

ORIGINAL ARTICLE

Effects of Intrauterine and Extrauterine GSM-like Radiofrequency on Distortion Product Otoacoustic Emissions in Infant Female Rabbits

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Objective: We investigated the effects of 1,800 MHz GSM-like Radiofrequency (RF) on the cochlear functions of female infant rabbits by Distortion Product Otoacoustic Emission (DPOAE) recordings upon intrauterine (IU) and/or extrauterine (EU) exposure.

Materials and Methods: Thirty-six infant female New Zealand White rabbits, each one-month-old, were randomly divided into four groups, Groups 1-4, each having 9 rabbits. Group 1 animals were remained untreated (Control), whereas other groups were treated by virtue of extrauterine (Group 2, EU), intrauterine (Group 3, IU), or both type of exposure (Group 4, IU and EU) to 1800 MHz GSM-like Radiofrequency (RF). EU exposure started after they reached to one-month of age, and IU was at intrauterine period which was in between 15th to 22nd days of gestation. The duration for exposures was 7 days with 15 minutes per day protocol. Cochlear functions were assessed by DPOAEs at 1.0-8.0 kHz.

Results: DPOAE amplitudes for EU-RF group at 1.0 and 1.5 kHz was found higher with respect to control. At 2.0 kHz, the recordings for EU-RF group were higher than control and IU-RF groups. At 6.0 kHz, EU-RF group were higher than control, IU-RF, and (IU+EU)-RF groups. At 8.0 kHz, EU-RF group were higher than IU-RF and (IU+EU)-RF groups.

Conclusions: We conclude that if the anatomical growth of exposed animate is completed and the extrauterine GSM-like RF exposure is not prolonged, there will not be much harmful effects on inner ear of female rabbits, measured by DPOAEs. The observed increase in DPOAE amplitudes at 1.0-2.0 kHz shows the increase in cochlear activity and outer hair cell electromotility. Prolonged exposure and hyperthermia related to the power density of applied RFR, increasing the temperature in the ear canal, may affect the DPOAE amplitudes especially during intrauterine period. When pregnant women use earphone or even carry their mobile phones in their bags or pockets, foetus may expose to GSM-like RF from the very short distance with the exposure level higher than the standard limits, which may cause ear toxicity with decreased DPOAEs.

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The extensive use of mobile phones with increasing number of users raised concerns about the exposure to electromagnetic (EM) waves emitted by phones which may lead to adverse health effects. This concern is further magnified by the fact that the most common human exposure is by the head of the handset user. It has been further speculated that children would be more vulnerable to the radio frequency (RF) energy absorption from mobile phones^[1].

Electromagnetic fields in the frequency range used by mobile phone and similar technologies do not penetrate deeply into the body. Most of the field energy is absorbed by the skin and by directly underlying tissue, mostly due to the high electric conductivity of the skin. The resulting heat generated in the tissue is readily distributed, mainly by the blood flow. The parameter describing the amount of energy absorbed per unit of tissue within a given time is called

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the specific absorption rate (SAR, units: W/kg). Four W/kg SAR value was shown to result in a temperature increase by 1 °C^[2].

The SAR difference of larger RF energy absorption by the heads of children compared to that of adults is more likely caused by the general variations in the head anatomy and geometry of the individuals, rather than their age. It seems that the external shape of the head and the distribution of different tissues within the head play a significant role in the RF energy absorption^[1]. Since DNA damage is closely related to every aspect of physical and pathological activity of cells, one of the most active areas of RF investigation is the assessment of direct and indirect effects on DNA^[3]. In this context, it has been shown that exposure to 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in Chinese hamster lung cells^[4].

Final results of the INTERPHONE study on the risk of brain tumours, acoustic neurinoma and parotid gland tumours associated with the use of mobile phones will be soon available. Preliminary results do not seem to indicate a substantial increase in risk. Although the concept of precautionary measures adapted to such concerns is critically discussed in detail^[2], there are presently no scientific data supporting the concept of a special vulnerability of children and adolescents to high-frequency EMF, even if the usual caveats are considered.

