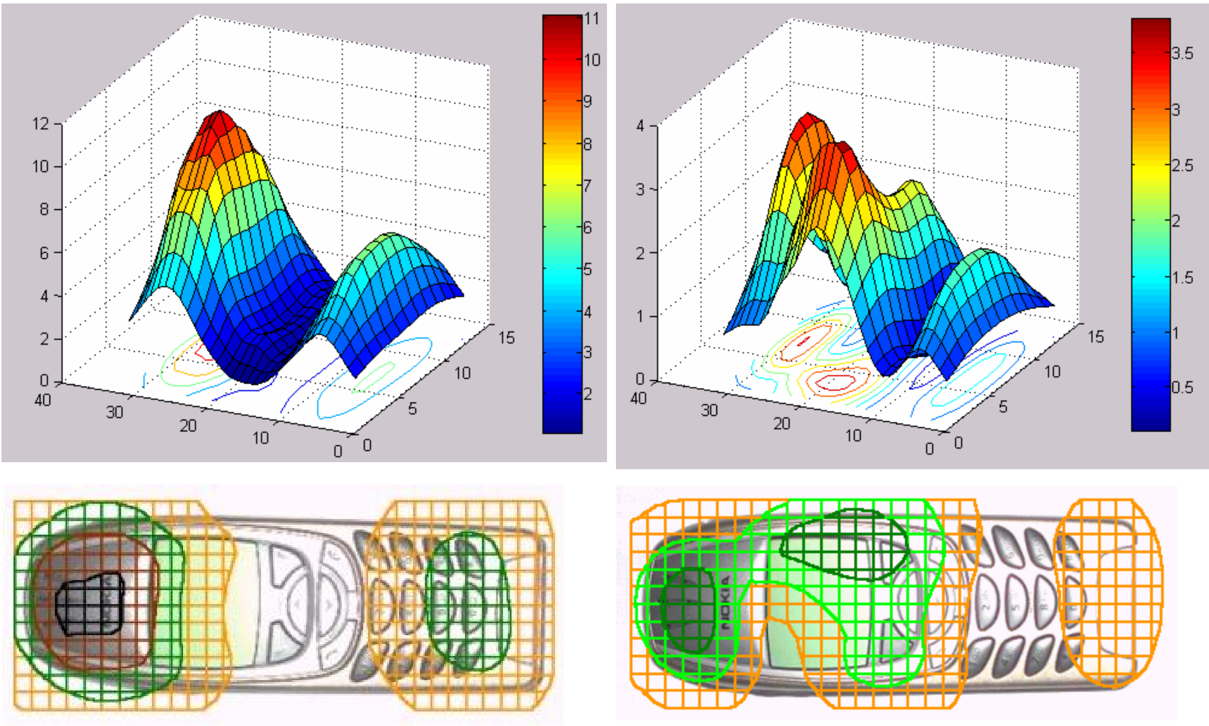

Fig . 2.14 Near-field power density distribution at 900 MHz (left) and 1800 MHz (right). The 3D scale indicates the power density in W/m2.

Fig. 2.15 Two views of the load; the maximum dimension is of about 2 cm

SAR measurements

The SAR measurements were made in the CENELEC liquid phantom using the "touch position" of the phone (Fig. 2.16). In the measurements, a 3-D step motor robot system with internal E-field probe (Kuster-probe) and non metallic phone positioning system was applied according to the CENELEC standard. The SAR measurements were performed with and without modelling the ear tube in the liquid phantom.

Fig. 2.16 SAR measurement system with liquid phantom and 3-D motor driver system.

All measurements were performed at 900 and 1800 MHz. The mobile phone under test was controlled by PC program user interface and powered at the maximum RF level (2 W peak at 900 MHz, 1 W peak at 1800 MHz respectively). After the measurement of the internal E-field in the liquid phantom the results were converted to SAR (W/kg) according to the CENELEC standard requirements (Fig. 2.17, 2.18, and Table *c*).

