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Review

Effects of acute exposure to WIFI signals (2.45 GHz) on heart variability and blood pressure in Albinos rabbit

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ABSTRACT

Electrocardiogram and arterial pressure measurements were studied under acute exposures to WIFI (2.45 GHz) during one hour in adult male rabbits. Antennas of WIFI were placed at 25 cm at the right side near the heart. Acute exposure of rabbits to WIFI increased heart frequency (+22%) and arterial blood pressure (+14%). Moreover, analysis of ECG revealed that WIFI induced a combined increase of PR and QT intervals. By contrast, the same exposure failed to alter maximum amplitude and P waves. After intravenously injection of dopamine (0.50 ml/kg) and epinephrine (0.50 ml/kg) under acute exposure to RF we found that, WIFI alter catecholamines (dopamine, epinephrine) action on heart variability and blood pressure compared to control. These results suggest for the first time, as far as we know, that exposure to WIFI affect heart rhythm, blood pressure, and catecholamines efficacy on cardiovascular system; indicating that radiofrequency can act directly and/or indirectly on cardiovascular system.

Contents

1. Introduction

Due to the constant evolution of new technologies more and more people are exposed at home or at work to different frequencies of electromagnetic fields (Feychting et al., 2005). In fact, there is an increase in the use of WIFI (wireless fidelity) devices 2.40 GHz by local networks (Brunel, 2004). Increasing evidence suggests that electromagnetic field (EMF) in the environment have many bioeffects (Lahbib et al., 2014; Ghodbane et al., 2015) that could affect cardiovascular system (Gmitrov, 2007) and induce oxidative stress (Salah et al., 2013). Besides that, Abdelmelek et al. (2006) showed an increase in norepinephrine in skeletal muscle after static magnetic field (SMF) exposure, indicating sympathetic hyperactivity. Interestingly, Heart rate variability was usually used for

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quantifying the autonomic nervous system activities (Acharya et al., 2002). Previous studies on animals and humans, demonstrated that EMF induced changes in heart activities. Pawlak et al. (2013) show that the exposure of animals to EMF increased heart rate, in chick embryos especially from 17 days of incubation (Gaffey and Tenforde, 1981), in rats, (Togawa et al., 1967) in rabbits, (Gaffey et al., 1980) in baboons, (Tenforde et al., 1983) in monkeys, and (Jehensen et al., 1988) in humans. Moreover, Thomas and Tenforde (2005) demonstrated the rise of blood flow under magnetic field environment. Interestingly, magnetic exposure induced electrocardiogram (ECG) abnormalities (Bortkiewicz et al., 1997). Creasey and Goldberg (1993) showed increase in heart rate and arrhythmia in people working around electrical trains (26 kV/m). In addition, Braune et al. (1998) reported that exposure to GSM signals for 35 min increased (+10%) blood pressure in volunteers. Vangelova et al. (2005) found that the radiofrequency may enhance hypertension and dyslipidemia. Cai et al. (2006) reported that radar exposure increased the rate of ECG changes in soldiers. Interestingly, Israel and Tomov (2000) showed high rates of hypertension in broadcast and TV station operators. The chronotropic effects, classically observed under radiofrequencies RF could be related to heart's excitability characteristic and rhythm or contraction (Elmas et al., 2012).

The present study aimed to evaluate (i) firstly the effects of WIFI on heart rate variability and blood pressure, (ii) secondly the physiological effects of catecholamines (dopamine and epinephrine) on heart rate under WIFI in rabbit.

2. Materiel and methods

2.1. Animals

In the present investigation we used adult male rabbit weighing 2.00 ± 0.50 kg (Central Pharmacy, Tunis, Tunisia). Animals were housed in groups of six in cages at +25 °C, under a 12:12 h light/dark cycle, with free access to water and commercial mash. Animals were cared for, under the Tunisian code of practice for the care and use of animals for scientific purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

2.2. Exposure system

The animals were exposed to an access point (AP) from WIFI device (D-Link DWL-3200 AP with 802.11 g mode and WPA2 network protection) as previously described in Salah et al. (2013). WIFI integrated two omnidirectional antennas that were setup for internet broadcast via wireless at 2.45 GHz. The sham control rabbits were placed under the same condition without applying RF (0 Hz). Antennas of WIFI were placed at 25 cm at the right side near the heart (animal in dorsal decubitus).

2.3. Experimental design

The rabbits were divided into six groups and for each group six rabbits and treated by intravenous injection as follows:

Group 1. Normal healthy control.
Group 2. Normal healthy: rabbits were exposed to WIFI one hour (between 9 h and 13 h).
Group 3. Rabbits were intravenously injected once with epinephrine (0.50 ml/kg).
Group 4. Rabbits were exposed to WIFI one hour (between 9 h and 13 h) following once intravenous injection of epinephrine (0.50 ml/kg).
Group 5. Rabbits were intravenously injected once with dopamine (0.50 ml/kg).
Group 6. Rabbits were exposed to WIFI one hour (between 9 h and 13 h) following once intravenous injection (iv) of dopamine (0.50 ml/kg).

The variation of the frequency and the cardiac rhythm were measured with an electrocardiogram" ECG". The ECG was recorded using Biopac® (MP35/30). Changes in blood pressure were measured using a pressure transducer connected to a chart recorder.

2.4. Analytical procedures

Records of changes in heart rate were done using a device consists of a software Biopac Student Lab 3.7.1, Biopac acquisition unit (MP35/30) with the associated cables, transformer BIOPAC, BIOPAC of electrode cables (SS2L), a computer, three vinyl disposable electrodes subject (EL503). The electric phenomena hearts materialize on the ECG by a base line broken by a P wave, a complex QRS and a T-wave. We measured the intervals PR, QT, RR, and P wave, beats heart per minute (BPM) and maximum amplitude after each exposure to WIFI (2.45 GHz, 1 h) and before each injection of catecholamines (dopamine, epinephrine).

2.5. Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) for comparison between groups. Values for (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ were considered statistically significant. The data are shown as a mean ± standard error of the mean (SEM).

3. Results

Our investigation reported that acute exposure to WIFI device induced an important reduction of the RR interval duration compared to controls, indicating an increase of heart frequencies. Moreover, we observe an increase PR and QT intervals (Fig. 1A–C). WIFI may influence the activity of nodal tissues especially auriculoventricular nodes. By contrast, the same exposure failed to alter P wave (Fig. 1D).

The present data showed that WIFI radiation (2.45 GHz) induced an increase of heart beats of animals. However, amplitude of the electrocardiogram remained unchanged during WIFI exposure compared to controls (Fig. 2A and B). In addition, we observe that acute exposure of rabbits to WIFI (2.45 GHz) during one hour induced an important increase in blood pressure compared to controls as shown in Fig. 3.

Our investigation showed that the single injection of dopamine (0.50 ml/kg, iv) induced an increase of the RR, QT intervals duration and decreased the PR interval and P wave of electrocardiogram compared to controls. Moreover, injection of dopamine under acute exposure to RF (2.45 GHz, 1 h) induced an important decrease in the length of the RR and QT intervals and an important decrease in the duration of the interval PR compared with rabbits given only dopamine, whereas P wave remained unchanged (Fig. 4A–D).

The administration of dopamine under WIFI exposure (2.45 GHz, 1 h) induced an important increase of beats per minute and decreased the maximum amplitude, compared to the rabbits injected only dopamine as reported in Fig. 5.

The epinephrine injection induced a decrease of RR, PR, QT intervals duration and P wave of electrocardiogram compared to control. Contrary, combined treatment with epinephrine (0.50 ml/kg, iv) and WIFI induced an important increase in the length of RR and QT intervals. The same treatment provoke an important decrease of the duration of interval PR and P wave compared to control (Fig. 6A–D).

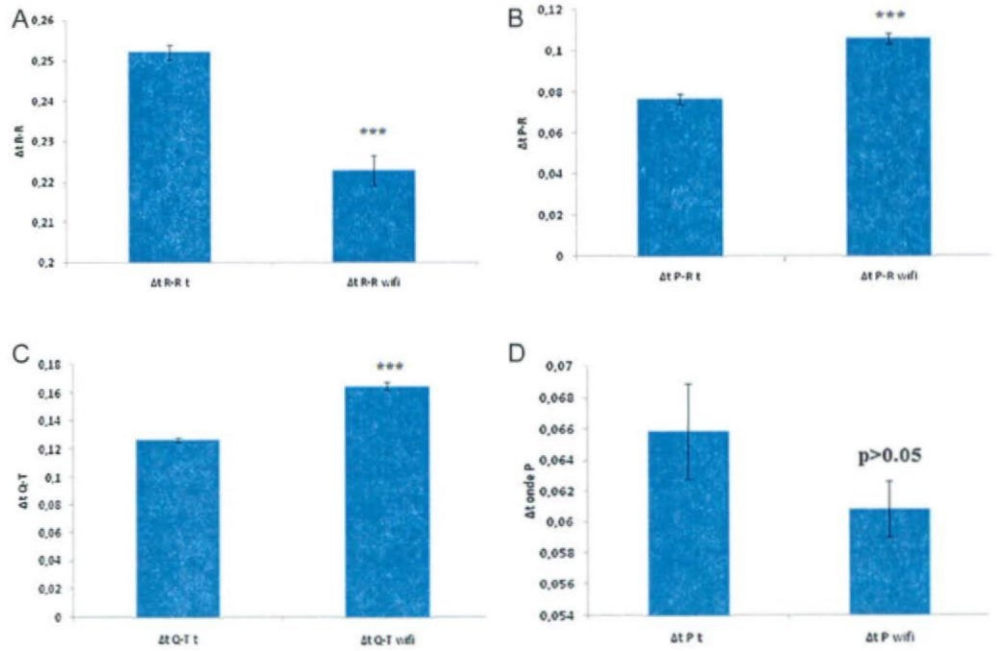

Fig. 1. The effects of acute exposure to WIFI (2.45 GHz, 1 h) on: (A) the RR intervals (Δt R-R) and (Δt R-R WIFI), (B) PR intervals (Δt P-R) and (Δt P-R WIFI), (C) QT intervals (Δt Q-T) and (Δt Q-T WIFI), (D) P wave of electrocardiogram in rabbits (Δt P) and (Δt P WIFI). Values are given as the mean ± SEM for groups of six animals. WIFI exposed rabbits were compared with control rabbits.

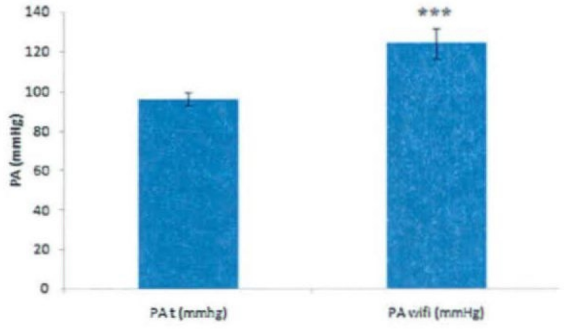

Fig. 2. The effect of acute exposure to WIFI (2.45 GHz, 1 h) on: (A) BPM control and BPM WIFI, (B) max control and max WIFI. Values are given as the mean ± SEM for groups of six animals.

The epinephrine injection induced an important increase in beats per minute and decreases the maximum amplitude compared to control. In case of rabbits treated with epinephrine (0.50 ml/kg, iv) under RF radiation (2.45 GHz), we observed an important decrease of beats per minute and the maximum amplitude compared to the rabbits injected only epinephrine (Fig. 7A and B).

Single injection of dopamine (0.50 ml/kg) decreased the arterial pressure compared to control. By contrast, single administration of epinephrine (0.50 ml/kg) increased the arterial pressure. In case of rabbits treated with dopamine and epinephrine under RF exposure the arterial pressure return to the normal state. WIFI (2.45 GHz, 1 h) exposure alters the classical responses observed following the administration of both catecholamines as showing in Fig. 8

4. Discussion

Our investigation point that acute exposure to WIFI induced an increase in heart rate and arterial blood pressure; showing a modulatory effects of RF on the cardiovascular system regulation. Interestingly, catecholamines (dopamine and epinephrine) bioeffects on cardiac rhythm and vasomotricity were altered by WIFI in rabbits.

Exposure to new wireless technologies will be inevitable in our domestic life. The development of new technologies such as WIFI, which allows data transfer through a microwave field (2.4 GHz) and

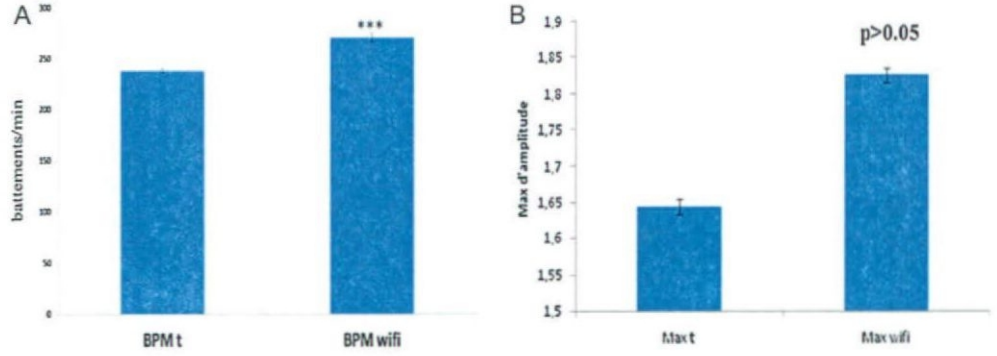

Fig. 3. Effects of acute exposure to WIFI on blood pressure of rabbits on: PAt control and PA WIFI. Values are given as the mean ± SEM for groups of six animals.

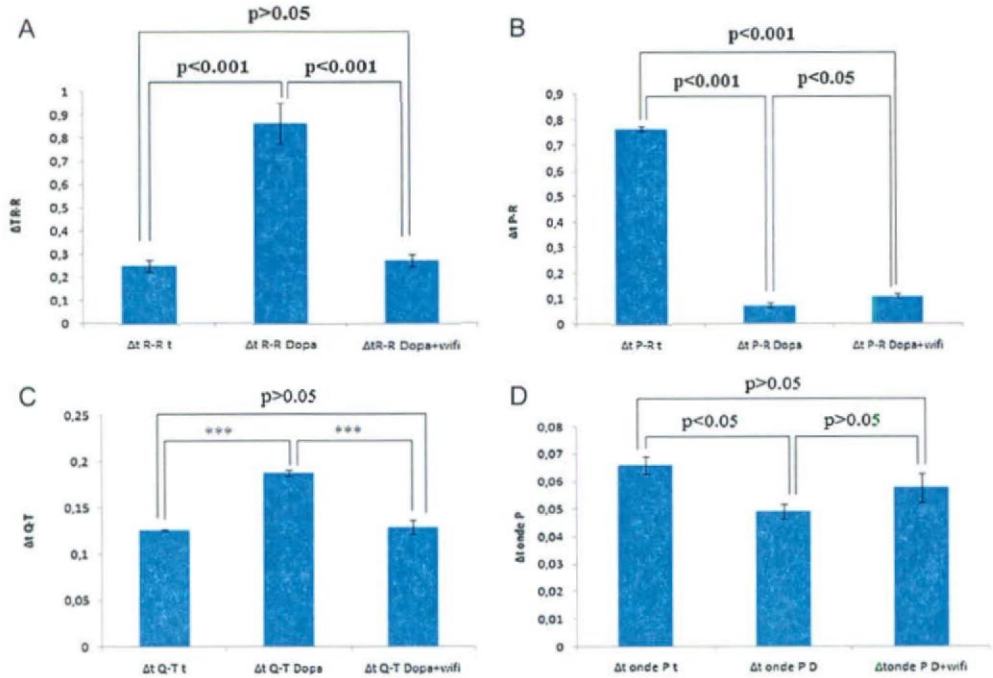

Fig. 4. Effects of single injection of dopamine on the component of electrocardiogram in rabbits under acute exposure to WIFI (2.45 GHz, 1 h). Values are given as the mean ± SEM for groups of six animals.

with a transmission rate from 1 to 50 Mbps (Valberg et al., 2007); can have serious consequences on public health and multiple bioeffects (Sage and Carpenter, 2009). Our results show clearly that WIFI increased heart rate and arterial blood pressure probably via direct and/or indirect pathways. The direct effects of RF could be related to their action on Ca^{++} and Zn^{++} homeostasis especially on divalent mineral flux modulated by EMF as shown previously by Amara et al. (2007). Interestingly, Pilla et al. (1999) demonstrated that static magnetic fields (SMFs) in the range of 0–200 mT accelerate Ca^{++}/calmodulin-dependent myosin light chain phosphorylation. In order to demonstrate the whole mechanism and the implication of K^+ channel in the heart responses induced by RF, electrophysiological studies of RF will be programmed for future investigations.

The indirect pathway will deal with the modulatory effects of RF on autonomic nervous system, plasma catecholamines, and glucocorticoides.

In fact, Abdelmelek et al. (2006) showed an increase in norepinephrine in skeletal muscle following SMF (128 mT) exposure; indicating sympathetic hyperactivity. The sympathetic hyperactivity classically observed following EMF exposure explain in part our data reporting an increase of heart rate (HR) and arterial blood pressure under WIFI exposure. Similar investigation by Braune et al. (1998) pointed for the implication of sympathetic tone induced by EMF in hypertension. The present investigation reported that acute exposure to WIFI provoked a decrease in the RR interval, indicating a tachycardia explaining the hypertension. By contrast, Jehensen et al. (1988) showed an increase (+17%) of the length of the RR interval after ten minutes of SMF exposure in healthy volunteers. The PR interval reflects the time that electrical impulse takes to travel from the auriculo-ventricular node and enters to the ventricle. The increase in the PR interval supported that the conduction system of the heart was altered. It is in accordance with Blanchi et al. (1973) when mice were exposed to 100 kv/m (50 Hz); the QRS duration and the PR interval were each lengthened by 19.50%. While, the acute exposure to WIFI increased the QT interval; showing that the time for both ventricular depolarization and repolarisation was larger. Otherwise, the heart is a contractile organ that can generate its own rhythm (Elmas et al., 2012). Arber and Lin (1985) showed that continuous exposure of neurons to microwaves for 60 min inhibited spontaneous activity and prolong the refractory period following depolarization. An increase of K^+ current could be implicated as a cause for prolongation of refractory period (Seaman and Wachtel, 1978; Arber and Lin, 1985) as reported in our investigation by the prolonged PR and QT intervals.