The potential hazardous effects of 1800 MHz GSM-like Radiofrequency (RF) on the cochlear functions of infancy period is another important question, must be answered by researchers. To investigate the potential hazardous effects of intrauterine and/or extrauterine exposure to 1,800 MHz GSM-like Radiofrequency (RF) on the cochlear functions of infant female rabbits by Distortion Product Otoacoustic Emission (DPOAE) recordings, as well as the probable effects of female hormonal factors upon to 1,800 MHz GSM-like (RF) exposed inner ear and DPOAEs, here, we tailored an experimental study with female rabbits, for the first time in the literature, to our best knowledge. In this study the animals were randomly divided into four

groups, Groups 1-4, each having 9 rabbits. Group 1 animals were remained untreated (Control), whereas other groups were treated by virtue of extrauterine (Group 2, EU), intrauterine (Group 3, IU), or both type of exposure (Group 4, IU and EU) to 1,800 MHz GSM-like Radiofrequency (RF). EU exposure started after they reached to one-month of age, and IU was at intrauterine period which is in between 15th to 22nd days of gestation. The duration for exposures was 7 days with 15 minutes per day protocol. Cochlear functions were assessed by DPOAEs at 1.0-8.0 kHz.

Material and Methods

The study was assessed in Gazi University Faculty of Medicine. Adaptation and care of the animals were taken by Experimental Animal Breeding and Experimental Studies Center of Gazi University. During both the adaptation and experimentation periods, the animals were treated in compliance with the principles of the Declaration of Helsinki^[5].

Animal Subjects

Each one-month old, thirty-six New Zealand white female rabbits were used in the present study. Rabbits were obtained from Laboratory Animals Breeding and Experimental Researches Center of Gazi University. The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All the animal procedures were performed in accordance with the approved protocol.

Baby rabbits were housed under the same conditions, that is the temperature and humidity controlled room (20±1°C, 50±10% relative humidity), and 14-16 h light/dark cycle conditions. Except RF exposure periods, tap water and standard food pellets are provided ad libitum. Since placing more than one animal in a cage would create a stress factor, only one animal was placed in each cage during each RF exposure period. Eighteen infant rabbits had been exposed to 1,800 MHz GSM-like RF, 15 minutes daily for 7 days at intrauterine period (Between 15th to 22nd days of gestational period). Eighteen infant rabbits had not been exposed to 1,800 MHz GSM-like

RF, 15 minutes daily for 7 days at intrauterine period (Between 15th to 22nd days of gestational period). All of these 36 infant rabbits was placed and lived with their mothers until they became one-month old. They had breastfeeding and their optimum growth was obtained during this one-month-period.

Experimental Design

Thirty-six one-month old female New Zealand White rabbits were randomly divided into four groups:

1. Group 1: Nine one-month old infant rabbits were not exposed to 1,800 MHz GSM-like RF (Control-C). The DPOAE measurements were managed when they became one-month old after birth.
2. Group 2: Nine one-month old infant rabbits were exposed to 1,800 MHz GSM-like RF. They were exposed to RF in 15 minutes daily for 7 days after they reached to one-month old (Extrauterine-EU). The DPOAE measurements were managed after RF application.
3. Group 3: Nine infant rabbits were exposed to 1,800 MHz GSM-like RF, 15 minutes daily for 7 days at intrauterine period (Between 15th to 22nd days of gestational period) (Intrauterine-IU). The DPOAE measurements were managed when they reached to one-month old after birth.
4. Group 4: Nine infant rabbits were exposed to 1,800 MHz GSM-like RF twice. The first RF application was 15 minutes daily for 7 days at intrauterine period (between 15th to 22nd days of gestational period). The second RF application was 15 minutes daily for 7 days after they reached to one-month old after birth (IU + EU). The DPOAE measurements were managed after the second RF application.

Intrauterine RF exposure was between 15th to 22nd days of gestational period, that is the transition period from embryogenesis to organogenesis.

Exposure Level and Quality Control

GSM like signals in 1,800 MHz frequency are formed by using a signal generator (Agilent Technologies 8648C, 9 kHz- 3.2 GHz) with the integrated pulse

modulation unit and horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5 - 2.8 GHz) in a shielded room. The length of the cable which connects the signal generator to the antenna is 2 m long, that is, the lateral distance between the signal generator and the antenna is approximately 2 meters. On the other hand, it should be emphasized that the distance between the ear region of the rabbits' heads and the directional horn antenna which generates the RF energy is 10 cm.

The generated power was controlled by a spectrum analyzer (Agilent Technologies N9320A, 9 kHz - 3 GHz) integrated to a signal generator. The signals are amplitude modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 ms), corresponding to the dominant modulation component of GSM (Figure 1).