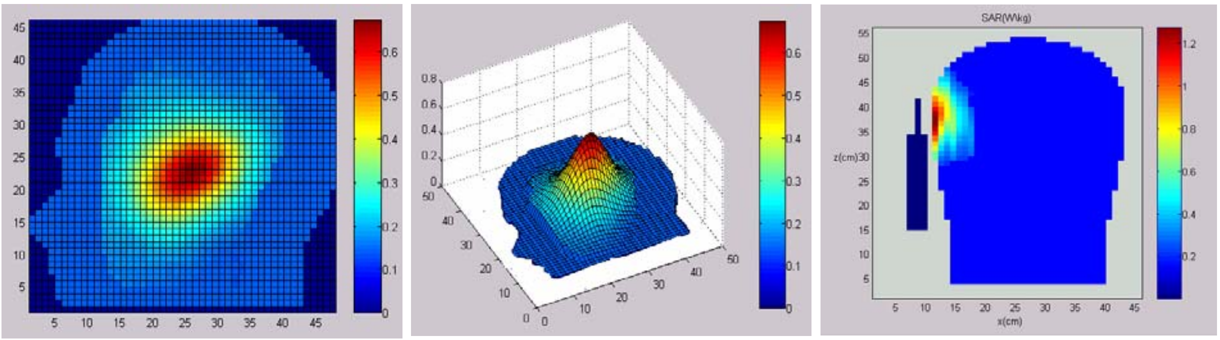

Fig. 2.17 SAR distribution within the head phantom at 900 MHz.

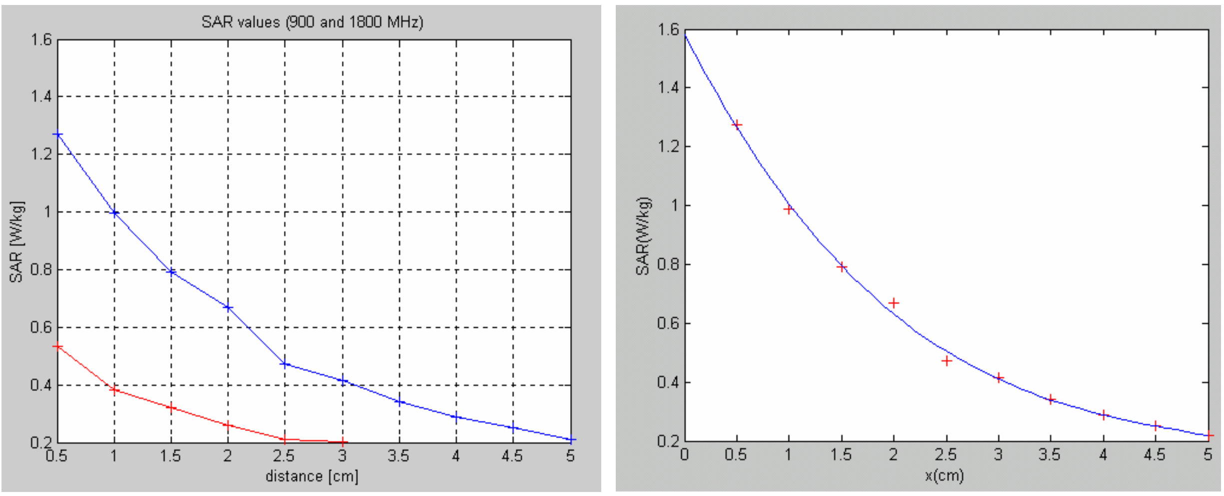

Fig.2.18 Measured SAR values (W/kg) vs. the distance from the phantom surface in the liquid phantom at 900 MHz (blue) and 1800 MHz (red) (left) and the numerical extension of the SAR to the phantom surface (right).

According to the anatomical position of the cochlea the relevant distance from the surface equates to 3 cm. The SAR values at the location of interest in this study were 0.413 W/kg at 900 MHz and 0.190 W/kg at 1800 MHz respectively.

Table c. The maximum SAR values at different layers in the liquid phantom, at 900/1800 MHz.