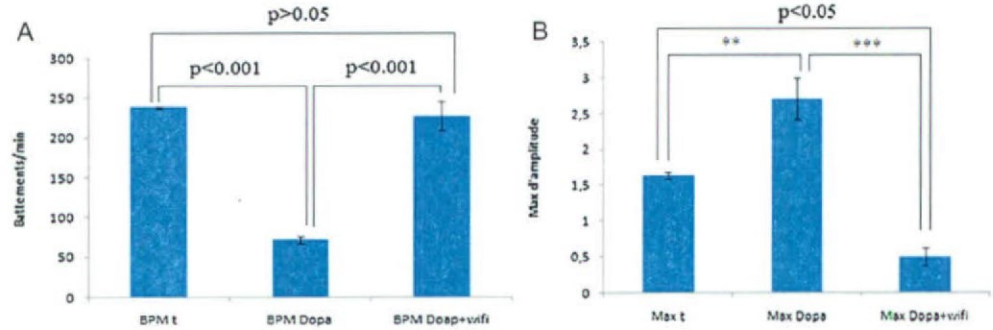

Fig. 5. Effects of single injection of dopamine on the beats per minute (BPM) and maximum amplitude (max) in rabbits under WIFI (2.45 GHz, 1 h).

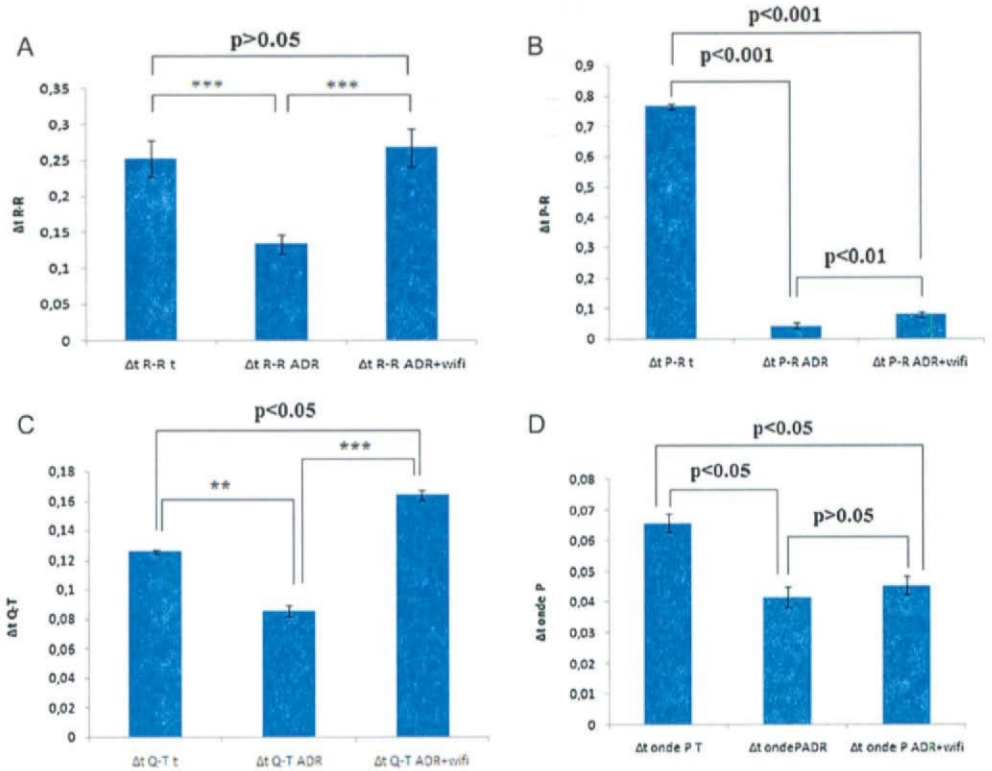

Fig. 6. Effects of single injection of epinephrine on the component of electrocardiogram under WIFI (2.45 GHz, 1 h). Values are given as the mean ± SEM for groups of six animals.

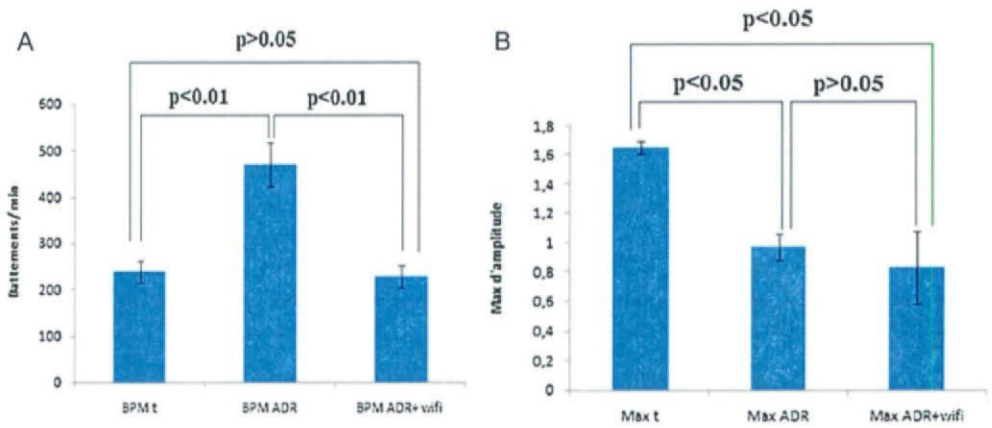

Fig. 7. Effects of single injection of epinephrine on the beats per minute (BMP) and maximum amplitude (max) in rabbits under acute exposure to WIFI (2.45 GHz, 1 h). Values are given as the mean ± SEM for groups of six animals.

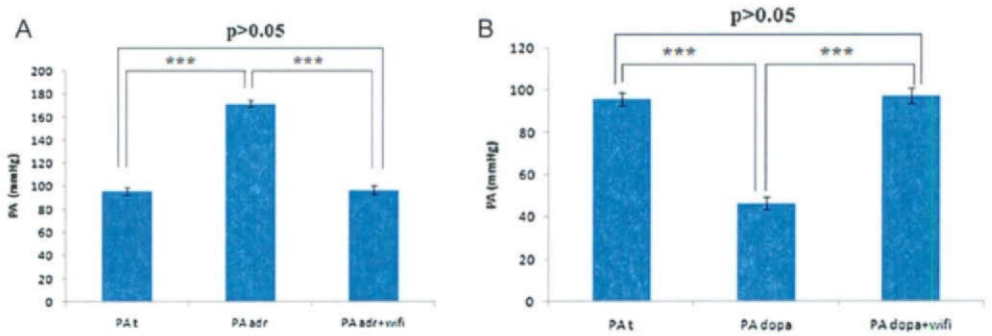

Fig. 8. Effects of epinephrine and dopamine injections on blood pressure under acute exposure to WIFI (2.45 GHz, 1 h). Values are given as the mean ± SEM for groups of six animals.

Our data demonstrate that single injection of epinephrine increase heart rate associated to a decrease in the RR, QT, PR intervals and even the P-wave. Moreover, single injection of dopamine decreased heart rate and increased RR, QT, PR intervals and even the P-wave. Acute exposure to WIFI (2.45 GHz during one hour) alters epinephrine and dopamine effects classically observed on heart rate, arterial blood pressure and the most studied intervals. This data report clearly that the action mechanism of epinephrine was abolished through RF. We can therefore say that RF emitted by WIFI act probably on the receptors, thereby altering the ligand–receptor binding. In fact, Chiabrera et al. (2000) showed that the probability of binding could be modified by the electric component of the RF. Previous study reported that exposure to 50-Hz magnetic field decreased the binding affinity of the 1B receptor subtype of serotonin (Masuda et al., 2010). Behari et al. (1998) shows that AM radio radiation alters Ca^{2+} binding in the membrane, $Na^{+}K^{+}$-ATPase activity.

Our studies point that WIFI is not completely safe at home near the animal or human body because it employ harmful radio waves. But it is safer compared to cellphone that it is close to our brain during communications. WIFI signals are everywhere. If you switched off your WiFi at night, you are still exposed to the WIFI signals coming in from neighbors but we have a significant reduction of the bioeffects of WIFI with distance from the router. Future investigations will focus on the long term bioeffects of WIFI placed at an important distance from the animal or the human.

5. Conclusion

These results suggested that exposure to WIFI (2.45 GHz) affect HR variability leading to tachycardia and hypertension. The WIFI alter the physiological action of catecholamines on cardiovascular system perhaps via the disruption of the interaction between ligand-receptors in rabbit.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgment

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References

Abdelmelek, H., Molnar, S., Servais, S., Cottet-Emard, J.M., Pequignot, J.M., Favier, R., Sakly, M., 2006. Skeletal muscle HSP72 and norepinephrine response to static magnetic field in rat. J. Neural Transm. 113, 821–827.

Acharya, R.U., Lim, C., Joseph, P., 2002. Heart rate variability analysis using correlation dimension and detrended fluctuation analysis. ITBM-RBM 23, 333–339.

Amara, S., Abdelmelek, H., Garrel, C., Guiraud, P., Douki, T., Ravanat, J.L., Favier, A., Sakly, M., Ben Rhouma, K., 2007. Zinc supplementation ameliorates static magnetic field-induced oxidative stress in rat tissues. Environ. Toxicol. Pharmacol. 23 (2), 193–197, http://dx.doi.org/10.1016/j.etap.2006.09.001.

Arber, S.L., Lin, J.C., 1985. Microwave-induced changes in nerve cells: effects of modulation and temperature. Bioelectromagnetics 6, 257–270.

Behari, J., Kunjilwar, K.K., Pyne, S., 1998. Interaction of low level modulated RF radiation with Na^{+}-K^{+}-ATPase. Bioelectrochem. Bioenerg. 47, 247–252.

Blanchi, D., Cedrini, L., Ceria, F., Meda, E., Re, G.G., 1973. Exposure of mammals to strong 50-Hz electric fields. Arch. Fisiol. 70, 33.

Bortkiewicz, A., Zmyslony, M., Gadzicka, E., Palczynski, S., 1997. Ambulatory ECG monitoring in workers exposed to electromagnetic fields. J. Med. Eng. Technol. 21 (2), 41–46.

Braune, S., Wrocklage, C., Raczek, J., Gailus, T., Lucking, C.H., 1998. Resting blood pressure increase during exposure to radio-frequency electromagnetic field. Lancet 351, 1857–1858.

Brunel, J.L., 2004. Les risques liés au WiFi. Observatoire Académique de la Sécurité Informatique: études/actualités.

Cai, G., Li, Q.Y., Wang, D.W., Qu, H.Y., Wang, S.M., Li, H., 2006. The ECG effection of exposure to radar. Chin. Heart J. 18 (3), 325.

Chiabrera, A., Bianco, B., Moggia, E., Kaufman, J.J., 2000. Zeeman–Stark modeling of the RF EMF interaction with ligand binding. Bioelectromagnetics 21 (4), 312–324.

Creasey, W.A., Goldberg, R.B., 1993, August. Safety of High Speed Guided Ground Transportation Systems: Potential Health Effects of Low Frequency Electromagnetic Fields Due to Maglev and Other Electric Rail Systems. US Department of Transportation, Federal Railroad Administration. Report DOT-FRA/ORD 93/31, Washington, DC.

Elmas, O., Comlekci, S., Koylu, H., 2012. Effects of short-term exposure to powerline-frequency electromagnetic field on the electrical activity of the heart. Arch. Environ. Occup. Health 67 (2), 65–71, http://dx.doi.org/10.1080/19338244.2011.578680.

Feychting, M., Anders, A., Leeka, K., 2005. EMF AND HEALTH – Annual Review of Public Health, vol. 26., pp. 165–189.

Gaffey, C.T., Tenforde, T.S., 1981. Alterations in the rat electrocardiogram induced by stationary magnetic fields. Bioelectromagnetics 2, 357–370.

Gaffey, C.T., Tenforde, T.S., Dean, E.E., 1980. Alterations in the electrocardiograms of baboons exposed to DC magnetic fields. Bioelectromagnetics 1, 209.

Ghodbane, S., Lahbib, A., Ammari, M., Sakly, M., Abdelmelek, H., 2015. static magnetic field-exposure induced oxidative stress and apoptosis in rat kidney and muscle? Effect of vitamin E and selenium supplementations. Gen. Physiol. Biophys. 34 (1), 23–32, http://dx.doi.org/10.4149/gpb_2014027.

Gmitrov, J., 2007. Static magnetic field effect on the arterial baroreflex-mediated control of microcirculation: implications for cardiovascular effects due to environmental magnetic fields. Radiat. Environ. Biophys. 46 (3), 281–290.

Israel, M., Tomov, P., 2000. Epidemiological study of the effect radiofrequency radiation on operators in radio, TV and relay stations. In: Israel, M., Repacholi, M. (Eds.), Proceedings of Eastern European Regional EMF Meeting and Workshop. VM-OFSET, Sofia. , pp. 145–154.

Jehensen, P., Duboc, D., Lavergne, T., Guize, L., Gueirin, F., Degeorges, M., Syrota, A., 1988. Change in human cardiac rhythm induced by a 2-T static magnetic field. Radiology 166, 227–230.

Lahbib, A., Ghodbane, S., Sakly, M., Abdelmelek, H., 2014. Vitamins and glucose metabolism: the role of static magnetic fields. Int. J. Radiat. Biol. 90 (12), 1240–1245, http://dx.doi.org/10.3109/09553002.2014.930537.

Masuda, H., de Gannes, F.P., Haro, E., Billaudel, B., Ruffié, G., Lagroye, I., Veyret, B., 2010. Lack of effect of 50-Hz magnetic field exposure on the binding affinity of serotonin for the 5-HT 1B receptor subtype. Brain Res. 1368, 44–51.

Pawlak, K., Sechman, A., Nieckarz, Z., Wojtysiak, D., 2013. Effect of weak electromagnetic field on cardiac work, concentration of thyroid hormones and blood aminotransferase level in the chick embryo. Electromagn. Biol. Med. 32 (2), 173–181, http://dx.doi.org/10.3109/15368378.2013.776424.

Pilla, A.A., Muehsam, D.J., Markov, M.S., Sisken, B.F., 1999. EMF signals and ion/ligand binding kinetics: prediction of bioeffective waveform parameters. Bioelectrochem. Bioenerg. 48, 27–34.

Salah, M.B., Abdelmelek, H., Abderraba, M., 2013. Effects of olive leave extract on metabolic disorders and oxidative stress induced by 2.45 GHz WIFI signals. Environ. Toxicol. Pharmacol. 36 (3), 826–834, http://dx.doi.org/10.1016/j.etap.2013.07.013.

Sage, C., Carpenter, D.O., 2009. Public health implications of wireless technologies. Pathophysiology 16, 233–246.

Seaman, R., Wachtel, H., 1978. Slow and rapid responses to CW and pulsed microwave radiation by individual Aplysia pacemakers. J. Microw. Power 13, 77–86.

Tenforde, T.S., Gaffey, C.T., Moyer, B.R., Budinger, T.F., 1983. Cardiovascular alterations in Macaca monkeys exposed to stationary magnetic fields: experimental observations and theoretical analysis. Bioelectromagnetics 4, 1–9.

Thomas, S., Tenforde, 2005. Magnetically induced electric fields and currents in the circulatory system. Progress Biophys. Mol. Biol. 87, 279–288.

Togawa, T., Okai, O., Oshima, M., 1967. Observation of blood flow E.M.F. in externally applied strong magnetic fields by surface electrodes. Med. Biol. Eng. 5, 169–170.

Valberg, P.A., van Deventer, T.E., Repacholi, 2007. Workgroup report: base stations and wireless networks – radiofrequency (RF) exposures and health consequences. Environ. Health Perspect. 115 (3), 416–424, http://dx.doi.org/10.1289/ehp.9633.

Vangelova, K., Deyanov, C., Israel, M., 2005. Cardiovascular risk in operators under radiofrequency electromagnetic radiation. Int. J. Hyg. Environ. Health 209 (2), 133–138, http://dx.doi.org/10.1016/j.ijheh.2005.09.008.

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Cellular Phone Irradiation of the Head Affects Heart Rate Variability Depending on Inspiration/Expiration Ratio

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Abstract. *Background: Mobile phones may have harmful health effects and clinical examinations report ambiguous results of exposure concerning neurophysiological and cardiovascular actions. Materials and Methods: This study investigated heart rate asymmetry (HRA) and heart rate variability (HRV) parameters with 1:2 and 1:1 metronome-paced inspiration/expiration ratios during short-term 1,800MHz GSM cellular phone exposure in 20 healthy volunteers. Results: Significant HRA changes by Porta and Guzik indices were not found on exposure compared to sham exposure. Time-domain HRV parameters on exposure showed significant differences at 1:1 paced, but not at 1:2 paced breathing compared to sham exposure. A mild post-exposure effect was observed regarding root mean square of successive RR-differences. Conclusion: The findings reflect persisting acute effects of GSM handset emission on the autonomic nervous system. Exploring its influences on health status and survival needs further studies. Symmetrical breathing can be used as a sensitizing factor in other HRV/HRA analysis studies.*

Evolution of species has taken place in the presence of natural ionizing- and non-ionizing electromagnetic radiation, including sunlight, cosmic radiation, natural radioactivity and atmospheric electromagnetic phenomena, among others (1). There is increasing concern regarding the potentially harmful biological effects of exposure to electromagnetic fields (EMF), especially on humans, from military, industrial and commercial wireless telecommunication systems. Mobile phones have become popular and indispensable in our everyday life. The first generation analogue network was replaced in 1991 by the digital Global System for Mobile Communication (GSM), which had more than 90% market share still in 2014 in spite of the introduction of the third-generation Universal Mobile Telecommunication System (UMTS) in 2001 and the fourth-generation Long-term Evolution (LTE) in 2009, with increasingly improved transmission capacity (2). All three generations of mobile communication standards are simultaneously present in Hungary in 2017 (3).

The mobile communication system consists of a cellular network of base and mobile stations (cellular phone or handset). The latter emits significantly less microwave power, however, its long-term close proximity to the head and torso results in more significant exposure in the general (not professional) population. The possible biological effects of microwave radiation are related to the energy absorption by living cells and their interactions at the molecular, cellular, tissue and organism level. Since the relative permeability of biological materials is around unity, independently of the exposure frequency, whereas their relative permittivity is 10-200, the magnetic rather than electric component of EMF may be responsible for any biological effects (4).