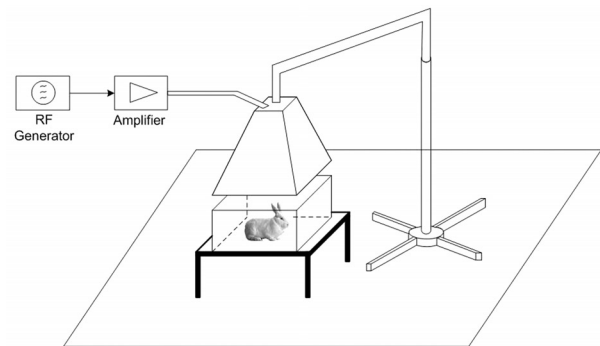


Figure 1. GSM-like Radiofrequency exposure system.

RF Generator provided 20 dB (0.1 W) power during the exposure period. The signal controlled by a spectrum analyzer connected to the signal generator, where NARDA EMR 300 and type 26.1 probe is used for the measurement of output radiation. The data was collected as measurements during the course of the experiment and stored in a computer connected to device via fiber optic cable.

The design of the exposure system and quality controls were performed in Bioelectromagnetics Laboratory of Biophysics Discipline of Gazi University Faculty of Medicine.

DPOAE Recordings

Prior to the experimental DPOAE measurements, ear examination of the infant rabbits was managed by otoscope and any foreign body, if present, was removed from the external auditory canal by curette. The animals having external auditory canal or eardrum pathologies that could prevent noise transmission were excluded from the study. Included animals were anesthetized both during examinations and experiments via intramuscular injection of 40 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, USA) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany). Eye-blink reflexes and respiratory rhythms were followed during the experiments and deep anesthesia was achieved by repeated doses.

DPOAE is generated in 72 ears of 36 rabbits by ILO 288 USB II (Otodynamics Ltd Clinical OAE System, England) cochlear emission analyzer. The acoustic stimulus consisted of two simultaneous continuous pure tones at different frequencies; F1 and F2 (F2/F1: 1.22). The general trend indicates that (for the 2F1-F2 DPOAE) there tends to be a predominance of the wave-fixed component if F2/F1 is equal to 1.22^[6]. Intensities are L1 [dB sound pressure level (SPL)] and L2 (dB SPL)^[7]. DP-grams were recorded over a restricted frequency range and using frequency-scaled stimuli. Frequency sweeps were performed with the primary-frequency ratio F2/F1 and the F1 frequency step held constant^[6]. Specifically, the following stimulus parameters were usually used: L1/L2 of 65 dB SPL/55 dB SPL, F2/F1 ratio of 1.22^[7]. F1 frequency ranges of 1,216-2,432, with sweeps having steps of 32 Hz^[6].

In our study, with L1=65 dB SPL and L2=55 dB SPL, the responses received were not sufficient. It is known that the variations in the external physical dimensions, such as the length and width of the ear canal (a wider ear canal in rabbits than in humans) and the differences in the depth of probe insertion, lead to complicating problems throughout the otoacoustic measurement; thus, they should be corrected^[8-10]. In view of this fact, the intensity of the stimuli was increased to L1=80 dB SPL and L2=70 dB SPL.

The recordings were performed in an isolated quiet environment and the female rabbits were followed to be totally sedated and motionless condition with regular spontaneous breathing, in order to minimize the noise contamination originating from the environment or the muscular activity of the animals. The plastic tubing adapters that presented the optimum fit to the external auditory canal were attached to the emission probe and a closed cavity was formed by placement of the probe into the external auditory canal.

DPOAE recordings and assessments were performed at the laboratories of Physiology Discipline of Gazi University Faculty of Medicine.

Method

In four groups (Group 1, 2, 3 and 4), after infant female rabbits were kept in plexiglas cage; the rabbits were anesthetized, otoscopic examinations were performed; and DPOAE measurements were assessed both ears (Right and left) of each rabbits; and DPOAE amplitudes were detected.

Study were planned and continued in accordance with Gazi University Faculty of Medicine Local Ethics Committee.

Statistical Analysis: Statistical packet for SPSS (Version 9.0) was used for statistical evaluation.

At each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of Group1-4 was analyzed by "Kruskal Wallis Variance Analysis". When statistically significant result was present, "Wilcoxon Matched-Pairs Signed-Ranks Test " with Bonferroni correction was used to detect the time of value which had caused difference.

p value (0.05 was considered as statistically significant.

Results

Table 1 and Figure 2 demonstrates DPOAE amplitudes of all groups (Group 1-4) at 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 kHz.