Distance from the surface of the phone [cm]	**SAR_{max} [W/kg]**	
	900 MHz	**1800 MHz**
0.5	1.273	0.532
1	0.998	0.381
0.5	0.792	0.319
2	0.670	0.258
2.5	0.474	0.201
3	***0.413***	***0.190***
3.5	0.341	
4	0.288	
4.5	0.251	
5	0.212	

ELF magnetic fields

The mobile phones usually generate low-level Extremely Low Frequency (ELF) magnetic fields due to the battery, which supplies the power for working during the RF radiation. The frequency range is usually below 300 Hz. ELF magnetic field measurements were performed by an Wandel & Goltermann EMF Analyser system between 5 Hz and 300 kHz with a small isotropic probe.

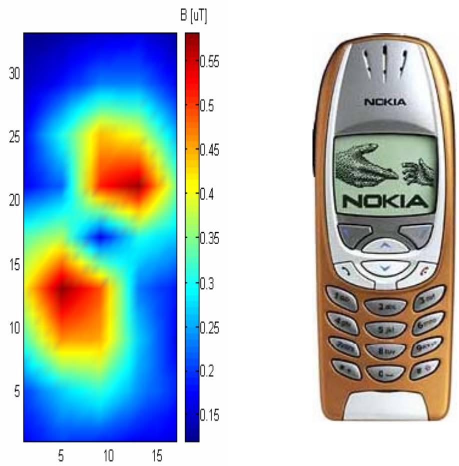

Fig.2.19 ELF magnetic field distribution on the surface of the phone (maximum: 0.58 (T), minimum: 0.12 (T), surface average: 0.26 (T), background: 0.1 (T)

The maximum ELF magnetic field was 0.58 (T), the minimum was 0.12 (T) respectively. The surface average was 0.26 (T) over the whole mobile phone surface (Fig. 2.19). Therefore the ELF field was negligible in the present study.

Analysis of results

Statistical analysis was performed initially at each laboratory for the local data and finally at ISVR, NSS and CNR.ISIB Laboratories for the pooled data across laboratories. As there was some variation in data format across laboratories, the pooled analysis was limited to a common subset of data. For both local and pooled data analysis, the data sets were encoded such that the person performing the analysis was unaware of the exposure condition (genuine or sham) or the group membership (*heavy user* or *light user*) for each data set.

Within-subject study

In addition to descriptive analysis of the data, differences in the measures obtained before and after exposure were compared between the genuine and the sham exposure. Statistical analysis methods included Student's t-test for related samples and repeated-measures analysis of variance. Hearing threshold levels and other data obtained at the screening stage were used as covariates.

Between-subject study

In addition to descriptive analysis of the data, the two groups were compared (*heavy user* and *light user*). Statistical analysis methods included Student's t-test for independent samples and analysis of covariance. Hearing threshold levels and other data obtained at the screening stage, and detailed noise exposure estimates, were used as covariates.

3. RESULTS

Animal experimentation

During the lifetime of the project, three different types of experiment involving a total of more than 400 animals, were performed:

- study of the acute effects of EMF exposure at 900 and 1800 MHz on the auditory system of Sprague-Dawley rats and Guinea-Pigs (*acute effects*);
- study of the combined effects of ototoxic drugs and microwave exposure on the auditory system of Sprague-Dawley rats and Guinea Pigs (*combined effects*);
- in-vitro study on hair cells in newborn rats (*in-vitro effect*).

Table *a* reports in details the number of animals and the type of effects found after the statistical analysis of the data collected according to the protocols described in the previous Chapter 2.

Table a: Scheme of the number of animals tested and type of effect.

	Number of animals	**Effects**
ACUTE	168	No effects
COMBINED	230	No effects
IN-VITRO	90	No effects
TOTAL	488	

Human experimentation

During the lifetime of the project, three different types of experiments involving a total of more than 500 subjects, were performed:

- study of the acute effects of GSM mobile phone at 900 and 1800 MHz on the auditory system of humans (*within-subject* study);
- study of the chronic effects of GSM mobile phone on the auditory system of humans (*between-subject* study);
- study of the contralateral acoustic stimulation effects (*CAS Analysis*) on TEOAE both in the within- and between-subject approach;

Table *b* reports in detail the number of human subjects and the type of effects found after the statistical analysis of the pooled data collected according to the protocols described in the previous Chapter 2.