The direct biophysical mechanism of the interaction of EMF and living tissues is still not clear; however, there are several theories (1). Membranes play a crucial role in biology by ensuring compartmentalization and interaction of the inner and outer cellular spaces at the same time. The interplay between membrane potential and membrane conductivity regulates cell functions, and excitation of neurons and contractile cells among others. A direct membrane-polarizing effect of microwave radiation can be excluded since the average capacity of the cell membrane (1 μF/cm) is considered as a short circuit above ~1 MHz, hence there is no voltage drop and energy dissipation induced across the membrane itself (5). However, a low-frequency modulated radiofrequency field can act differently. Moreover,

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Key Words: Heart rate variability, heart rate asymmetry, cellular phone, microwave radiation.

considering the resting membrane potential of −70mV results in an electric field gradient across the 5 nm-thick cell membrane of $E \approx 14 \times 10^6$V/m, the external EMF used in communication are negligibly weak to be able to directly act on membrane potential (6). Furthermore, the quantum (ionizing) effect of microwave radiation at the molecular level can be disregarded considering the energy carried by photons with a frequency of 0.300-300GHz would be 1.24×10^{-6} to 1.24×10^{-3} eV. This amount of energy is well below the ionizing threshold of biological molecules (~13.6 eV) and even smaller than the 0.027eV energy of Brownian motion at 37°C (4). Today the most widely accepted and explored theory for interaction is based on the thermal effects of microwave radiation. The current safety limits are established based on this; however, there are several biological phenomena that cannot be explained simply by warming of tissues, and temperature changes cannot be detected at all behind some observed biological effects (1, 7). The physical basis for thermal effects is excitation by rotational resonance of dipole molecules induced by sinusoidal EMF, which is resisted by the molecular moment of inertia and friction with the surrounding particles, which increases the thermal energy of the object, resulting in its warming up. In the microwave range, water and similar small dipolar molecules are the target of resonant frequency, although conformational changes of proteins *via* their bound water or molecular arms can also be considered targets. Depending on the inhomogeneity of the tissues, local hot-spots can arise, and due to the temperature-dependent nature of relative permittivity, focal thermal breakdown can occur (4).

The present applicable standard of maximal transmission power of a hand unit is 1.0 W at GSM 1800/1900 and 2.0 W at GSM 850/900 (8). The European Union specific absorption rate (SAR) limit for the public is 2.0 W/kg averaged over 10 g of tissue, but in a few countries the limit is 1.6 W/kg averaged over 1 g of tissue (9).

Exposure to EMF may have negative health effects even below the power defined in safety standards. Investigations into possible adverse biological effect of cellular phones are focused on the central nervous system (CNS) and inner ear, because most of the microwave radiation is absorbed here due to the anatomical proximity to the signal source (10-15). Conclusions of human trials on the impact of EMF on the autonomic nervous system are controversial (16-19).

HRV analysis is a non-invasive assessment of the actions of the autonomic neuroendocrine system on the sinus node *via* the beat-to-beat fluctuations of the heart rate. This method is considered valuable in the prediction of progression and outcome in several diseases (20-22). Atlas *et al.* found no difference in HRV in 35 healthy volunteers breathing spontaneously during 10-minute exposure to EMF from a commercial mobile phone forced at 2 W output peak power at 900 MHz (23). Heart rate asymmetry (HRA) is a relatively novel marker of HRV, based on the Poincaré-plot. This asymmetry can be quantified as Guzik index (24) or Porta index (25), among others. HRA reflects the dynamics of respiratory arrhythmia, namely temporal asymmetry of pulse rate acceleration and deceleration as a consequence of inspiration/expiration period ratio, first published by our research group in 2012 (26): Change in inspiration/expiration ratio by double-paced breathing from 1:2 to 1:1 or 2:1 is followed by corresponding change of HRA parameters. HRA measures can be more sensitive markers of respiration–heart rate coupling than standard HRV parameters; additionally, paced breathing 'stress-situation' requiring auditory and cortical functions also acts as a sensitizing factor.

In the available literature, there is no publication on the effect of cellular phone exposure on HRA parameters and at different paced inspiration/expiration period ratios. The aim of present study was to examine the influence of the acute effects of pulsed microwave irradiation from a commercial cellular phone on HRV and HRA parameters during various double-paced breathing patterns in healthy volunteers in randomized double-blind repeated-measures crossover design.

Materials and Methods

The study was approved by the Regional Research Ethics Committee (approval number: 2013/4747) and was conducted in accordance with the Declaration of Helsinki and its later amendments. Twenty healthy volunteers (14 female and six male) were enrolled. Informed consent was obtained from all individual participants included in the study. The mean age was 25.2 (range 21 to 32) years. The majority of the volunteers did not smoke (17 altogether), two people smoked fewer than 10 cigarettes/day, and one person smoked more than 10 cigarettes/day. Sixteen volunteers consumed alcohol occasionally, while four did not consume alcohol at all. Regarding coffee consumption, the population was heterogeneous: six did not consume at all, seven occasionally and seven regularly drank coffee. The mean body mass index (BMI) of the group was within the normal range: 21.8±2.5 kg/m^2. The participants were not allowed to consume alcohol, coffee or cigarettes for 4 hours before the test.

A commercial Nokia 6230i mobile telephone (Nokia Corporation, Helsinki, Finland) was used for radiofrequency exposure working on an 1,800 MHz GSM network (217 Hz pulse rate; 0.577 μs pulse width, actual emissions during the experiment were not measured) with SAR value of 0.70 W/kg at the head (27). The call to the mobile phone was initiated from a landline unit in a neighbouring room until automatic disconnection at about 1 minute and 25 sec, and then the number was redialled three more times over a 6-minute period. The participants had no information on the emission of the phone since it was in silent mode and was totally covered by an elastic bandage.

During each experimental stage, a 6-min-long single channel electrocardiogram (ECG) and respiration signal were recorded by a microcontroller-based, battery-powered, handheld data acquisition system (László Hejjel, Pécs, Hungary). An ultra-rapid thermistor probe was fixed under the volunteer's nose for monitoring respiration. The thermistor sensed the cooling and warming of the

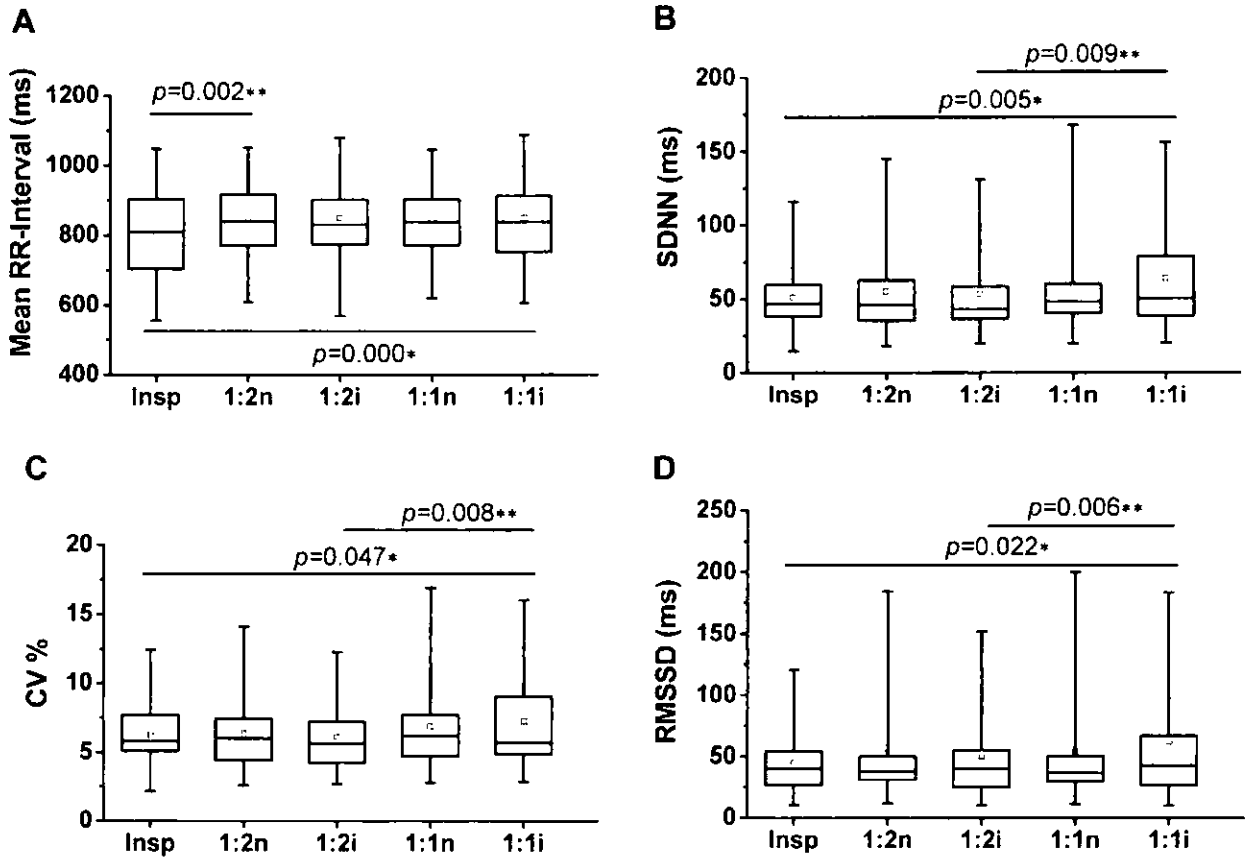

Figure 1. *Box and whisker diagrams of the time domain parameters. A: Mean time between two R-waves on the ECG (RR interval). B: Standard deviation of RR intervals (SDNN). C: Coefficient of variation (CV%). D: Root mean square of successive RR interval differences (RMSSD). Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. *Friedman test p-value. **post-hoc Wilcoxon's paired-sample test p-values, only significant differences are indicated. Insp: Only inspiration paced without exposure; 1:1 symmetrically paced inspiration/expiration; 1:2 asymmetrically paced inspiration/expiration; i: with irradiation; n: without irradiation.*

flowing air during inhalation and exhalation, respectively. The instrument also contained a metronome to trigger inspiration and expiration with two different frequency beeps. Standard 300-second tachograms (22) were extracted from the ECG records by ECGRdet v2.4 (László Hejjel), while the HRV analysis was carried out with Varian v2.2 (László Hejjel). The mean time between two R-waves on the ECG (RR interval; MeanRR), standard deviation of normal-to-normal RR-intervals (SDNN), coefficient of variation (CV%=SDNN/MeanRR), root mean square of successive RR-interval differences (RMSSD) in the time domain (21, 22); high-frequency (HF) integral in the range of 0.15-0.40 Hz (in normalized units), low-frequency (LF) component at 0.01-0.15 5Hz (in normalized units) and LF/HF were analysed by fast Fourier transformation in the frequency domain (21, 22); Porta index and Guzik index (24, 25) were also computed as HRA parameters.

The study was carried out at our Institution in 2014 in the late afternoon, in quiet circumstances. The volunteers lay in a comfortable supine position with head elevated at 30° 15 minutes prior to data acquisition to allow orthostatic adaptation. The ECG electrodes along with the thermistor probe were placed on the subject, and the mobile phone was fixed on their right ear with an elastic bandage imitating its position during use. The subjects were educated regarding metronome-controlled respiration during this adaptation period. Talking and movements were not allowed, since these can influence HRV parameters (28).

Five consecutive 360-second-long stages were recorded for each volunteer. A T=4.5s breathing cycle (0.22 Hz) was ensured by participants breathing according to the built-in beeper. Since previous studies have already demonstrated that single metronome-controlled *versus* spontaneous breathing does not differ as regards of HRV parameters, we did not investigate spontaneous respiration here in order to reduce the study duration and hence the stress on the participants (29, 30). During the first stage, only inhalation was according to the metronome. In the second and fourth stages, inhalation and exhalation were triggered with beeps of two different frequencies, in an asymmetrical manner at a 1:2 (physiological) inspiration:expiration ratio. During the third and fifth stages, symmetrical breathing was paced, *i.e.* 1:1 inhalation:exhalation

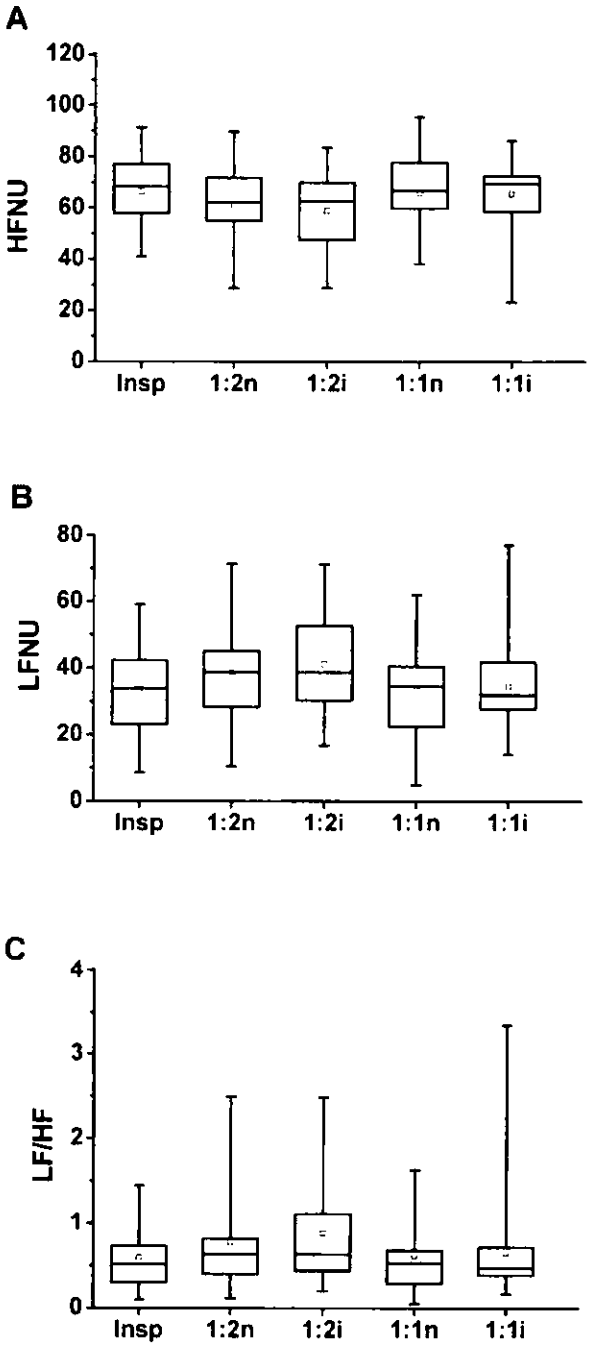

Figure 2. *Box and whisker diagrams of the frequency domain parameters. A: High frequency power in normalized units (HFNU). B: Low frequency band power in normalized units (LFNU). C: Low frequency/high frequency power ratio (LF/HF). Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. By Friedman test p=0.107 for each frequency domain parameter, no significant changes. Insp: Only inspiration paced without exposure; 1:1 symmetrically paced inspiration/expiration; 1:2 asymmetrically paced inspiration/expiration; i: with irradiation; n: without irradiation.*

ratio. The radiofrequency exposure from the mobile phone took place randomly either during the second and third, or during the fourth and fifth stages, blinded to both the volunteers and the person who analyzed the records. Valentini *et al.* stated in their meta-analysis that GSM-irradiation causes only transient cortical phenomena by EEG studies, therefore only the instantaneous effects of radiofrequency exposure were analysed (10). For this reason and in order to minimize the mental load of our investigation, only two 6-minute exposures were applied with 1:2 and 1:1 inspiration/expiration ratios in contrast to the frequently applied 30-minute microwave exposure. The entire measurement needed about 45 minutes per volunteer.

Statistical analysis was prepared using the statistiXL v1.8 (statistiXL, Broadway Nedlands, Western Australia) plug-in in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). The figures were constructed with OriginPro v2017 (OriginLab Corporation, Northampton, MA, USA). Non-parametric repeated-measures ANOVA by Friedman was applied for primary assessment. Values of $p \leq 0.05$ were considered statistically significant. Wilcoxon paired-sample tests with the Holm-Bonferroni correction of significance level were applied *post-hoc*. In pairs, the following were compared: A: only inspiration controlled by metronome (Insp) with sham-exposed (*i.e.* not exposed) asymmetrically double-triggered breathing (1:2n); B: 1:2n pattern with the pattern of non-irradiated symmetrical (1:1n); C: irradiated asymmetrical (1:2i) with irradiated symmetrical breathing (1:1i); D: 1:2n with 1:2i; and E: 1:1n with 1:1i.

Results

On manual checking of the ECG and respiration recordings, no records were rejected due to poor signal quality, inappropriate breathing, arrhythmia or any other reason. Moreover, spectral analysis by fast Fourier transformation clearly showed the respiratory peak at around 0.22 Hz for each individual.

Time domain analysis. The mean RR-interval changed significantly according to Friedman's test. Wilcoxon's paired-sample test *post-hoc* showed a significant difference (p=0.002) between the Insp and 1:2 paced sham-exposed measurements even after Holm–Bonferroni correction. Further comparisons of the mean RR-interval showed no significant changes (Figure 1A).

The Friedman test of SDNN parameters was statistically significant (Figure 1B). *post-hoc* paired-sample assessment resulted in significant differences even after p-value correction between 1:2 and 1:1 breathing measurements under EMF exposure (p=0.009). The other comparisons regarding SDNN demonstrated no significant changes. Interestingly, the mean and median of SDNN were separated at 1:1 breathing with EMF irradiation. The CV% behaved similarly giving a statistically significant p=0.008 value on comparing 1:2i with 1:1i series by *post-hoc* Wilcoxon's paired-sample test (Figure 1C).