At each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of Group1-4

was analyzed by “Kruskal Wallis Variance Analysis”. The statistically significant difference were present at frequencies, namely 1.0 kHz (p=0.008); 1.5 kHz (p=0.001); 2.0 kHz (p=0.000); 6.0 kHz (p=0.000); and 8.0 kHz (p=0.000). At 3.0 (p=0.151) and 4.0 kHz, (p=0.093) no significant difference was present (Table 1)

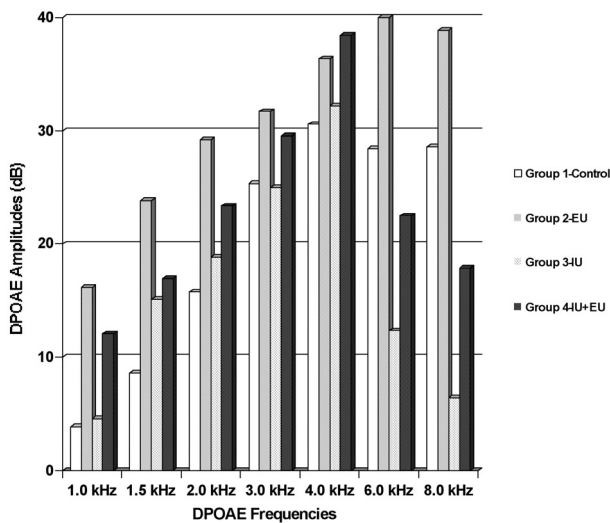


Figure 2. DPOAEs of the Groups 1-4 at 1.0-8.0 kHz

As statistically significant result were present at all DPOAE frequencies of 1.0-2.0 kHz and 6.0-8.0 kHz,

“Wilcoxon Matched-Pairs Signed-Ranks Test “ with Bonferroni correction was used to detect the time of value which had caused difference.

At 1.0 kHz:

-Group 2-EU (16.19 dB) value was significantly higher than Group 1-C value (3.86 dB) (p=0.006).

At 1.5 kHz:

-Group 2-EU (23.80 dB) value was significantly higher than Group 1-C value (8.56 dB) (p=0.006).

At 2.0 kHz:

-Group 2-EU (29.20 dB) value was significantly higher than Group 1-C value (15.78 dB) (p=0.002).

-Group 2-EU (29.20 dB) value was significantly higher than Group 3-IU value (15.09 dB) (p=0.012).

At 6.0 kHz:

-Group 2-EU (43.57 dB) value was significantly higher than Group 1-C value (28.40 dB) (p=0.001).

-Group 2-EU (43.57 dB) value was significantly higher than Group 3-IU value (12.33 dB) (p=0.000).

-Group 2-EU (43.57 dB) value was significantly higher than Group 4-IU+EU value (22.47 dB) (p=0.001).

Table 1. DPOAE amplitudes for the frequencies 1.0-8.0 kHz of the Groups 1-4

Groups	DPOAE Results (dB)						
	1.0 kHz	1.5 kHz	2.0 kHz	3.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
Group 1- Control (C) n=18 ears of 9 rabbits	3.86±7.29	8.56±7.44	15.78±10.11	25.34±12.47	30.56±13.42	28.40±14.74	28.59±19.10
Group 2 Extrauterine RF (EU) n=18 ears of 9 rabbits	16.19±14.31	23.80±15.43	29.20±9.32	31.71±5.48	36.38±5.98	43.57±6.03	38.90±5.12
Group 3 Intrauterine RF (IU) n=18 ears of 9 rabbits	4.54±9.55	15.09±8.60	18.82±9.92	24.95±9.61	32.21±12.52	12.33±21.06	6.39±23.99
Group 4 Intrauterine + Extrauterine RF(+) (IU + EU) n=18 ears of 9 rabbits	12.07±13.35	16.95±10.66	23.34±8.45	29.54±6.17	38.47±7.58	22.47±13.97	17.86±18.65
p*	0.008	0.001	0.000	0.151	0.093	0.000	0.000

*p value shows the results of “Kruskal Wallis Variance Analysis”

At 8.0 kHz:

-Group 2-EU (38.90 dB) value was significantly higher than Group 3-IU value (6.39 dB) (p=0.001).

-Group 2-EU (38.90 dB) value was significantly higher than Group 4-IU+EU value (17.86 dB) (p=0.002).