Table b: Scheme of the number of humans tested and type of effect.

	Number of subjects	**Effects**
WITHIN	169	Sporadic
CAS ANALYSIS WITHIN	95	Some effects
CAS ANALYSIS BETWEEN	65	No effects
BETWEEN	213	No effects
TOTAL	542	

Summary of the results

No effects on the main measures of the status of the auditory system were found, either in animals or humans.

Sporadic effects found here and there in the human within-subject study were not consistent enough to come to any reliable conclusion about presence of an effect.

Similarly, some statistically significant effects found during the final weeks of the project in an advanced analysis related to study of the CAS effects in the within-subject investigation, on possible differences in males and females; these findings are not regarded as reliable without further verification due to the small number of the subjects in the two sub-population involved. This must be investigated more thoroughly in future, before coming to any conclusion.

In order to summarise the results of GUARD, in the following, a list of the scientific papers (in press, submitted and in a final stage of preparation) on GUARD results is included. The full GUARD publication list is attached to this report as ANNEX I, whereas the copies of the manuscripts listed below are attached to this report as Annex II “Guard Publication Copies” to this report.

Animal Experimentations

1. Galloni P., Lovisolo G. A., Mancini S., Parazzini M., Pinto R., Piscitelli M., Ravazzani P., Marino C.: Effects of 900 MHz electromagnetic fields exposure on cochlear cells' functionality in rats: evaluation of Distortion Product OtoAcoustic Emissions. *Bioelectromagnetics, in press*;
2. Galloni, P., Parazzini, M., Piscitelli, M., Pinto, R., Lovisolo, G. A., Tognola, G., Marino, C. and Ravazzani, P.: Effects of GSM cellular phone radiation on the inner auditory system of Sprague-Dawley rats. *Submitted to Radiation Research.*

3. Brazzale A. R, Galloni P., Parazzini M., Marino C., Ravazzani P.: Assessing the repeatability and reproducibility of Distortion product Otoacoustic emissions in Sprague-Dawley rats by hierarchical modelling. *Submitted to Journal of Applied Statistics.*
4. Parazzini M., Galloni P., Brazzale A. R., Tognola G., Marino C., Ravazzani P.: Assessment of the stimulus level influence on the repeatability of low frequency distortion product otoacoustic emissions in laboratory animals. *Submitted to IEEE Trans. Biomedical. Engineering.*

Human Experimentations

1. Parazzini M., Interactions between radio frequency electromagnetic fields produced by mobile phones and the auditory system: biological effects and numerical dosimetry*, PhD Thesis, Polytechnic of Milan,* 2004*.*
2. Parazzini M., Bell S., Thuroczy G., Tognola G., Lutman M. E., Ravazzani P.: Influence on the mechanisms of generation of distortion product otoacoustic emissions of mobile phone exposure, m*anuscript in final stage of preparation, to be submitted to Hear. Res..*

<u>*Future Actions*</u>

- Dissemination activities, continuously pursued during the three years of the project, will continue also during the next two years, considering the continuing major interest on this topic and the time needed to reach publication of papers in scientific journals.
- The full processing of the huge mass of recorded data (more than 7,000 recordings, considering animals an humans) needs to be continued into 2005, also considering that some additional processing was defined only in the last two months of the project (e.g., wavelet analysis of CAS TEOAE), as a consequence of some human results..
- The scientific book on “Electromagnetic field exposure and hearing” is in preparation. A preliminary agreement was reached with IOS Press, in the framework of the possible publication series Electromagnetic Fields and Health under the auspices of the Coordination Action EMF- NET.