The RMSSD resulted in significant differences by Friedman test (p=0.022) and again, only 1:2i with 1:1i series

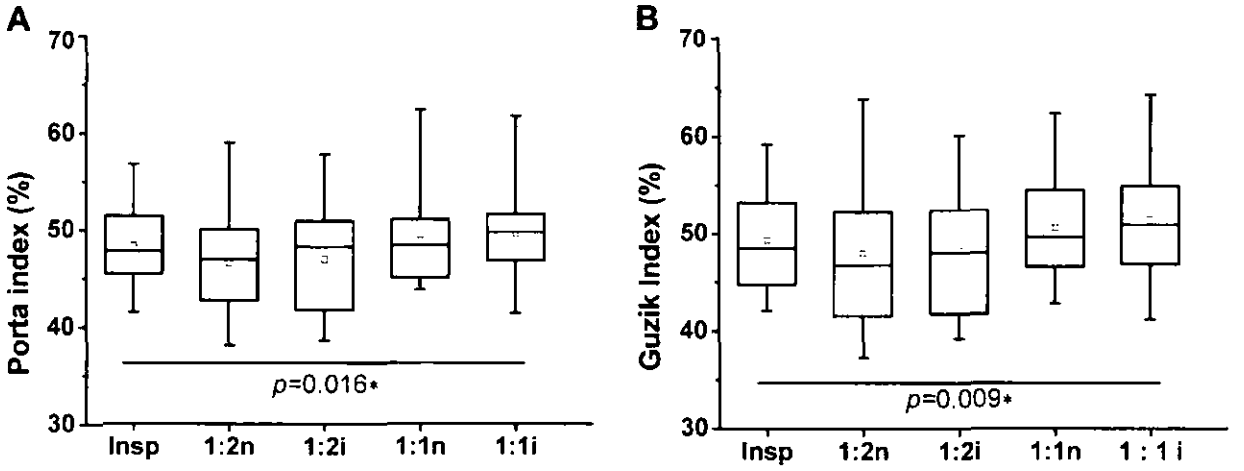

Figure 3. *Box and whisker diagrams of heart rate asymmetry (HRA) parameters. A: Porta index. B: Guzik index. Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. *Friedman test p-value, only significant differences are indicated: the examined post-hoc Wilcoxon's paired sample tests resulted in non-significant p-values after Holm-Bonferroni correction. Insp: Only inspiration paced without exposure; 1:1 symmetrically paced inspiration/expiration; 1:2 asymmetrically paced inspiration/expiration; i: with irradiation; n: without irradiation.*

gave significant differences by *post-hoc* Wilcoxon's paired-sample test (p=0.008, Figure 1D). The increase of the interquartile distance is evident here as well, representing a possible separation of the population during cellular phone EMF exposure at symmetrical but not asymmetrical breathing together.

Frequency domain analysis. Among frequency domain parameters (LF, HF, LF/HF) no statistically significant differences were found by Friedman test (Figure 2). Due to the complementary nature of LF and HF power and the ranking in Friedman test, the p-values proved to be identical.

HRA analysis. The Friedman test resulted in statistically significant p-values for both Porta (p=0.016; Figure 3A) and Guzik (p=0.009; Figure 3B) indices. However, *post-hoc* Wilcoxon's paired-sample test of Porta index demonstrated only a tendency for differences between 1:2 and 1:1 breathing patterns in both comparisons sham-exposed and irradiated pairs (p=0.011 for 1:2n *vs.* 1:1n; p=0.024 at 1:2i for 1:1i) after Holm–Bonferroni correction of the significance level. Wilcoxon's paired-sample test for Guzik's index showed similar results: p=0.027 for 1:1n *vs.* 1:2n; p=0.011 for 1:1i *vs.* 1:2i. There were no significant differences in either index between sham-exposed and irradiated groups comparing the same breathing patterns: 1:1n *vs.* 1:1i and 1:2n *vs.* 1:2i (Figure 3). Both Pearson's correlation and Spearman's rank correlation confirmed a

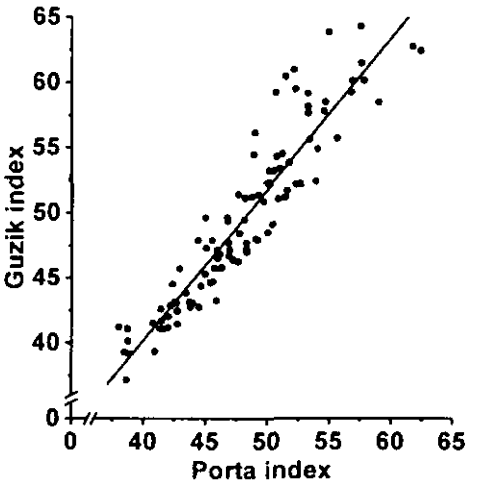

Figure 4. *Pearson's correlation and Spearman's rank correlation tests between Porta and Guzik indices. The tests showed a significantly strong positive correlation. Pearson correlation: r_P=0.932, p<0.0001; Spearman rank correlation: r_S=0.948, p=1.022×10^{-18}.*

significantly strong positive correlation above 0.9 between Porta and Guzik indices with p<0.001 and p=1.022×10^{-18}, respectively (Figure 4).

Recombination of the time domain measurements. In order to reject or confirm order-dependent effects, the five stages

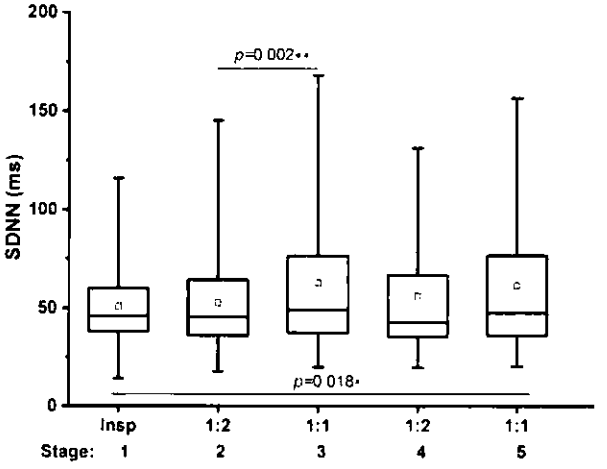

Figure 5. *Standard deviation (SDNN) changes according to the order of measurement and breathing pattern. Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. *Friedman test p-value, **post-hoc Wilcoxon's paired-sample test p-values, only significant differences are indicated. Insp: Only inspiration paced without exposure; 1:1 symmetrically paced inspiration/expiration; 1:2 asymmetrically paced inspiration/expiration; i: with irradiation; n: without irradiation.*

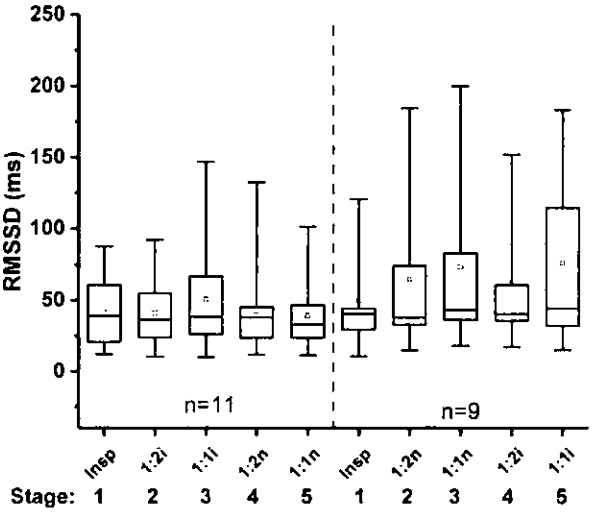

Figure 6. *Root mean square of successive differences (RMSSD) changes after separation of the first-exposed and second-exposed volunteers. Boxes: Lower and upper quartiles, horizontal line: median value, whiskers: minimum and maximum values, square: mean value. Horizontal axis with the breathing patterns, the serial number, and the presence of exposure. Insp – Only inspiration paced without exposure, 1:1 symmetrically paced inspiration/expiration; 1:2 asymmetrically paced inspiration/expiration; i: with irradiation; n: without irradiation, bottom line: stage serial number.*

were regrouped in the order of data acquisition ignoring irradiation: 11 volunteers were exposed in the 2nd and 3rd stage, while nine were exposed in the 4th and 5th stage. Figure 5 demonstrates that there were no significant differences in SDNN among the groups with identical breathing patterns independently of the EMF exposure. Between the 1:1 and 1:2 groups, SDNN in the 2nd and 3rd stages differed, p=0.002 is considered a statistically significant difference even after Holm-Bonferroni correction.

Exploring possible post-exposure effects, the 2nd and 3rd irradiation (n=11) and 4th and 5th stage irradiation (n=9) groups were analyzed separately according to exposure *versus* sham-exposure, given that RMSSD reflects parasympathetic influence on heart rate. The 2nd and 3rd stage exposed groups gave the chance to examine post-exposure effects up to 12 minutes (during the 4th and 5th stages) following a 12-minute EMF exposure. During exposure (n=11+9) and before exposure (n=9), the 1:1 breathing resulted in greater median and mean RMSSD than with 1:2 breathing (Figure 6). Paradoxically, the post-exposure analysis (n=11) showed lower mean and median RMSSD with 1:1 breathing. Due to the small number of people in the split groups, we did not perform further statistical analysis. However, this may be the explanation for the significant differences according to breathing patterns only between the 2nd and 3rd but not between the 4th and 6th stages apparent in Figure 5.

Discussion

Additionally to electric inhomogeneity and anisotropy of the human body, local EMF effects are influenced by the wavelength relative to the target size, penetration/damping and intracorporal reflections of the microwave besides passive and active thermoregulation processes, such as instantaneous regional blood flow. All these factors call for sophisticated numerical simulation studies, phantom model tests, careful extrapolation of experimental results, and further epidemiological investigations (4, 6). In the present study, the possible effects of pulsed, low-frequency-modulated EMF emitted by mobile phones on the autonomic modulation of the heart rate was investigated by HRV and HRA analysis at different paced breathing patterns. We supposed altered breathing–respiratory sinus arrhythmia coupling on exposure which could be detected by HRA analysis. We did not find statistically significant differences in HRA parameters due to exposure, and even the earlier published (26) reporting on 1:1 *vs.* 1:2 breathing did not reach statistically significant differences after Holm–Bonferroni correction of the significance level in spite of the similar study population and pace-adhered breathing verified by recordings. The powerful correlation of Guzik and Porta indices observed in this study also calls attention to their

redundancy, since assuming a constant heart rate during the examination period, the number of heart beats and the inter-beat differences in the accelerating and decelerating set of points of the Poincaré plot are reciprocal, as detailed in our previous investigation (26).

On the other hand, we detected statistically significant elevation of the SDNN, CV% and RMSSD parameters during irradiation in symmetrical but not in asymmetrical breathing patterns. Thus 1:1 paced breathing seems to be a sensitizing factor in HRV analysis, at least concerning the effects of EMF. The higher inter-individual variation of HRV in the 1:1i group (Figure 1B-D) may be the result of the presence of some individuals with electromagnetic hypersensitivity, or due to the non-uniform power of exposure since actual emissions during the experiment were not measured; this is a limitation of the study. The power output of mobile phones is automatically set to the necessary minimum (31) in order to increase battery life and reduce emission. Individuals with electromagnetic hypersensitivity only comprise a minority of the population: they report symptoms that may be related to exposition to weak EMF (32). However, Hietanen and co-workers did not ascribe subjective complaints of adverse effects of mobile phone emission in a blind study on 20 healthy volunteers (33). In a meta-analysis (34) no evidence was found of a causal association of electrosensitivity and EMF. Eltiti and colleagues conducted a study similar in volume on EMF-sensitive and healthy volunteers: combined GSM900, GSM1800 and UMTS 2000MHz base station-like exposure did not affect subjective well-being, skin conductance or pulse rate (35). Another limitation of our study is the fixed 1:2 and 1:1 sequence of breathing patterns, resulting in longer (altogether 12 minutes) exposure or a pre-exposed state at 1:1 breathing, however, we considered the negative results or only a rapidly recovering transient effect of previous authors (10, 18, 23, 35, 36) regarding cellular phone exposition and HRV, hence we focused on the immediate effects of exposure on HRV and HRA parameters. However, randomization made it possible to examine post-exposure effects in 11 volunteers, where we found opposite changes of RMSSD with 1:2 *versus* 1:1 breathing patterns compared to the exposed (the same group n=11) or pre-exposed (other group n=9) cohort (Figure 6). This can reflect vagal withdrawal following 12-minute-long mobile phone EMF irradiation.

HRV analysis is a relatively frequently used non-invasive method to analyse the acute impact of mobile phone irradiation on the autonomous modulation of cardiac functions. In our previous study (23), we did not find any changes in standard HRV parameters during short-term 900 MHz GSM EMF exposure in healthy volunteers. Parazzini *et al*. concluded that short-term RF irradiation of healthy young persons by mobile phone does not result in statistically significant changes but suggested a weak interaction of time domain parameters and LF power (36). Other researchers came to similar conclusions regarding blood pressure and spectral HRV parameters in 120 volunteers (18). Upon habitual exposure to mobile phone radiation, the time domain HRV parameters were lower and the LF/HF ratio was higher, suggesting a shift to sympathetic activation compared to controls not using cellular phone at all (37).

The mean RR-interval at the inspiration-only controlled first run was significantly shorter (corresponding to higher pulse rate) compared to 1:2 paced sham-exposed measurements. The latter was the second stage only in about the half of the participants considering the double-blind randomized exposure. The 15 minutes of orthostatic adaptation prior to recording excludes orthostatic effects; a stress reaction caused by the beginning of the recording or starting the paced breathing might be one explanation.

Szmigielski and co-workers detected altered diurnal rhythm of blood pressure and heart rate in people regularly working exposed to EMF compared to not exposed controls with the same working pattern (16). The effect was additionally proportional to the electric field strength, ranging from 20 to 550 V/m at 0.738-1.503 MHz. Braune and colleagues used a remote-controlled 900 MHz GSM phone at the right hemisphere for 35 minutes in 10 healthy volunteers and found an increase in capillary vasoconstriction due to increased efferent sympathetic activity resulting in higher systolic and diastolic blood pressures but there were no changes in standard baroreflex tests (17). On the other hand, Barker *et al*. excluded any significant effect in mean arterial pressure and heart rate variability (HRV) parameters on short-term exposure by simulating GSM and TETRA handsets in 120 healthy subjects (18). Tahvanainen and co-workers did not find any change in arterial blood pressure or heart rate during or after 35 minutes of exposure at 900 or 1800 MHz at maximal allowed antenna power in a double-blind placebo-controlled crossover trial in 32 healthy volunteers (19).

Braune *et al*. suspected blood pressure elevation and increased efferent sympathetic activity due to cellular phone irradiation central effects since there were no changes in standard baroreflex tests (17). Our experimental setup demands more complex interaction between auditory (beep for pacing), cortical (recognition and differentiation of beep frequency and intentional breathing in and out), and autonomic centres (intentional breathing and reflex responses to intrathoracal and blood pressure changes), all of them localized in the vicinity of the microwave signal source. Valentini and co-workers concluded in their meta-analysis of the neurophysiological effects of mobile phone exposure that not only the cortex but also subcortical structures such as the hypothalamus and thalamus can be affected by pulsed microwave radiation since the alpha and sigma bands of EEG are enhanced during exposure (10). However, in our study we cannot exclude the role of

baroreflexes following intrathoracal pressure changes due to breathing either at the receptor level or at processing in the brain stem. In addition, the carotid body is close to a mobile phone during use, so its function may also vary with exposure. We believe an interaction exists between the affected centres resulting in a delay of feedback which can increase HRV under 1:1 breathing and EMF exposure. This might be due to the excitation effect of cellular phone exposure on the brain (10), resulting in increased sympathetic activity in accordance with Braune *et al.* (17) and Ekici and co-workers (37), considering also the relatively longer inspiratory period during the 1:1 breathing compared to the 1:2 pattern. During inhalation, respiratory sinus acceleration is a result of vagal withdrawal and increased sympathetic flow. In our investigation, HF power (vagal effect) decreased and LF power (vagal and sympathetic interactions) increased upon exposure during 1:2 breathing (Figure 2), however, it did not reach statistical significance by the Friedman test. We suspect that microwave exposure acts mainly during inspiration.

Conclusion

To our knowledge, the present examination was the first investigating HRA and HRV parameters with different paced inspiration/expiration ratios during short-term GSM cellular phone exposure in a double-blind repeated-measures design crossover trial. Significant HRA changes were not found on exposure compared to sham exposure, however, time domain HRV parameters on exposure were significantly different at 1:1 paced but not at 1:2 paced breathing, considering symmetrical breathing as a sensitizing factor. We observed a mild post-exposure effect regarding RMSSD. This finding reflects persisting acute effects of GSM handset emission on the autonomic nervous system, although exploring its real effects on health status and on survival, as well as the elucidation of any underlying physiopathology, call for further higher-volume double-blind studies focused on respiration–circulation coupling. Our study reproduced the HRA changes accordingly to the metronome-controlled inspiration/expiration ratios. The strong correlation of Porta and Guzik indices was also demonstrated in the overall study raising their redundant nature. Symmetrical breathing (1:1 inspiration:expiration ratio) can be used as sensitizing factor or 'stress test' in other HRV/HRA analysis studies.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

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Ethical Approval

All procedures performed in the study were in accordance with the ethical standards of the Regional Research Ethics Committee and with the 1964 Helsinki declaration and its later amendments.

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References

1 Funk RH, Monsees T and Ozkucur N: Electromagnetic effects – From cell biology to medicine. Prog Histochem Cytochem. *43(4)*: 177-264, 2009.

2 GSMA. About us. History – Brief history of GSM and the GSMA. Available from: https://www.gsma.com/aboutus/history Last accessed on 28 September 2017.

3 Maros D and Temesvári Z: Capacity of mobile networks in emergency case. Hadmérnök (The War Engineer) *XII/1*: 247-254, 2017.

4 Mátay G and Zombory L: Physiological effects and biomedical applications of radiofrequency radiation. Budapest, Műegyetemi Kiadó, pp. 31-148, 2000. (Hungarian).

5 Szalay L and Ringler A: Biophysics. Budapest, Tankönyvkiadó, pp. 126-138, 1985.

6 Stavroulakis P: Mechanisms of Action of EMFs on Biological Systems. Introduction. *In*: Biological Effects of Electromagnetic Fields. Stavroulakis P (ed). Springer-Verlag, Berlin-Heidelberg, p. 4, 2003.

7 Kim KE, Park SK, Nam SY, Han TJ and Cho IY: Potential therapeutic mechanism of extremely low-frequency high-voltage electric fields in cells. Technol Health Care *24(3)*: 415-427, 2016.