Discussion

Biological effects of electromagnetic (EM) radiation rely greatly on wavelength and other physical parameters. Exposure to EM fields induces body currents and energy absorption in tissues, depending on frequencies. Thermal effects caused by temperature rise are basically understood^[11]. The most prominent issue is mobile communication including mobile phones (cellular phones), their base stations, cordless phones operating according to the DECT standard and some of the baby phone devices^[2].

The eye is considered to be a critical organ when determining safety standards for radiofrequency radiation. The specific absorption rates (SARs) and thermal effects were determined in a whole-head model. The highest temperature is present in the anterior part of the lens at 900 MHz; and deeper part of the eye at higher frequencies. In the posterior part of the lens, at 1,500 MHz and near to the centre of the eyeball at 1,800 MHz, the peak temperature is detected. The SAR values are not in compliance with safety guidelines, the maximum temperature rises in the eye are too small to give harmful effects^[12]. Higher SAR in the brains of children can be expected depending on whether the thickness of their skulls and surrounding tissues actually depends on age^[13].

Tahvanainen K, et al^[14] suggested that RF exposure to a cellular phone, either using 900 or 1,800 MHz with their maximal allowed antenna powers, increases the temperature in the ear canal. The reason for the ear canal temperature rising is a consequence of mobile phone battery warming during maximal antenna power use.

In the present study, we investigated the potential hazardous effects of IU and/or EU exposure to 1,800 MHz GSM-like RF on the cochlear functions of infant female rabbits by DPOAE recordings. DPOAE amplitudes were found that at 1.0 and 1.5 kHz, EU-RF group were higher than control group. At 2.0 kHz, EU-RF group were higher than control; and IU-RF groups. At 6.0 kHz, EU-RF group were higher than control; and IU-RF; and (IU+EU)-RF groups. At 8.0 kHz, EU-RF group were higher than IU-RF; and (IU+EU)-RF groups.

In the present study, there were increase in the DPOAE amplitudes after extra uterine exposure of 1,800 MHz GSM-like RF compared to control (at 1.0-2.0 kHz); and IU exposure groups (2.0 kHz). Increased DPOAE amplitudes may show increase in the cochlear activity and in the outer hair cell electromotility. According to the Helmholtz's Resonance Theory, if the resonance frequency of the stimulus and cochlear structure is nearer to each other; vibration amplitudes at basilar membrane get higher^[15-17]. And also, as the water content increases in the tissue, affecting ratio from the electric field also increases^[16-18]. In our study, at the apical regions of cochlea, water content increases and motility of outer hair cells also increases. Since it was reported that the

Table 2. Wilcoxon Signed Ranks with Bonferroni Correction Results

Groups	p value				
	1.0 kHz	1.5 kHz	2.0 kHz	6.0 kHz	8.0 kHz
Group 1-Group 2	0.006	0.006	0.002	0.001	0.035
Group 1-Group 3	0.845	0.053	0.446	0.028	0.013
Group 1-Group 4	0.043	0.016	0.020	0.267	0.102
Group 2-Group 3	0.020	0.094	0.012	0.000	0.001
Group 2-Group 4	0.542	0.240	0.102	0.001	0.002
Group 3-Group 4	0.064	0.420	0.231	0.102	0.206

adverse effects of RF are mainly result of hyperthermia related to the power density of applied RFR^[3,17] or prolonged exposure^[17,19] it could be said that hyperthermia due to RF may be lower due to higher water content. Therefore by EU-RF exposure, DPOAE amplitudes were observed as increased at 1.0-2.0 kHz. Extrauterine GSM-like RF exposure, if not prolonged; and anatomical growth of exposed animate is completed, there is no much harmful effects on inner ear of the female rabbits, measured by DPOAEs.

DPOAE amplitudes were significantly higher after extrauterine exposure compared to IU exposure (at 2.0, 6.0 and 8.0 kHz); and IU+EU exposure (at 6.0-8.0 kHz). The reduction in DPOAE amplitudes seen after IU-RF exposure may be due to the organogenesis process which was going on during intrauterine period, where the inner ear damage of RF was more effective.

In this research, the reason that measurements were not realized for the foetuses exposed to RF energy in their mother's womb is due to the inconvenience of performing measurements in the inner ear of foetuses having very little ears as their whole body are only 14 cm long. Therefore, a month spent to allow infants to grow up for the development of the ears, and then the measurements are implemented. As we stated in the introduction section, electromagnetic energy do not penetrate deeply into the tissues and most of the energy is absorbed by the skin and underlying tissues. Furthermore, it is known that respiration rate and ear lobe temperature of the rabbits are the major physiological processes which regulate body temperature against any environmental changes^[20]. Namely, ear is the most vulnerable tissue of the rabbit against the thermal effect of RF exposure.