4. DISCUSSION AND CONCLUSIONS

On the basis of the GUARD activities, obtained results and related analysis, the following conclusions can be drawn:

- No effects on the main measures of the status of the auditory system were found;
- Potential effects of GSM on the auditory efferent system (*CAS analysis*) should be further investigated;
- The obtained results need at least one additional year for dissemination and further data analysis;
- GUARD results cannot be extrapolated directly to others RF signals and modulation patterns (e.g., 3G UMTS, Wi-Fi, radio base stations);
- GUARD proceeded as planned, on time and within budget;
- The activities and results of the project during its lifetime have provided some informative answers to public concern about mobile phones and health, having an important impact on concerned stakeholders and general public;
- The activities of the project have influenced the scientific field, helping by focusing EMF and health research on the study of the effects on sensory systems;
- The results of GUARD may be used as an input to industry and other stakeholders to assess potential impact mobile phones on health (see also the following Chapters);
- GUARD activities have generated a continuous process of requests for and exchange of information from consumer associations in Europe, media and European citizens about the possible effects of mobile phones on hearing in particular, and on sensory systems in general.
- GUARD has established a network of researchers that will continue to function after the project.

5. EXPLOITATION AND DISSEMINATION OF RESULTS

GUARD activities and results were disseminated throughout the project lifetime, both in the scientific arena and to the general public. Attached as Annex I is the full list of GUARD publications. Moreover, a continuous process of requests for and exchange of information developed with consumer associations, European citizen and media.

Scientific Dissemination

The results of GUARD were disseminated in the two scientific areas mainly interested to GUARD research (i.e., the audiological field and the study of the health effects of EMF), throughout the project lifetime.

Six manuscripts have already been prepared and submitted to leading scientific journals of both fields. Five other manuscripts are already planned on the basis of the final analysis of the data.

A scientific book “EMF effects on hearing” is currently under preparation, and should be published by IOS Press, including contributions from all GUARD activities but also from other research related to EMF and hearing, including microwave hearing and the study of the effects on the vestibular system.

Moreover, more than 40 presentations to Scientific Conferences, both at European, International and national level, on GUARD results and activities were given (6 as *Guest Speakers*).

The GUARD Consortium organized two main scientific events about EMF and hearing. The first one was in the course of the EBEA 2003, 6th International Congress of the European Bioelectromagnetics Association (EBEA), that was held in Budapest in November 13-15, 2003: a Special Session on “Potential effects of cellular phones on the hearing system” was organized by the GUARD Consortium, presenting not only GUARD results, but also inviting other external experts to present their results on the effects of GSM.

The second one was in the course of the EUFOS 2004 5th European Congress of Oto-Rhino-Laryngology Head and Neck Surgery, Rhodes, Greece: a Special Session on “Effects of Mobile Phones on Hearing” was organized by the GUARD Consortium on September 12, 2004, to an audience of about 50 delegates.

In the course of GUARD, a special effort was made to disseminate the results of the project in the whole European area beyond the borders of the European Union. The presence in the consortium of three laboratories coming from Associated and Third States facilitated this task.

Dissemination towards general public and other disseminations

The results of GUARD were also disseminated to the general public.

First of all the GUARD Website (http://www.guard.polimi.it) was developed and maintained. Moreover, a leaflet describing GUARD objectives, partnership, social relevance, were prepared and disseminated widely to both scientific societies and international bodies and to the general public, together with the series (four issues) of the GUARD News, that is newsletter on GUARD activities, published on ad hoc basis, when something relevant occurred. The GUARD News issues were downloadable from the GUARD Website and were distributed by electronic media to research centres, key people in public health services and administrations, European mobile telephony manufacturers, libraries, editors of international scientific journals, representatives of National and International societies of both scientific areas involved in these research, and all main Consumers Associations, Environment Protection Agencies and Authorities in Europe.

Further, GUARD activities were disseminated by media (such as BBC Online, Sky Italia), by some national press releases (Italy, UK) and websites across Europe (Germany, Hungary, Italy, UK, Lithuania).