8 William AS: Techmind. GSM phone signal analysis. Available from: http://www.techmind.org/gsm/ Last accessed on 28 September 2017.

9 World Health Organisation (WHO). Global Health Observatory data repository. Exposure limits for radio-frequency fields (public). Data by country. Available from: http://apps.who.int/gho/data/view.main.EMFLIMITSPUBCRADIOFREQUENCYv Last accessed on 28 September 2017.

10 Valentini E, Curcio G, Moroni F, Ferrara M, De Gennaro L and Bertini M: Neurophysiological effects of mobile phone electromagnetic fields on humans: a comprehensive review. Bioelectromagnetics *28(6)*: 415-432, 2007.

11 Krause CM, Sillanmaki L, Koivisto M, Haggqvist A, Saarela C, Revonsuo A, Laine M and Hamalainen H: Effects of electromagnetic field emitted by cellular phones on the EEG during a memory task. Neuroreport *11(4)*: 761-764, 2000.

12 Koivisto M, Revonsuo A, Krause C, Haarala C, Sillanmaki L, Laine M and Hamalainen H: Effects of 902 MHz electromagnetic field emitted by cellular telephones on response times in humans. Neuroreport *11(2)*: 413-415, 2000.
13 Croft RJ, Chandler JS, Burgess AP, Barry RJ, Williams JD and Clark AR: Acute mobile phone operation affects neural function in humans. Clin Neurophysiol *113(10)*: 1623-1632, 2002.
14 Preece AW, Iwi G, Davies-Smith A, Wesnes K, Butler S, Lim E and Varey A: Effect of a 915-MHz simulated mobile phone signal on cognitive function in man. Int J Radiat Biol *75(4)*: 447-456, 1999.
15 Ferreri F, Curcio G, Pasqualetti P, Gennaro LD, Fini R and Rossini PM: Mobile phone emissions and human brain excitability. Ann Neurol *60(2)*: 188-196, 2006.
16 Szmigielski S, Bortkiewicz A, Gadzicka E, Zmyslony M and Kubacki R: Alteration of diurnal rhythms of blood pressure and heart rate to workers exposed to radiofrequency electromagnetic fields. Blood Press Monit *3(6)*: 323-330, 1998.
17 Braune S, Wrocklage C, Raczek J, Gailus T and Lucking CH: Resting blood pressure increase during exposure to a radio-frequency electromagnetic field. Lancet *351(9119)*: 1857-1858, 1998.
18 Barker AT, Jackson PR, Parry H, Coulton LA, Cook GG and Wood SM: The effect of GSM and TETRA mobile handset signals on blood pressure, catechol levels and heart rate variability. Bioelectromagnetics *28(6)*: 433-438, 2007.
19 Tahvanainen K, Nino J, Halonen P, Kuusela T, Laitinen T, Lansimies E, Hartikainen J, Hietanen M and Lindholm H: Cellular phone use does not acutely affect blood pressure or heart rate of humans. Bioelectromagnetics *25(2)*: 73-83, 2004.
20 Chattipakorn N, Incharoen T, Kanlop N and Chattipakorn S: Heart rate variability in myocardial infarction and heart failure. Int J Cardiol *120(3)*: 289-296, 2007.
21 Hejjel L and Gal I: Heart rate variability analysis. Acta Physiol Hung *88(3-4)*: 219-230, 2001.
22 Task Force: Heart rate variability: Standards of measurements physiological interpretation and clinical use. Task Force of ESC/NASPE (European Society of Cardiology/North American Society of Pacing and Electrophysiology). Circulation *93*: 1043-1065, 1996.
23 Atlasz T, Kellenyi L, Kovacs P, Babai N, Thuroczy G, Hejjel L and Hernadi I: The application of surface plethysmography for heart rate variability analysis after GSM radiofrequency exposure. J Biochem Biophys Methods *69(1-2)*: 233-236, 2006.
24 Guzik P, Piskorski J, Krauze T, Wykretowicz A and Wysocki H: Heart rate asymmetry by Poincaré plots of RR intervals. Biomed Tech *51(4)*: 272-275, 2006.
25 Porta A, Casali KR, Casali AG, Gnecchi-Ruscone T, Tobaldini E, Montano N, Lange S, Geue D, Cysarz D and Van Leeuwen P: Temporal asymmetries of short-term heart period variability are linked to autonomic regulation. Am J Physiol Regul Integr Comp Physiol *295(2)*: 550-557, 2008.
26 Klintworth A, Ajtay Z, Paljunite A, Szabados S and Hejjel L: Heart rate asymmetry follows the inspiration/expiration ratio in healthy volunteers. Physiol Meas *33(10)*: 1717-1731, 2012.
27 Nokia 6230i user guide. 2006; Issue 3. Available from: www.rtv.co.uk/users_manuals/Nokia_6230i_UG_en.pdf. Last accessed on 28 September 2017.
28 Bernardi L, Wdowczyk-Szulc J, Valenti C, Castoldi S, Passino C, Spadacini G and Sleight P: Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability. J Am Coll Cardiol *35(6)*: 1462-1469, 2000.
29 Pinna GD, Maestri R, La Rovere MT, Gobbi E and Fanfulla F: Effect of paced breathing on ventilatory and cardiovascular variability parameters during short-term investigations of autonomic function. Am J Physiol Heart Circ Physiol *290(1)*: H424-433, 2006.
30 Stark R, Schienle A, Walter B and Vaitl D: Effects of paced respiration on heart period and heart period variability. Psychophysiology *37(3)*: 302-309, 2000.
31 Lönn S, Forssén U, Vecchia P, Ahlbom A and Feychting M: Output power levels from mobile phones in different geographical areas; implications for exposure assessment. Occup Environ Med *61(9)*: 769-772, 2004.
32 Bergqvist U, Vogel E, Aringer L, Cunningham J, Gobba F, Leitgeb N, Miro L, Neubauer G, Ruppe I, Vecchia P and Wadman C: Possible health implications of subjective symptoms and electromagnetic fields. A report prepared by a European group of experts for the European Commission. DG V. Solna: Arbete och Halsa; 1997. https://gupea.ub.gu.se/bitstream/2077/4156/1/ah1997_19.pdf accessed on 12 June 2017.
33 Hietanen M, Hamalainen AM and Husman T: Hypersensitivity symptoms associated with exposure to cellular telephones: no causal link. Bioelectromagnetics *23(4)*: 264-270, 2002.
34 Rubin GJ, Das Munshi J and Wessely S: Electromagnetic hypersensitivity: a systematic review of provocation studies. Psychosom Med *67(2)*: 224-232, 2005.
35 Eltiti S, Wallace D, Ridgewell A, Zougkou K, Russo R, Sepulveda F, Mirshekar-Syahkal D, Rasor P, Deeble R and Fox E: Does short-term exposure to mobile phone base station signals increase symptoms in individuals who report sensitivity to electromagnetic fields? A double-blind randomized provocation study. Environ Health Perspect *115(11)*: 1603-1608, 2007.
36 Parazzini M, Ravazzani P, Tognola G, Thuroczy G, Molnar FB, Sacchettini A, Ardesi G and Mainardi LT: Electromagnetic fields which are produced by GSM cellular phones and heart rate variability. Bioelectromagnetics *28(2)*: 1221-29, 2007.
37 Ekici B, Tanindi A, Ekici G and Diker E: The effects of the duration of mobile phone use on heart rate variability parameters in healthy subjects. Anatol J Cardiol *16(11)*: 833-838, 2016.

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REVIEW ARTICLE

Self-Reporting of Symptom Development From Exposure to Radiofrequency Fields of Wireless Smart Meters in Victoria, Australia: A Case Series

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ABSTRACT

Context • In 2006, the government in the state of Victoria, Australia, mandated the rollout of smart meters in Victoria, which effectively removed a whole population's ability to avoid exposure to human-made high-frequency nonionizing radiation. This issue appears to constitute an unprecedented public health challenge for Victoria. By August 2013, 142 people had reported adverse health effects from wireless smart meters by submitting information on an Australian public Web site using its health and legal registers.

Objective • The study evaluated the information in the registers to determine the types of symptoms that Victorian residents were developing from exposure to wireless smart meters.

Design • In this case series, the registers' managers eliminated those cases that did not clearly identify the people providing information by name, surname, postal address, and/or e-mail to make sure that they were genuine registrants. Then they obtained consent from participants to have their deidentified data used to compile the data for the case series. The author later removed any individual from outside of Victoria.

Participants • The study included 92 residents of Victoria, Australia.

Outcome Measures • The author used her medical experience and judgment to group symptoms into clinically relevant clusters (eg, pain in the head was grouped with headache, tinnitus was grouped with ringing in the ears). The author stayed quite close to the wording used in the original entries. She then calculated total numbers and percentages for each symptom cluster. Percentages were rounded to the nearest whole number.

Results • The most frequently reported symptoms from exposure to smart meters were (1) insomnia, (2) headaches, (3) tinnitus, (4) fatigue, (5) cognitive disturbances, (6) dysesthesias (abnormal sensation), and (7) dizziness. The effects of these symptoms on people's lives were significant.

Conclusions • Review of some key studies, both recent and old (1971), reveals that the participants' symptoms were the same as those reported by people exposed to radiofrequency fields emitted by devices other than smart meters. Interestingly, the vast majority of Victorian cases did not state that they had been sufferers of electromagnetic hypersensitivity syndrome (EHS) prior to exposure to the wireless meters, which points to the possibility that smart meters may have unique characteristics that lower people's threshold for symptom development. (*Altern Ther Health Med.* 2014;20(6):28-39.)

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The Victorian Auditor-General's November 2009 report[1] criticized the rollout of smart meters, which had commenced in 2009 under a previous government's mandate from 2006. As a result, a freshly elected Victorian Premier announced in 2010 that his government would review the program. Following a number of reports, including those by Deloitte,[2] EMC Technologies,[3] and Lockstep Consulting,[4] the new Victorian government announced on December 14, 2011, that it would continue with the program. Although the program would result in an overall net cost to consumers of $319 million dollars (NPV at

2008 values), Deloitte's analysis of the costs and benefits of the program had concluded that it made economic sense to continue given that a large portion of the costs had already been sunk into the project.[2] The rollout was scheduled to conclude by the end of 2013, but the deadline has been extended because of delays caused by technical difficulties, inaccessible sites, and customer refusals.

Issues Surrounding Rollout

After installation of wireless smart meters began, anecdotes of people developing symptoms started to be reported in mainstream media. For example, an article in the *Herald Sun* in Melbourne reported that Marc and Maureen Florio and their 4 children had left their home, claiming that they had been experiencing constant headaches and sleep deprivation since a neighbor's smart meter had been installed 3 weeks earlier.[5]

Public concerns over a number of issues with the compulsory rollout of smart meters have since intensified and multiplied. They have included (1) adverse health effects; (2) safety issues, such as a possible increased risk of house fires; (3) the incompatibility of the smart meter with existing wiring and appliances, possibly causing damage to electrical devices in the home; (4) privacy issues surrounding the collection and on-selling of vast amounts of data that reveal customers' energy usage patterns; (5) security issues, such as those inherent in any type of wireless communication (ie, a vulnerability to hacking and to cyber-attacks); (6) cost concerns; and (7) a perceived lack of democratic process because of the way in which the rollout had proceeded.[6] In response to these concerns, Energy Safe Victoria (ESV) released a report in July 2012, "Safety of Advanced Metering Infrastructure in Victoria," which stated that "smart meters are safe,"[7] notwithstanding the fact that ESV had mentioned in their draft in May 2012 that the issue of possible health effects was "beyond the detailed scope" of the report.[8]

Victoria's smart meters are electronic meters that are capable of measuring electricity consumption in 30-minute intervals and have a transmitter/antenna that is able to broadcast the collected data wirelessly to the base.[6] Victoria's smart meters also have a second internal antenna for the Home Area Network (HAN) radio, which can be turned on when requested by the customer.[3] The electronic meter is all that is needed to implement time-of-use tariffs (ie, charging different rates for electricity at different times); however, the remote-reading function means that meter readers are no longer required and that the power companies can disconnect and reconnect power remotely.[6] In effect, a smart grid, as opposed to deployment of electronic meters, constitutes the power companies' communication system. The bulk of Victoria's power distributors use wireless mesh networks that rely on the smart meters to act as relay stations, with households' data hopping unpredictably from meter to meter, thus forming a mesh.[6] Any reflective surface can cause a deviation in the transmission route of the radiofrequency signal. One distributor has deployed a WiMax network, which involves transmission from each meter directly to a collection tower in a star-like configuration.[6,9]

Smart meters do not have to be wireless. Italy has completed the largest smart meter rollout to date. Their smart meters are hard-wired and communicate over the existing power lines.[10] Other options have been proposed, such as communication via telephone lines, whereas fiber optic cabling has already been successfully deployed in other parts of the world.[11] Claims have been made that all types of electronic meters, including wired smart meters, can introduce dirty electricity (ie, high-frequency voltage transients and harmonics) along the wiring of a house, because of their switching-mode power supply, as well as back into the main powerline.[12] The function of the switching-mode power supply is to convert alternating current (AC) coming in from the power lines to direct current (DC), which is required to run the electronic meter. This process creates high frequency voltage spikes, which are emitted constantly, 24/7, and which travel along building wires and radiate outward from them. Critics claim that this dirty electricity can lead to short- and long-term, adverse health effects.[12,13]

Sources of Radiation

Electromagnetic fields (EMFs) is a broad term that encompasses both natural and human-made sources of radiation. The electromagnetic spectrum describes the continuum of different frequencies put together with the associated wavelength of each frequency.[14,15] The frequency is the number of oscillations or cycles per second, whereas wavelength describes the distance between successive peaks of a wave.[16] As a result, wavelength and frequency are inseparably intertwined: The higher the frequency, the shorter the wavelength is.[14] The electromagnetic spectrum is divided into 2 main types: (1) ionizing radiation, which comprises cosmic and gamma rays, X-rays, and ultraviolet rays; and (2) nonionizing radiation.[14,15,17]

Ionizing radiation has so much energy per quantum that it is able to break chemical bonds between molecules.[14] The negative effect on health of ionizing radiation is well recognized.[17] In this report, however, the term *radiation* will be used to describe nonionizing radiation, which does not carry sufficient energy to break molecular bonds.[14]

Nonionizing radiation includes (1) extremely low-frequency fields, such as those emitted by electrical appliances and power lines; (2) intermediate-frequency fields, such as those used in some antitheft and security systems; and (3) high-frequency radiation, which includes radiofrequency fields, such as those produced by mobile telephones, television and radio transmitters, and radar, as well as microwaves, a subset of radiofrequency radiation, which have frequencies in the 300 MHz to 300 GHz range.[16] The last are used in microwave ovens and for wireless Internet.[14,15]

These definitions are arbitrary but represent a useful way of describing different parts of the nonionizing component of the spectrum. Discussions of and research on the effects of nonionizing radiation revolve around thermal and

nonthermal effects.[17] According to the main regulatory agencies in Australia and the United States, only thermal effects are capable of affecting human health[17]; however, this article will deal exclusively with the nonthermal, or biological, effects on humans of nonionizing radiation. For this reason, the author has used the terms *radiation*, *radiofrequency*, and *microwaves* interchangeably in this article.

As societies industrialize, an unprecedented increase in the number and diversity of EMF sources occurs.[18] These sources include (1) video display units (VDUs) associated with computers and mobile phones and their base stations,[18] (2) wireless Internet, (3) digital television and radio, and—more recently—(4) wireless utility meters and their associated infrastructure. For some time, individuals have reported a variety of health problems that they relate to exposure to EMF.[18]

Electromagnetic Hypersensitivity Syndrome

Electromagnetic hypersensitivity syndrome (EHS) is characterized by a variety of nonspecific symptoms. The most common ones include dermatological symptoms—redness, tingling, and burning sensations—as well as neurasthenic and vegetative symptoms—fatigue, tiredness, concentration difficulties, dizziness, nausea, heart palpitations, and digestive disturbances.[18] This syndrome was first described by Russian researchers in the 1950s, who called it microwave sickness.[17]

Although the range of estimates of the EHS prevalence in the general population is broad, a survey of self-help groups has indicated that approximately 10% of reported cases have been considered severe.[18] The World Health Organization (WHO) has expressed a willingness to consider professional and public input on evidence supporting the inclusion of EHS into the 11th version of the International Classification of Diseases (ICD), to be released in 2015.[15] Various national governments have also recognized EHS as an emerging public problem. Sweden classifies EHS as a functional impairment,[15] whereas the Council of Europe Resolution 1815 calls for particular attention to be paid to the needs of electrosensitive people and for the introduction of special measures to protect them, including the creation of wave-free areas not covered by the wireless network.[19]

In May 2013, the author of the current study became aware that people were registering adverse health effects from smart meters on a public Web site. Two ways existed for people to register: (1) a health register and (2) a legal register. The health register requested that people send their data to a specific e-mail address if they believed that their health had been affected following installation of smart meters, asking 2 questions: (1) "Are you hypersensitive to electromagnetic radiation from sources such as smart meters and mobile phones?" and (2) "Has your health been affected following the installation of smart meters?" The legal register contained 1 similarly worded open-ended question: "Do you believe your health has been affected by the installation of smart meters?" If the answer was "yes," people were asked to state the symptoms from which they were suffering that they believed had resulted from exposure to electromagnetic radiation (EMR) that had been emitted from smart meters. The information could be submitted online or the form could be printed and filled in by hand, then sent to a designated postal address. Neither form of registration posed direct questions about types of symptoms or offered any form of tick-a-box questionnaire, thereby avoiding the suggestion of various symptoms, and both steered clear of a recruitment-style approach to the collection of information.

The author subsequently approached the managers of the Web site and the registers, and based on her status as a medical practitioner, she received permission to view people's deidentified data in both registers in hard-copy form. It was immediately apparent to the author that people from disparate parts of Victoria were listing the same or similar symptoms from exposure to smart meters. The majority of people could not possibly have known each other, and they certainly had no access to information that had been registered by others, as data sent to the registers had been kept strictly private and confidential. Because the information appeared to point to a new and ongoing public health problem for Victoria, the author decided that a case series report, based on the cases in the registers, was warranted.