Moreover, during pregnancy, since the mother's abdomen is soft tissue, an amount of RF energy is absorbed deeper, and is likely to reach foetus. Since the foetus is within the amnion fluid, it may be thought that this fluid environment may largely neutralize the damage depending on heat generated by RF exposure.

When we consider about the EU period, the rabbit is one month old; and ear's and head's bone structure's

growth and and maturation are completed; and get thickened. Therefore heat related harmful RF adverse effects are observed less during EU exposure. Whereas, at the IU period, inner ear bone structure is in the cartilaginous constitution and maturation of the cranial bones; and also myelinisation of 8th cranial nerve are not finished yet.

In Martínez-Búrdalo M, et al.'s^[13] study, the specific absorption rate (SAR) in scaled human head models is analyzed to study possible differences between SAR in the heads of adults and children and for assessment of compliance with the international safety guidelines, while using a mobile phone. As head size decreases, the percentage of energy absorbed in the brain increases. So, higher SAR in children's brains can be expected depending on whether the thickness of their skulls and surrounding tissues actually depends on age.

When we consider the Martínez-Búrdalo M, et al.'s study^[13], as head size decreases, the percentage of energy absorbed in the brain increases. Therefore, during IU period, size of the foetus is small and SAR values for per gram of tissue is higher and damage on the cochlear functions are found as higher. As a result, DPOAE amplitudes decreased at 2.0, 6.0 and 8.0 kHz. As maternal estrogen increased during the pregnancy, endolymph production by stria vascularis also increase, hyperthermia related to the power density of applied RF may be lower; Estrogen Receptor beta-mediated neuroprotection involving brain-derived neurotrophic factor (BDNF) in the auditory system protects the inner ear functions; and increased maternal corticosteroid levels during intrauterine period also improve hearing system and prevent ear form the harmful effects of GSM-like RF^[16,17,21-24]. These maternal protective factors may also help to prevent inner ear.

DPOAE amplitudes were significantly higher after extrauterine exposure compared to IU+EU exposure (at 6.0-8.0 kHz). When female rabbits were exposed to IU+EU RF, they took twice as much RF than only EU exposure. Double RF dosage affects mainly basal turn of the cochlea related to higher frequencies. A study

Parazzini et al. ^[25] shows that basal turn of the cochlea exposes higher intensity of RF. Therefore, as exposure duration to RF increased, higher frequencies were affected more; and IU exposure cause more decrease at DPOAE amplitudes of 6.0-8.0 kHz.

Dimbylow P ^[26] described the finite-difference time-domain calculation of SAR from 20 MHz to 3 GHz in hybrid voxel-mathematical models of the pregnant female. Whole-body averaged SAR in the mother is presented as well as the average over the foetus, over the foetal brain and in 10 g of the fetus. Comparison suggests that the ICNIRP public reference level is a conservative predictor of local SAR in the fetus. For eye toxicity due to RF, it is reported that standard limits can only be exceeded in the unpractical situation where the antenna is located at a very short distance in front of the eye ^[13].

In our study, IU-RF exposure may cause to decrease in outer hair cell motility and DPOAE amplitudes. SAR in the foetus may exceed on to the standard safe limits and cause inner ear toxicity. Because of that, pregnant women must be very careful for not to expose GSM-like RF. When they use earphone or even carry their mobile phones in their bags or pockets; foetus may expose to GSM-like RF from the very short distance above to the standard limits and had ear toxicity with decreased DPOAEs.

By EU short time RF exposure in early childhood, it seems that DPOAE amplitudes increased with increased and induced electromotility in outer hair cells of the cochlea (OHCs). But if there will be extrauterine long-time exposure, we don't know the behavior of OHCs. We must be very careful with child having prolonged RF exposure; DPOAE amplitudes may decrease due to harmful effects of RF with hyperthermia related to the power density.

We conclude that prolonged exposure and hyperthermia related to the power density of applied RFR, increasing the temperature in the ear canal, may affect the DPOAE amplitudes especially during intrauterine period. Therefore child must be protected from the RF by precautionary principles. We

recommend not using mobile phones from short distances to the ear because of lower thickness of anatomical structures in intrauterine period and infancy.

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