The GUARD project is also participating through its Coordinator, Paolo Ravazzani, in the EC Coordination Action EMF-NET, aimed to provide a common framework for the interpretation of the results of the research activities on EMF and health in Europe, including a coordinated dissemination of the results of the EC projects. To this end, an agreement about the publication of a Series on EMF and Health was reached with IOS Press. The first volume of the Series should be the one on GUARD mentioned above.

6. POLICY RELATED BENEFITS

In the last decade European public concern has been growing on the potential adverse health effects due to the use of mobile phones. Although many studies have addressed the biological effects of high-level microwaves, so far low-level microwaves, such as the EMF produced by mobile communications, have not been studied with the same intensity and conclusions about health hazards are not based on reliable scientific evidences. In particular, no studies have investigated the effects on hearing. This is difficult to understand, bearing in mind that the hearing system, for simple geometrical considerations, should be the biological system most affected by the mobile phones. The cochlear hair cells are also known to be highly sensitive to exogenous and endogenous agents. These gaps in knowledge need to be addressed with urgency.

Over a period of just a few years, mobile phones have produced a revolution, involving not only communication systems and the technological sphere, but also the whole social and environmental domains. Their widespread use has produced a huge and irreversible modification in the everyday life of all Europeans, irrespective of whether they are among the high percentage of mobile phone users, quickly becoming irreplaceable as communication tools. Due to their widespread use, the environment is subjected to irradiation by their electromagnetic fields (EMF) at GSM radiofrequencies, and in that sense the microwaves represent, now and even more in the near future, an ever-present potential environmental hazard. Moreover, the number of new applications of wireless communications is increasing. Hence, use of wireless communications, and the consequent biological effects and/or risks, cannot be restricted to the domain of personal lifestyle but involves the whole population, and should be considered as a high-priority environmentally related health issue.

These simple considerations about the spread of microwave applications lead to the conclusions that i) mobile phone microwaves are in Europe not only a problem of the mobile users, as personal lifestyle issue, but also impact on the general environment . In that sense, this represents an European environmentally related health issue. ii) This issue should be considered as an environmentally related health priority and hence approached at European level, taking also into account that this topic addresses problems concerned with standardization and regulation of safety standards, which should be approached at an EU level.

GUARD was the first study addressing the effects of GSM mobile phones on hearing. The results of this research represent the first international and European statement about GSM mobile phone hazards for hearing. This project, bringing together a European multidisclipinary research consortium, created and exploited at a European level synergies between disciplines joining together expertise in physics, engineering and medicine, linking technology with environmental and public health concerns.

GUARD provided the first coordinated European response to the concerns about these possible environmentally related health effects. Its results support European health and environmental policy making and public information, contributing by answering the health concerns about hearing hazards due to GSM mobile phones. It has contributed to the process of improving knowledge and providing tools to assess and manage the potential risks, and allowed the identification and formalization of new prevention strategies.

In that respect, GUARD has contributed to the process of improving European public awareness about the links between exposure, health outcome and risk for hearing so that European citizens can receive clear advice about the use of mobile phones and a clear risk assessment.

As to the input to European and International standardization and regulation bodies and other concerned stakeholders such as industry, GUARD has produced a series of feedback issues that can be summarised as follows:

- No need to change the carrier frequency (900 and 1800 MHz) and the GSM modulation;
- No need to change, in particular to reduce, the maximum power emitted by the mobile phones. However, the GUARD Consortium does not recommend increasing the maximum power.
- The results of GUARD on GSM and hearing cannot be directly extrapolated to others RF signals and modulation patterns. Therefore, the effects on hearing of other sources such as 3G UMTS phones, Wi-Fi and radio base stations must be investigated separately.
- Need to include in the numerical or experimental head models used in dosimetry a more realistic representation of the human head. In particular, models should consider in their biological characteristics, which structures may be potentially more affected by electromagnetic field exposure, such as sensory systems and, in particular, the vestibular and hearing systems.
- Need to move towards a "system specific" dosimetry, which must be considered crucial both from the scientific and regulatory points of view.

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