METHODOLOGY

The author began by enlisting the agreement and cooperation of the managers of the public Web site and registers and by instructing them on her planned methodology. The managers were given the task of selecting appropriate cases from both their health register and legal register. The cases were included when the managers could clearly identify the person by name, surname, postal address, and/or e-mail address to make sure that they were genuine registrants. In the case of children, name and surname, together with postal address and/or e-mail address of at least 1 parent, were considered sufficient for identification of the child.

The managers then proceeded to print or photocopy each qualifying individual's entry and to deidentify each case, providing the author with each person's gender, date of birth, and the name of his or her residential suburb. The author considered these details important for statistical purposes. Children's symptoms were reported by their parents. E-mail addresses and phone numbers were hidden by the registers' managers, and the author made no attempt to contact any person to obtain additional details or ask for clarification(s). This practice was judged by the author to be appropriate, not only for the maintenance of anonymity but also because any further questioning would have had the potential to introduce biases in reporting and interfere with its spontaneous and unsolicited nature. What was not written or written clearly was simply omitted from the report. This fact must be kept in mind when reading the case series.

The Web site's managers then proceeded to seek signed written consent to use people's deidentified data to compile a report. This request was done by sending a letter to each

individual, mainly via post, but in a few cases in which postal addresses were not available, via e-mail. In the case of children, consent had to be signed by 1 of the parents. One case was drawn directly from the public side of the earlier-mentioned Web site, and for this reason, consent was not sought for that case because it was already available in the public domain. The Web site contained a significant number of publicly available cases of symptoms from smart meters; however, the chosen case was included because it was the only one that provided fully identifiable details: name, surname, residential address, and phone number. The author subsequently removed 1 case from outside the state of Victoria and 1 from a resident of New Zealand.

Of 142 fully identifiable cases before this removal, 91 consented, with the 1 additional case being in the public domain and not requiring consent. Therefore, the sample size was 92, and the author received all deidentified submissions in hard-copy form only. They were stored in her home office under lock and key. The author intends to keep all documents for a period of 5 years after publication of this article. At the end of this period, the documents will be destroyed.

For the results, the author has used her medical experience and judgment to group symptoms into clinically relevant clusters (eg, pain in the head was grouped with headache; tinnitus was grouped with ringing in the ears). The author has stayed quite close to the wording used in the original entries. Total numbers and percentages were calculated for each symptom cluster. Percentage values were rounded to the nearest whole number.

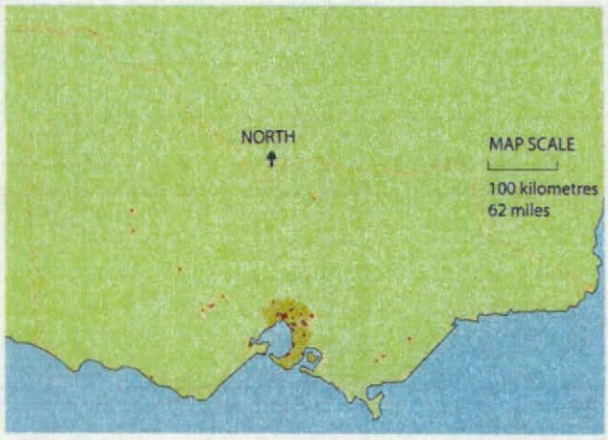
RESULTS

Of the 92 participants reporting symptoms from exposure to wireless smart meters, 87 were adults and 5 were children. Of the adults, the youngest person was 23 years of age and the oldest was 74; 55 (63%) were female and 32 (37%) were male. The children were aged 6, 10, and 14 years, with the ages of the remaining 2 children unknown. The children's group was composed of 2 females and 3 males. Therefore, for the total group, 57 (62%) were female and 35 (38%) were male.

Of all the individuals, 39 (42%) did not specify whether their symptoms were caused by their neighbors' or their own smart meters. This lack of information was not surprising, because that kind of information was not sought in either the health or the legal registers. Therefore, it is of note that a total of 53 people (58%) volunteered this data: (1) 27 (29%) claimed that their symptoms were from exposure to their neighbors' smart meters, (2) 20 (22%) thought the adverse health effects were from a smart meter at their own homes, and (3) 2 wrote that their symptoms were from both their neighbors' and their own smart meters. It is also interesting that 3 people stated that they experienced symptoms when visiting friends or relatives who had a smart meter, and 1 person became ill after exposure to a smart meter at work.

Only 7 people (8%) stated that they considered themselves to have been suffering from EHS prior to smart meter exposure. Of these, 2 felt that radiation from smart meters had aggravated their conditions. The place of residence of the person representing each case study was important, because the locations illustrate that individuals reporting symptoms were not concentrated in 1 geographical area but were from different and varied parts of metropolitan and rural Victoria. Figure 1 shows the residential locations of the current study's cases marked with red dots; 67% of the Victorians in this study lived within Melbourne's metropolitan area (ie, Melbourne's suburbs), which is shaded a darker green on the map. This correlates almost perfectly with current demographics for the state, which show more than 70% of all Victorians living in Melbourne's suburbs.

Figure 1. Map of Victoria and Places of Residence of the People in the Study's Cases



As Figure 2 shows, the most common symptoms were (1) insomnia, sleep disturbance, or sleep disruption—44 people (48%); (2) headaches, head pain, or dull head—41 people (45%); (3) tinnitus, ringing in the ears, or buzzing/noises in the ears—30 people (33%); (4) tiredness, lethargy, or fatigue, including chronic fatigue, exhaustion, or weakness—29 people (32%); and (5) cognitive disturbances, inability to concentrate or think, disorientation, or memory loss—28 people (30%). Table 1 identifies the symptoms that were experienced by participants, other than the 5 most common, with their incidence.

Figure 2. Five Most Common Symptoms

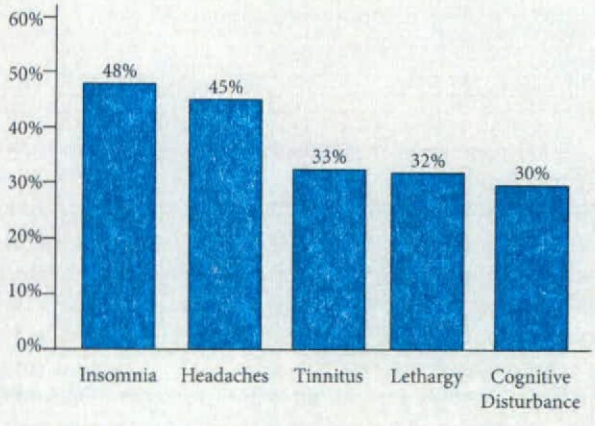


Table 1. Other Symptoms

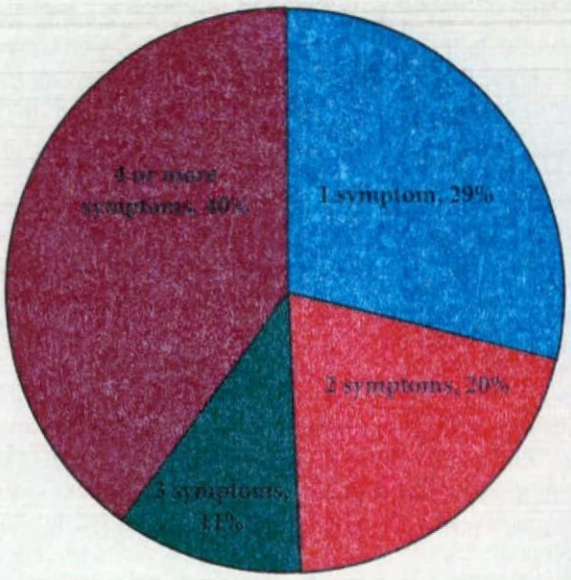
Symptom/Symptom Cluster	n (%)
Dysesthesias, including nerve pain, neuropathy, burning sensations, tremors, cold extremities, and poor circulation	20 (22%)
Dizziness/loss of balance	19 (21%)
Heart palpitations	16 (17%)
Nausea	15 (16%)
Onset of EHS	14 (15%)
Pain (in joints, bones, muscles, other and including arthritic changes)	13 (14%)
Pressure/heat/weird feeling in or on head	12 (13%)
Anxiety/agitation/irritability/restlessness	12 (13%)
Adverse health effects not otherwise specified	11 (12%)
Problems with eyes or eyesight/blurred vision	10 (11%)
Chest pain/pain in the heart	9 (10%)
Rashes/skin irritation/skin discoloration/dry skin	7 (8%)
Aggravation of pre-existing medical condition	6 (7%)
Digestive problems/bowel irritability/stomach pain	5 (5%)
Muscle spasms/cramps/twitches	5 (5%)
Nose bleeds	4 (4%)
Ear problems (ear pain, loss of hearing)	3 (3%)
Depression/loss of motivation	3 (3%)
Increased rate of infections/colds	3 (3%)
Allergies/food sensitivities	3 (3%)
Aggravation of EHS	2 (2%)
Sinus problems	2 (2%)
Lump in throat/sore throat	2 (2%)
Weight loss/loss of appetite	2 (2%)
Swollen face/lips	2 (2%)
Bladder infections/strains	2 (2%)
Flu-like symptoms	1 (1%)
Dehydration/thirst	1 (1%)
Weight gain	1 (1%)
Inability to talk	1 (1%)
Loss of motor skills	1 (1%)
Loss of feeling and movement from waist down	1 (1%)

Abbreviations: EHS = electromagnetic hypersensitivity syndrome.

It is concerning that 40% of all participants reported 4 or more symptoms, as this finding is very likely to be predictive of a greater level of disability (Figure 3). Eleven percent had developed only 3 symptoms, 20% only 2 symptoms, and 29% only 1 symptom. Note that the author counted "adverse health effect(s) not otherwise specified" as 1 symptom. She is of the opinion that even 1 symptom, depending on its type and severity, could result in significant disruption for an individual. An example of this result is the experience of the person in Case 82, an adult male who developed only 1 symptom—chronic, severe nerve pain—and had to go on a disability pension as a result.

It may reasonably be expected that a random sample of the population would also report a number of symptoms at any one time, but the difference in these cases is that all people in this study self-reported symptoms that they attributed directly to smart meters. Because EHS is a self-reported syndrome and given the current absence of a reliable assessment tool for identifying EHS in individuals, Eltiti et al[20] concluded that researchers have to rely on the

Figure 3. Number of Symptoms per Person



individual's self-diagnosis of their symptoms as caused by exposure to EMF. The researchers proposed an EHS screening tool that is centered on the fact that an individual explicitly attributes his or her symptoms to exposure to EMF-producing object(s).[20]

Similarly, a survey conducted by the Dutch Electrohypersensitivity Foundation in 2007 argues that EMF-affected individuals simply know, often by experimentation, that certain pieces of electrical equipment, installations, or facilities make them sick and that most of the problems are solved when these items are switched off or the EMF exposure is lowered by shielding or increasing the distance from a device.[21] This statement mirrors the experience of the majority of the Victorian cohort, who were specific in their description of their health problems as being directly related to smart meter exposure. A chronological relationship existed between the onset of exposure and symptom development.

A chronological relationship between length of exposure and an increase in the number or severity of symptoms, however, did not necessarily exist. This finding suggested a possible all-or-nothing mechanism, whereby smart meter exposure leads people to reach a personal threshold beyond which adverse health effects are consciously perceived. More than one-half (58%) of all the current participants also volunteered a statement with regard to the location of the smart meter(s) that they had identified as causing their symptom(s) and described clear alleviation of symptom(s) when they moved away from the smart meter(s) or when shielded from the smart meter(s).

As a consequence, a large number of people self-helped either by using shielding measures or by putting distance between themselves and the smart meter(s), which meant either relocating their bedrooms, moving to another residence, ceasing employment, restricting their movement in general, or moving out of the state of Victoria (Table 2).

Table 2. Effect on People's Lives

Effect
1. Having to go on a disability pension
2. Not being able to use part of one's house
3. Restricting freedom of movement
4. Spending a lot of money on shielding products
5. Causing financial problems
6. Causing relationship problems
7. Having to undergo otherwise unnecessary medical investigations
8. Needing to see a psychologist and doctors
9. Producing general deterioration in quality of life
10. Needing to restrict time spent using a computer
11. Needing to avoid all EMR-emitting devices
12. Being unable to drive
13. Causing secondary stress
14. Having to temporarily move out of one's home while it was being shielded
15. Developing concerns about long-term effects of exposure
16. Relocating bedroom
17. Decreased performance at work
18. Being unable to work
19. Being able to feel normal only when away from home
20. Causing several issues, such as lethargy or cognitive impairment, secondary to sleep disturbances
21. Needing to move into a caravan 25 km out of town
22. Sleeping in a van for 6 months
23. Relocating to another state

Abbreviation: EMR = electromagnetic radiation.

Figure 1 shows that people in this study were from disparate parts of the state of Victoria. They were from metropolitan as well as regional and rural areas and were not concentrated in any geographical area, which makes possible causes of symptoms related to a specific location unlikely (eg, proximity to airports, wind farms, open-cut coal mines, or chemicals used in agriculture). It is also unlikely for the reported symptoms to be associated with any seasonal factor (eg, extremes of temperatures, degree of humidity, bushfire smoke, or a high pollen count), because the reporting period stretched between September 2012 and August 2013, which meant that symptoms were reported during all 4 seasons.

Smart meters represent an ubiquitous presence throughout the state of Victoria, having been rolled out across the entire state. Their presence is not subject to seasonal variation. Therefore, they are a credible possible cause of the symptoms reported in this study, although a case series cannot prove causality. It can and does, however, offer a new hypothesis, one that will have to be tested by further research.

More than one-half (55) of all the cases did not state what effect the symptoms had had on their lives. This lack is possibly caused by the fact that the registration of their symptoms occurred in an open-ended style that did not

directly ask questions other than whether they thought that smart meters had affected their health. Moreover, participants had consented for their deidentified data to be used to compile a report at a time after their initial submission to the Web site's registers. This situation had the benefit of eliminating the likelihood of a real or perceived secondary gain for registrants but also led to the writing of short, simple statements that did not elaborate on how the symptoms had affected their lives. Table 2 provides details about the effect on the lives of the 37 people who made a statement about those effects..

DISCUSSION

Biological Effects of Radiation

With regard to the reported symptomatology related to wireless smart meters, it is interesting to look back at a research report by Dr Zorach R. Glaser for the Naval Medical Research Institute (NMRI) in the United States, completed in 1971 and revised in 1972.[22] The report lists in excess of 2300 references on the biological responses to radiofrequency and microwave radiation in its bibliography. What is immediately apparent is the fact that most of the symptoms reported in the current case series were also present in the NMRI report. This fact indicates that biological effects from nonionizing radiation are the same irrespective of the device that emits them—accounting for frequency, intensity, and duration—and that such biological effects were already known and reported to the public in 1971. In fact, Glaser mentions 2 even earlier studies that were both published in 1969.[22] The value of Glaser's report lies particularly in its lack of bias and conflict of interest because the sponsoring department was the Bureau of Medicine and Surgery (Navy) in Washington, DC.

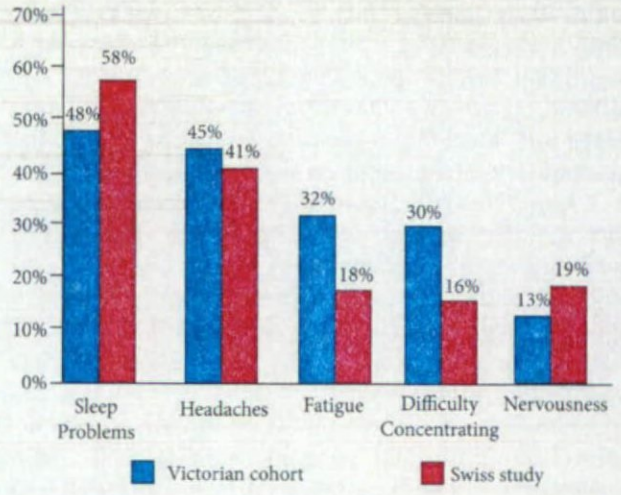
In terms of the biological symptoms listed, an almost complete overlap exists with symptoms reported in the current case series. All commonly reported symptoms in the current case series, such as insomnia, headaches, tinnitus (described as buzzing about the ears in the NMRI document), fatigue, cognitive disturbances, memory problems, dizziness, buzzing in the head, heart rate problems, eye problems, chest pain, dysesthesias, anxiety, and restlessness are very clearly biological symptoms that were listed in Glaser's report,[22] together with less common symptoms, such as heat/weird feeling in/on the head, skin problems, digestive problems, muscle cramps, sinus problems, depression, loss of appetite, and dehydration.[22]

The symptoms reported by Victorians but not mentioned in the 1971 report are (1) nausea; (2) pressure in the head; (3) pain other than head or chest pain, although the pain could be caused by changes in oxidative processes in tissues as listed by Glaser, and consequent tissue inflammation; (4) shortness of breath; (5) ear problems—pain and decreased hearing; (6) allergies and food sensitivities; (7) nose bleeds; (8) increased rate of infections/colds; (9) bladder infections/strains (10) flu-like symptoms; (11) lumps in the throat (the NMRI report instead mentions a peculiar metallic taste in the mouth); (12) swollen face or swollen lips; (13) weight gain; (14) inability to talk, which could be caused by electroencephalogram (EEG) changes and/or pyramidal tract lesions as mentioned in the 1971 report; and (15) loss of motor skills or loss of feeling and movement from the waist down, which are both consistent with pyramidal tract lesions and effects on locomotor nerves that are listed in the NMRI paper. In looking at these symptoms that were not obviously listed in the NMRI report, it is important to keep in mind that the language of that report was more technical and clinical compared with the current case series, in which the author has purposely stayed true to the wording and terms used by participants and which is, therefore, less technical and less interpretive.

In 1990, a study was commissioned in response to a petition that had been signed by a group of residents in Schwarzenburg, Switzerland, who claimed to be experiencing ill health from a shortwave-radio transmitter present in their small town. The Federal Office of Energy was charged with setting up a study group, which was chaired by Dr J. Cattin, head of the Section Energy Management, and which included the University of Berne and Swiss Telecom, among others.[23] The study was criticized, particularly because of Swiss Telecom's involvement and because of its 5-year duration, which was too short a time for any conclusive findings on long-term health effects, including cancer, to emerge.[24] It nevertheless revealed some impressive understandings on short-term effects from exposure to radiofrequency fields. The most important of these effects was that of sleep disruption, which was very common, affecting 55% of those older than 45 years, and which was directly associated with the electromagnetic-field strength of the transmitter.[23] Other symptoms reported by residents included headaches, tiredness, general weakness, irritability, nervousness, limb pain, lower-back pain, and palpitations. Most important, personality studies were carried out that showed that symptoms were not related to a health-worrying personality but displayed a dose-response relationship with logistic regression. The strong correlation between the type of symptoms experienced by the Victorian cohort and by the residents of Schwarzenburg, together with the shared high prevalence of sleep disruptions in both groups, should further inform assessment of the significance of the findings of the current case series.

A consensus paper of the Austrian Medical Association's EMF Working Group, adopted on March 3, 2012, in Vienna and titled "Guideline of the Austrian Medical Association for the Diagnosis and Treatment of EMF-related Health Problems and Illnesses (EMF Syndrome)," mentions a survey carried out in Switzerland in 2001.[25] In it, 394 respondents attributed specific health problems to EMF exposure. The following symptoms were reported: (1) sleep problems (58%), (2) headaches (41%), (3) nervousness (19%), (4) fatigue (18%), and (5) difficulty concentrating (16%). It is apparent at first glance that the first 2 symptoms are of the same order of frequency as for the Victorians in the current case series (Figure 4). A very similar percentage of people complained of headaches in both the current study (45%) and the Swiss one (41%). A similar, albeit slightly lower, number of participants reported sleep problems, such as insomnia and frequent waking, in Victoria (48%) versus those reported in the Swiss study (58%). All 5 symptoms

Figure 4. Victorian Cohort Versus Swiss Study



reported in the Swiss survey corresponded to symptoms experienced by the Victorian cohort, with fatigue (32%) and difficulty concentrating (30%) being more common in Victoria and nervousness (anxiety/agitation) (13%) being less common.

The Austrian Guidelines also list a number of what their authors consider to be EMF-related symptoms: sleep problems, fatigue, exhaustion, lack of energy, restlessness, heart palpitations, muscle and joint pain, headaches, depression, difficulty concentrating, forgetfulness, anxiety, urinary urgency, anomia, dizziness, tinnitus, and a sensation of pressure in the head and the ears.[25] All listed symptoms were experienced by Victorians in the current study, if the reader accepts that anomia corresponds with inability to talk and urinary urgency to bladder infections/strains.

Short-term effects from exposure to radiofrequency fields are also mentioned in another recent publication, the BioInitiative 2012 report prepared by 29 independent scientists and health experts from around the world. It documents bioeffects (ie, adverse health effects) and public health conclusions about effects of nonionizing radiation, including radiofrequency microwave fields. It replaces the BioInitiative 2007 report.[26] These effects involve cognition; memory and learning; behavior; reaction time; attention and concentration; and altered brainwave activity (altered EEG), as well as insomnia; discomfort; loss of well-being; sleep disruption; aberrant immune, allergic, and inflammatory responses in tissues; interference with normal cardiac function; alteration of circadian rhythms; and desynchronization of neural activity that regulates critical functions in the brain, gut, and heart. Radiofrequencies can act as disrupters of synchronized neural activity.

The BioInitiative report offers a detailed explanation on how environmental exposures to artificial EMFs can interact with fundamental biological processes in the human body.[26] This finding should not be unexpected because "human beings are bioelectrical systems."[26] In addition to short-term effects, the report dwells on the long-term sequelae (pathological conditions) from chronic exposure to nonionizing radiation, which include genotoxicity and DNA breakages among others.[26] It is not strictly within the scope of this case series to explain the biophysical mechanisms that may account for acute symptoms or effects or to discuss the long-term serious health endpoints associated with radiofrequency radiation; however, a summary of the nonthermal biological effects of nonionizing radiation is contained in Table 3. It is distilled from the BioInitiative report and intends to be a basic guide for clinicians.

It also needs to be mentioned that in 2011, the International Agency for Research on Cancer (IARC), which is part of the WHO, classified radiofrequency fields as a Group 2B Possible Human Carcinogen, based on an increased risk of glioma after 10 years or longer of cell phone use.[27] The IARC clarified that the evidence for carcinogenicity applies to exposures to radiofrequency radiation from all sources, not only cell phones (ie, it is not device-specific).[28] This finding has implications for the continued massive rollout of wireless technologies, in particular the wireless smart utility

Table 3. Summary of Biological Effects of Nonionizing Radiation

Effects
1. Pathological leakage of the blood-brain barrier, which allows toxins into brain tissues
2. Pathological leakage of the blood-gut barrier
3. Altered immune function, including increased allergic and inflammatory responses
4. Cardiovascular effects, particularly on blood pressure and heart rate
5. Disregulation of circadian rhythms and reduced melatonin production, which may account for insomnia
6. Nervous system effects, which include altered brainwave activity, changes in neuronal functioning and changes in autonomic nervous system electrophysiology
7. Desynchronization of neural activity that regulates critical functions in brain, gut, and heart
8. Lipid peroxidation of cell membranes
9. Elevated intracellular calcium with consequent disruption of cell metabolism
10. Poorly functioning mitochondria
11. Production of stress proteins as a result of the direct interaction of EMF with the DNA molecule, whereby DNA acts as a fractal antenna (because of its coiled-coil configuration)
12. Altered biochemical functions and production of hormones
13. Increased production of free radicals and deficiencies of antioxidants such as glutathione and melatonin leading to oxidative stress

Abbreviation: EMF = electromagnetic field.

meter, which was described in a recent statement to the UK Parliament as having triggered thousands of complaints of ill health and disabling symptoms worldwide.[29]

Mandated, Involuntary Exposure

With regard to smart meters, 2 unique features should be considered: (1) exposure may be involuntary and (2) exposure can be universal. In Victoria, smart meters were mandated, thereby removing the individual's choice to avoid exposure in his or her own home, and involuntary exposure also occurred to meters in neighboring homes. Each smart meter in the mesh networks transmits an unknown and variable number of burst transmissions per day, which typically reach into many thousands in number.[30] Meters on the WiMax network,[9] although not communicating with each other and deploying only bidirectional communication between a meter and the base station, nevertheless send hourly time synchronization signals in addition to their daily session transmissions.[3]

A submission by the Public Utilities Commission of California shows that only 45.3 seconds of transmissions per day (<0.1% duty cycle) still equates to 9600 transmissions.[30] Exposures are likely to be physiologically additive in nature.[25,26,31] Moreover, belief is increasing in the concept that intermittent pulses of radiofrequencies, such as those used in the smart grid, are more biologically significant compared with constant-type exposures, even when the time-averaged exposure is miniscule.[26,31] This kind of signal is biologically active and *not* invisible to the human body and its proper biological functioning, because the unpredictable pulses disrupt the synchronized biological oscillations within cells.[26] The Austrian Medical Association recommends that such periodic signals should be critically evaluated, whereas nonperiodic signals may be considered more leniently.[25]

In a 2012 memorandum titled "Health Risks Associated with SmartMeters," Dr Poki Namkung, public health officer of the County of Santa Cruz (CA, USA) stated that no scientific literature exists on the health risks of smart meters because they are a new technology.[31] This statement parallels the Austrian EMF Working Group's statement that "new technologies and applications have been introduced without certainty about their health effects."[25] Dr Namkung also explains that research on the potential health risks from radiofrequencies has been funded largely by industry because little funding is available for basic scientific research.[31]

The report indicates:

> ... exposure is additive and consumers may have already increased their exposures to radiofrequency radiation in the home through the voluntary use of wireless devices such as cell and cordless phones, personal digital assistants (PDAs), routers for internet access, home security systems, wireless baby surveillance monitors (baby monitors), and other emerging devices. It would be impossible to know how close a consumer might be to his or her limit, making safety a uncertainty if SmartMeters are mandatorily installed.[31]

Again, this statement correlates with the conclusion in the Austrian Guidelines that "multiple exposures to different EMF sources must be taken into account."[25] Dr Namkung's conclusion that "... governmental agencies are the only defense against such involuntary exposure" to mandated smart meters' nonionizing radiation emissions[31] applies in a particularly relevant way to the Victorian experience.

A similar view is also shared by Dr David O. Carpenter and 53 other scientists and doctors, who, in an article published in 2012, outline some of the effects of EMF exposure with the intent to correct some of the gross misinformation regarding wireless smart meters and advocate for the application of a precautionary principle, such as using wired meters.[32]

Although some of the studies discussed in this report offer recommendations regarding wireless smart meter deployment (Table 4), virtually no published studies are available with respect to smart meters and human health, and no long-term studies exist because of the newness of the technology.

Notably, an early voice of concern on this issue was that of Don Maisch, PhD, from Tasmania, who posed the question of whether smart meters would end up creating a public health nightmare in an article published in September 2012.[33] In it, he explained how current exposure standards are outdated and no longer relevant and warned that, given the sheer number of people exposed, simply dismissing anecdotal evidence of symptoms from smart meters as a nocebo (harmless) effect without a serious research effort would be inexcusable.

Incidence of Effects

This article has discussed the fact that people from various regional and metropolitan areas in the state of Victoria, of all ages and during all seasons, have reported symptoms from exposure to the radiofrequency fields of wireless smart meters as well as the onset or aggravation of EHS and the aggravation of pre-existing medical conditions after installation of the meters. Interestingly, only 8% of the participants in the current study stated that they had suffered from EHS prior to exposure to smart meters, which suggests that the threshold for symptom development appears to be significantly lower when it comes to wireless meters compared with that for other wireless devices.

Of an initial 142 people who had formally registered their adverse health effects from smart meters related to the current study, 92 consented to participation. The author considers this number to be significant and most likely to represent the tip of the iceberg in terms of total numbers. Underestimation could be caused by the fact that people do not associate their symptoms with smart meter exposure when the symptoms are not severe or do not occur concurrently. In addition, this underdiagnosis may be caused by a lack of knowledge about the effects of wireless technologies on the part of the general population and the majority of the medical fraternity. The ongoing campaign of

Table 4. Summary of Scientific Reports

Title	Author(s)	Country	Year	Subject Matter and Findings	Recommendations
"Bibliography of Reported Biological Phenomena and Clinical Manifestations Attributed to Microwave and Radio-frequency Radiation"	Glaser[22]	United States	1971	Provides more than 2000 references on the biological responses to radiofrequency radiation	No specific recommendation; prepared for the Naval Medical Research Institute, Bethesda, Maryland; approved for unlimited public release
"Study on Health Effects of the Shortwave Transmitter Station of Schwarzenburg, Berne, Switzerland"	Altpeter, Krebs, Pfluger, et al[23]	Switzerland	1995	Notes marked deterioration of sleep quality in persons exposed to radio transmitter	No urgent protection measures; review of current exposure guidelines; further research
"Guideline of the Austrian Medical Association for the Diagnosis and Treatment of EMF-related Health Problems and Illnesses (EMF Syndrome)"	Austrian Medical Association's EMF Working Group[25]	Austria	2012	Discusses EMF-related problems and outlines clinical-management approach	Primary method of treatment of EMF-related health problems to consist of prevention or reduction of EMF exposure
"BioInitiative 2012–A Rationale for Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation"	Prepared by 29 experts, edited by Sage & Carpenter[26]	Experts from more than 10 countries	2012	Reviews more than 1800 new scientific studies added to the BioInitiative Report 2007, which cited 2000 studies on adverse health effects from extremely low frequencies and radiofrequencies	New, biologically based public-exposure standard; precautionary approach to RF exposure levels
"Health Risks Associated with SmartMeters"	Namkung[31]	United States	2012	Indicates objective evidence supports EHS diagnosis; no scientific literature on health risks of smart meters	All available, peer-reviewed research data on EMF applicable to smart meters; governmental agencies to protect public health from involuntary exposure
"Smart Meters: Correcting the Gross Misinformation"	Carpenter et al[32]	Authors from a number of countries; published in Canada	2012	Summarizes long-term and short-term health effects of EMF exposure, in particular from smart meters	Application of Precautionary Principle, such as using wired meters
"Electromagnetic and Radiofrequency Fields Effect on Human Health"	Dean, Rea, Smith, Barrier (American Academy of Environmental Medicine)[17]	United States	2012	Discusses different types of radiation and effect of the increasing use of wireless technology on human health	Immediate caution on smart-meter installation; further research on effects of EMF and RF exposure; use of safer technology, including for smart meters

Abbreviations: EMF = electromagnetic field; RF = radiofrequency; EHS = electromagnetic hypersensitivity syndrome.

the state government and power distributors to portray smart meters as safe has also contributed to this lack of knowledge. Even when people believe that their new symptom(s) are caused by smart meters, some are not able to report or register their symptoms because they have no Internet access, and of those who do, not all are aware of Web sites or ways to make reports.

Limitations of Current Study

The main limitation of the current study is that, being a case series, it is a descriptive, retrospective study that does not have a control arm and can therefore help formulate a new hypothesis, but can only make limited statements on the causality of correlations observed.

Another limitation, which is specific to this type of noninterventional analysis of existing nonidentifiable data, is that the author was not able to contact individual case studies and was therefore unable to clarify or add to the information given by them. For the same reason, the author was also unable to follow up these cases longitudinally, which is something that could have potentially yielded valuable information.

CONCLUSIONS

This case series has discussed the most commonly reported symptoms from wireless smart meters. Although some of these symptoms are also reported in relationship to other environmental exposures, such as proximity to airports

or wind turbines, Victorians in this report claimed a direct chronological association between exposure to wireless smart meters and symptom development. A look at the place of residence of people reporting symptoms does not suggest a link to any possible environmental factors that are geographically specific. Seasonal factors are also excluded, because the reporting period stretched over all 4 seasons. The effect of these symptoms on people's lives is far-ranging, from stress, financial problems, and unnecessary investigations to needing to move out of one's home and even to another state.

The author of the current study offers the hypothesis that some people can develop symptoms from exposure to the radiofrequency fields of wireless smart meters. This hypothesis cannot be disproven without further assessment of the affected individuals and the electromagnetic fields in which they live. An evidence-based approach, such as the one used in all other areas of medicine, must be applied, which would mean the establishment of a postrollout surveillance study and funding for further research into the particular effects of wireless smart meters, in conjunction with research into the short-term and long-term consequences of EMR exposure. Until more knowledge is accumulated and until this type of wireless technology can be proven safe, the author believes that communities should use a cautionary approach, asking for a moratorium on deployment of wireless smart meters and smart grids and for the use of safer technologies for smart meters, such as hard-wiring, fiber optics, or other nonharmful methods of data transmission, including reading of meters by meter readers. Living in a wireless smart grid makes the Austrian Medical Association's recommendation to "take all reasonable measures to reduce exposure to electromagnetic fields" impossible to implement.

Dr Maisch's article title, "Smart Meter Health Concerns: Just a Nocebo (Harmless) Effect or an Emerging Public Health Nightmare?", resonates strongly with the Victorian experience so far. This question is very pertinent and one that must be urgently answered.

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REFERENCES

1. Pearson DD; Victorian Auditor-General. Towards a 'smart grid' – the rollout of Advanced Metering Infrastructure. http://www.audit.vic.gov.au/publications/2009-10/111109-AMI-Full-Report.pdf. Published November 2009. Accessed February 27, 2014.
2. Deloitte; WorleyParsons; Victoria Department of Treasury and Finance. Advanced Metering Infrastructure cost benefit analysis report. http://www.smartmeters.vic.gov.au/resources/reports-and-consultations/advanced-metering-infrastructure-cost-benefit-analysis. Published August 2, 2011. Accessed February 27, 2014.
3. EMC Technologies. AMI meter electromagnetic field survey. http://www.smartmeters.vic.gov.au/resources/reports-and-consultations/ami-meter-em-field-survey-repor. Published October 20, 2011. Accessed February 27, 2014.
4. Lockstep Consulting. Privacy impact assessment report: Advanced Metering Infrastructure (AMI). http://www.smartmeters.vic.gov.au/resources/reports-and-consultations/lockstep-dpi-ami-pia-report. Published August 2011. Accessed February 27, 2014.
5. Hosking W. Family forced to move out after suffering health problems. *Herald Sun*. November 1, 2011. www.heraldsun.com.au/archive/news/smart-meter-shock-forces-family-out/story-fn7x8me2-1226181915461. Accessed February 27, 2014.
6. Smith J. Smart meters in Victoria: Information and concerns. http://stopsmartmetersau.files.wordpress.com/2012/02/smart-meters-in-victoria_information-and-concerns_version_3.pdf. Published March 29, 2013. Accessed February 27, 2014.
7. Energy Safe Victoria. Safety of Advanced Metering Infrastructure in Victoria. http://www.esv.vic.gov.au/Portals/0/About%20ESV/Files/whats%20new/FINAL%20ESV%20smart%20meter%20safety%20report%2031%207%2012.pdf. Published July 31, 2012. Accessed February 27, 2014.
8. Energy Safe Victoria. Safety of Advanced Metering Infrastructure in Victoria [draft report]. http://www.esv.vic.gov.au/Portals/0/Consumers/Files/Safety%20of%20advanced%20metering%20infrastructure%20in%20Victoria%20%20170512.pdf. Published May 17, 2012. Accessed February 27, 2014.
9. SP AusNet communications network. SP AusNet Web site. http://www.sp-ausnet.com.au/?id=1010100C36913FCE7EB6037CA2579D1001C8D91. Accessed February 27, 2014.
10. International Confederation of Energy Regulators. Report on experiences on the regulatory approaches to the implementation of smart meters, annex 4: case study, smart meters in Italy. http://www.icer-regulators.net/portal/page/portal/ICER_HOME/publications_press/ICER_Reports_2009_2012/Reports_Annexes/Annex%204%20-%20Case%20study%20from%20Italy. ICER report 12-C&A-08-01. Published April 2012. Accessed February 27, 2014.
11. Smart alternatives. In: Jamieson I. Smart Meters-Smarter Practices: Solving Emerging Problems. Leicestershire, UK: EM-Radiation Research Trust; 2012:24-32. http://www.radiationresearch.org/component/content/article/10-uncategorised/173-smart-meters-smarter-practices-document. Accessed February 27, 2014.
12. Brangan MB, Heddle J. New critical problem with 'smart' meters: the Switching-Mode Power Supply (SMPS). eon3EMFblog.net Web site. http://eon3emfblog.net/?p=2180. Published March 30, 2011. Accessed February 27, 2014.
13. Havas M. Electromagnetic hypersensitivity: biological effects of dirty electricity with emphasis on diabetes and multiple sclerosis. *Electromagn Biol Med*. 2006;25(4):259-268.
14. Electromagnetic fields (EMF): what are electromagnetic fields? World Health Organization Web site. http://www.who.int/peh-emf/about/WhatisEMF/en/. Accessed March 11, 2014.
15. Genuis SJ, Lipp CT. Electromagnetic hypersensitivity: fact or fiction? *Sci Total Environ*. January 2012;414:103-112.
16. Exposure data. In: Non-ionizing Radiation, II: Radiofrequency Electromagnetic Fields. Lyon, France: International Agency for Research on Cancer; 2013:37-38. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; vol 102. http://monographs.iarc.fr/ENG/ Monographs/vol102/mono102-001.pdf. Accessed March 11, 2014.
17. Dean AL, Rea WJ, Smith CW, Barrier AL. Electromagnetic and radiofrequency fields effect on human health. American Academy of Environmental Medicine Web site. http://aaemonline.org/emf_rf_position.html. Accessed February 28, 2014.
18. World Health Organization. Electromagnetic fields and public health: fact sheet 296. http://www.who.int/peh-emf/publications/facts/fs296/en/. Published December 2005. Accessed February 28, 2014.
19. The potential dangers of electromagnetic fields and their effect on the environment. Resolution 1815, Council of Europe Parliamentary Assembly. Adopted May 27, 2011. http://www.assembly.coe.int/Documents/AdoptedText/ta11/ERES1815.htm. Accessed March 11, 2014.
20. Eltiti S, Wallace D, Zougkou K, et al. Development and evaluation of the electromagnetic hypersensitivity questionnaire. *Bioelectromagnetics*. 2007;28(2):137-151.
21. Schooneveld H, Kuiper J; Dutch Electrohypersensitivity (EHS) Foundation. Electrohypersensitivity (EHS) in the Netherlands—a questionnaire survey. http://www.electroallergie.org/downloads/EHS_in_the_Netherlands.pdf. Published December 2007. Accessed March 11, 2014.
22. Glaser ZR. Bibliography of Reported Biological Phenomena ('Effects') and Clinical Manifestations Attributed to Microwave and Radio-frequency Radiation. Bethesda, MD: Naval Medical Research Institute; 1972. Research report MF 12.524.015-0004B.
23. Altpeter ES, Krebs T, Pfluger DH, et al. Study on Health Effects of the Shortwave Transmitter Station of Schwarzenburg, Berne, Switzerland. Berne, Switzerland: Federal Office of Energy; 1995. Study 55.
24. Jakob HV. The Schwarzenbourg (Switzerland) short-wave transmitter: the scandal continues! *Franz Weber J*. July/August/September 1997;(41).
25. Austrian Medical Association's EMF Working Group. Guideline of the Austrian Medical Association for the diagnosis and treatment of EMF-related health problems and illnesses (EMF syndrome): consensus paper of the Austrian Medical Association's EMF Working Group. http://magdahavas.com/wordpress/wp-content/uploads/2012/06/Austrian-EMF-Guidelines-2012.pdf. Adopted March 3, 2012. Accessed February 27, 2014.
26. Carpenter D, Sage C, eds; BioInitiative Working Group 2012. BioInitiative 2012: a rationale for biologically-based exposure standards for low-intensity electromagnetic radiation. http://www.bioinitiative.org/report/wp-content/uploads/pdfs/BioInitiativeReport2012.pdf. Published December 31, 2012. Accessed February 27, 2014.

27. IARC classifies radiofrequency electromagnetic fields as possibly carcinogenic to humans [press release]. Lyon, France: International Agency for Research on Cancer; May 31, 2011. http://www.iarc.fr/en/media-centre/pr/2011/pdfs/pr208_E.pdf. Accessed February 27, 2014.
28. General remarks. In: Non-ionizing Radiation, II: Radiofrequency Electromagnetic Fields. Lyon, France: International Agency for Research on Cancer; 2013:33-34. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; vol 102. http://monographs.iarc.fr/ENG/ Monographs/vol102/mono102-F07.pdf. Accessed February 27, 2014.
29. Written evidence submitted by Stop Smart Meters! (SMR 124). Energy and Climate Change Committee, UK Parliament. http://www.publications.parliament.uk/pa/cm201314/ cmselect/cmenergy/161/161vw107.htm. Published May 2013. Accessed March 11, 2014.
30. Kim AH, Nwamu CJ; Pacific Gas and Electric Company. Pacific Gas and Electric Company's Response To Administrative Law Judge's October 18, 2011 Ruling Directing it to File Clarifying Radio Frequency Information. Public Utilities Commission of the State of California. http://emfsafetynetwork.org/wp-content/uploads/2011/11/PGERFDataOpt-outalternatives_11-1-11-3pm.pdf. Filed November 1, 2011. Accessed March 11, 2014.
31. Namkung PS. Health risks associated with SmartMeters [memorandum]. Santa Cruz, CA: County of Santa Cruz Health Services Agency; January 13, 2012. http://sccounty01.co.santa-cruz.ca.us/bds/Govstream/BDSvData/non_legacy/agendas/ 2012/20120124/PDF/041.pdf. Accessed February 28, 2014.
32. Carpenter DO, Adlkofer F, Al Salameh MS, et al. Smart Meters: correcting the gross misinformation. *Maison du 21e Siècle*. July 12, 2012. http://maisonsaine.ca/smart-meters-correcting-the-gross-misinformation/. Accessed February 28, 2014.
33. Maisch D. Smart meter health concerns: just a nocebo effect or an emerging public health nightmare? *Australasian Coll Nutr Environ Med J*. 2012;31(2):15-19.

International Agency for Research on Cancer

World Health Organization

IARC Monographs Questions and Answers

What does the IARC Monographs Programme do?

The Monographs Programme identifies and evaluates environmental causes of cancer in humans. To date, more than 900 agents have been reviewed.

What types of agents or substances are evaluated?

The Monographs Programme evaluates chemicals (e.g. formaldehyde), complex mixtures (e.g. air pollution), occupational exposures (e.g. work in coke production), physical agents (e.g. solar radiation), biological agents (e.g. hepatitis B virus), and personal habits (e.g. tobacco smoking).

How does IARC choose which agents to evaluate?

IARC works with international experts to identify priorities from among agents suspected of causing cancer, based on the availability of scientific evidence of carcinogenicity and evidence that people may be exposed to the agent. Priority can be given to a wide variety of agents or substances with different impacts on public health. For example, air pollution has a high public health impact because everyone is exposed, even if exposure levels are generally low. On the other hand, occupational exposures, such as those involving vinyl chloride, may be very high and can therefore have a marked impact even if very few workers are exposed.

How is the evaluation carried out?

The evaluation is carried out by a Working Group of independent international experts. The experts prepare draft documents in advance, based on the available scientific evidence, and subsequently gather for eight days at IARC in Lyon to discuss and finalize their assessment of whether a specific agent causes cancer. They critically review the scientific evidence according to strict criteria, which focus on determining the strength of the available evidence that the agent causes cancer. First, the experts work in subgroups to critically review four types of data:

- The situations in which people are exposed to the agent
- Epidemiological studies on cancer in humans exposed to the agent (scientific evidence of carcinogenicity in humans)
- Experimental studies on cancer in laboratory animals treated with the agent (scientific evidence of carcinogenicity in animals)
- Studies of how cancer develops in response to the agent (scientific evidence on cancer mechanisms).

During the second part of the meeting, the entire Working Group meets together to discuss the subgroup evaluations and to combine these into overall evaluations of carcinogenicity to humans.

IARC Monographs Questions and Answers

What are the different classifications?

IARC classifies carcinogens in five categories ranging from *carcinogenic to humans* (Group 1) to *probably not carcinogenic to humans* (Group 4). The classification indicates the weight of the evidence as to whether an agent is capable of causing cancer (technically called "hazard"), **but it does not measure the likelihood that cancer will occur (technically called "risk") as a result of exposure to the agent.**

How are these classifications used? Can IARC enforce regulations based on these classifications?

Health and regulatory agencies include IARC evaluations in their consideration of actions to prevent exposure to potential carcinogens. IARC does not recommend regulations, legislation, or public health interventions, which remain the responsibility of individual governments and other international organizations.

What are the different classifications of agents?

Group 1: The agent is *carcinogenic to humans.*
This category is used when there is sufficient evidence of carcinogenicity in humans. In other words, there is convincing evidence that the agent causes cancer. The evaluation is usually based on epidemiological studies showing development of cancer in exposed humans. Agents can also be classified in Group 1 based on sufficient evidence of carcinogenicity in experimental animals supported by strong evidence in exposed humans that the agent has effects that are important for cancer development.

Group 2
This category includes agents with a range of evidence of carcinogenicity in humans and in experimental animals. At one extreme are agents with positive but not conclusive evidence in humans. At the other extreme are agents for which evidence in humans is not available but for which there is sufficient evidence of carcinogenicity in experimental animals. There are two subcategories, indicating different levels of evidence.

Group 2A: The agent is *probably carcinogenic to humans.*
This category is used when there is limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. Limited evidence means that a positive association has been observed between exposure to the agent and cancer but that other explanations for the observations (technically termed chance, bias, or confounding) could not be ruled out.

Group 2B: The agent is *possibly carcinogenic to humans.*
This category is used when there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. It may also be used when the evidence of carcinogenicity in humans does not permit a conclusion to be drawn (referred to as "inadequate" evidence) but there is sufficient evidence of carcinogenicity in experimental animals.

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Group 3: The agent is *not classifiable as to its carcinogenicity to humans.*
This category is used most commonly when the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals. Limited evidence in experimental animals means that the available information suggests a carcinogenic effect but is not conclusive.

Group 4: The agent is *probably not carcinogenic to humans.*
This category is used when there is evidence suggesting lack of carcinogenicity in humans and in experimental animals.

What does the classification mean in terms of risk?

The classification indicates the strength of the evidence that a substance or agent causes cancer. The Monographs Programme seeks to identify cancer hazards, meaning the potential for the exposure to cause cancer. However, it does not indicate the level of risk associated with exposure. The cancer risk associated with substances or agents assigned the same classification may be very different, depending on factors such as the type and extent of exposure and the strength of the effect of the agent.

What is the difference between risk and hazard?

The IARC Monographs Programme evaluates **cancer hazards but not the risks associated with *exposure.***

The distinction between *hazard* and *risk* is important. An agent is considered a cancer *hazard* if it is capable of causing cancer under some circumstances. *Risk* measures the probability that cancer will occur, taking into account the level of exposure to the agent. The Monographs Programme may identify cancer hazards even when risks are very low with known patterns of use or exposure. Recognition of such carcinogenic hazards is important because new uses or unforeseen exposures could lead to risks that are much higher than those currently seen.

What do classifications in Groups 2A and 2B mean?

Group 2A means that the agent is **probably** carcinogenic to humans. For agents in this category, there is usually convincing evidence that the agent causes cancer in laboratory animals and some evidence that it could cause cancer in humans, but the evidence in humans is not conclusive.

Group 2B means that the agent is **possibly** carcinogenic to humans. Agents can be classified in Group 2B in several different ways. Usually a classification of Group 2B means that there is convincing evidence that the agent causes cancer in experimental animals but little or no information about whether it causes cancer in humans. This category can also be used when there is some evidence that the agent could cause cancer in humans and in experimental animals but neither the evidence in humans nor the evidence in animals is convincing enough to permit a definite conclusion to be drawn.

For example, radiofrequency electromagnetic fields are classified in Group 2B because there is evidence that falls short of being conclusive that exposure may cause cancer in humans and in

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animals. *Aloe vera* leaf extract is also classified in Group 2B, based on studies showing that it causes cancer in rats, but it has not been studied in humans.

Why should two substances or agents classified in the same Group not be compared?

The classifications reflect the strength of the scientific evidence as to whether an agent causes cancer in humans but do not reflect how strong the effect is on the risk of developing cancer. The types of exposures, the extent of risk, the people who may be at risk, and the cancer types linked with the agent can be very different across agents. Therefore, comparisons within a category can be misleading. First, exposures may vary widely. For example, there is widespread exposure to the Group 1 agent air pollution, whereas far fewer people would be exposed to certain Group 1 chemicals, such as 1,2-dichloropropane. Second, the magnitude of risk associated with exposure to two agents may be very different. Active smoking carries a much higher risk of lung cancer than does air pollution, although both are categorized in Group 1. Third, the number of resulting cancers can be different; for example, tobacco smoking causes some common cancers, whereas 1,2-dichloropropane causes a rare bile duct cancer. This also applies to Group 2 agents. For example, radiofrequency electromagnetic fields and the prescription drug digoxin are each classified in Group 2B.

In other words, because the Groups indicate the strength of the evidence regarding a cancer hazard and not the risk, the risk associated with two agents classified in the same Group may be very different.

Where can I find the list of agents evaluated and their categories?

The list of agents classified by the Monographs Programme can be found on IARC's webpage:

http://monographs.iarc.fr/ENG/Classification/index.php

More information about the Monographs Programme is available at

http://monographs.iarc.fr/index.php

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American Academy of Pediatrics
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August 29, 2013

The Honorable Mignon L. Clyburn
Acting Commissioner
Federal Communications Commission
445 12th Street SW
Washington, DC 20054

The Honorable Dr. Margaret A. Hamburg
Commissioner
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Dear Acting Chairwoman Clyburn and Commissioner Hamburg:

The American Academy of Pediatrics (AAP), a non-profit professional organization of 60,000 primary care pediatricians, pediatric medical sub-specialists, and pediatric surgical specialists dedicated to the health, safety and well-being of infants, children, adolescents, and young adults appreciates this opportunity to comment on the Proposed Rule "Reassessment of Exposure to Radiofrequency Electromagnetic Fields Limits and Policies" published in the Federal Register on June 4, 2013.

In the past few years, a number of American and international health and scientific bodies have contributed to the debate over cell phone radiation and its possible link to cancer. The International Agency for Research on Cancer (IARC), part of the United Nations' World Health Organization, said in June 2011 that a family of frequencies that includes mobile-phone emissions is "possibly carcinogenic to humans." The National Cancer Institute has stated that although studies have not demonstrated that RF energy from cell phones definitively causes cancer, more research is needed because cell phone technology and cell phone use are changing rapidly. These studies and others clearly demonstrate the need for further research into this area and highlight the importance of reassessing current policy to determine if it is adequately protective of human health.

As radiation standards are reassessed, the AAP urges the FCC to adopt radiation standards that:

> **Protect children's health and well-being.** Children are not little adults and are disproportionately impacted by all environmental exposures, including cell phone radiation. Current FCC standards do not account for the unique vulnerability and use patterns specific to pregnant women and children. It is essential that any new standard for cell phones or other wireless devices be based on

protecting the youngest and most vulnerable populations to ensure they are safeguarded throughout their lifetimes.

Reflect current use patterns. The FCC has not assessed the standard for cell phone radiation since 1996. Approximately 44 million people had mobile phones when the standard was set; today, there are more than 300 million mobile phones in use in the United States. While the prevalence of wireless phones and other devices has skyrocketed, the behaviors around cell phone uses have changed as well. The number of mobile phone calls per day, the length of each call, and the amount of time people use mobile phones has increased, while cell phone and wireless technology has undergone substantial changes. Many children, adolescents and young adults, now use cell phones as their only phone line and they begin using wireless phones at much younger ages. Pregnant women may carry their phones for many hours per day in a pocket that keeps the phone close to their uterus. Children born today will experience a longer period of exposure to radio-frequency fields from cellular phone use than will adults, because they start using cellular phones at earlier ages and will have longer lifetime exposures. FCC regulations should reflect how people are using their phones today.

Provide meaningful consumer disclosure. The FCC has noted that it does not provide consumers with sufficient information about the RF exposure profile of individual phones to allow consumers to make informed purchasing decisions. The current metric of RF exposure available to consumers, the Specific Absorption Rate, is not an accurate predictor of actual exposure. AAP is supportive of FCC developing standards that provide consumers with the information they need to make informed choices in selecting mobile phone purchases, and to help parents to better understand any potential risks for their children. To that end, we support the use of metrics that are specific to the exposure children will experience.

The AAP supports the reassessment of radiation standards for cell phones and other wireless products and the adoption of standards that are protective of children and reflect current use patterns. If you have questions, please contact Clara Filice in the AAP's Washington Office at 202/347-8600.

Sincerely,

Thomas McInerny MD

Thomas K. McInerny, MD FAAP
President

TKM/cf

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Effects of short-term radiation emitted by WCDMA mobile phones on teenagers and adults

Soo Beom Choi[1,2], Min Kyung Kwon[1,2], Jai Won Chung[1,3], Jee Soo Park[4], KilSoo Chung[5] and Deok Won Kim[1,3*]

Abstract

Background: With the rapid increasing use of third generation (3 G) mobile phones, social concerns have arisen concerning the possible health effects of radio frequency-electromagnetic fields (RF-EMFs) emitted by wideband code division multiple access (WCDMA) mobile phones in humans. The number of people, who complain of various symptoms such as headache, dizziness, and fatigue, has also increased. Recently, the importance of researches on teenagers has been on the rise. However, very few provocation studies have examined the health effects of WCDMA mobile phone radiation on teenagers.

Methods: In this double-blind study, two volunteer groups of 26 adults and 26 teenagers were simultaneously investigated by measuring physiological changes in heart rate, respiration rate, and heart rate variability for autonomic nervous system (ANS), eight subjective symptoms, and perception of RF-EMFs during sham and real exposure sessions to verify its effects on adults and teenagers. Experiments were conducted using a dummy phone containing a WCDMA module (average power, 250 mW at 1950 MHz; specific absorption rate, 1.57 W/kg) within a headset placed on the head for 32 min.

Results: Short-term WCDMA RF-EMFs generated no significant changes in ANS, subjective symptoms or the percentages of those who believed they were being exposed in either group.

Conclusions: Considering the analyzed physiological data, the subjective symptoms surveyed, and the percentages of those who believed they were being exposed, 32 min of RF radiation emitted by WCDMA mobile phones demonstrated no effects in either adult or teenager subjects.

Keywords: Physiological changes, Subjective symptoms, RF-EMFs perception, Provocation, ANS, Smart phones, Teenagers

Background

With the increasing use of third generation (3 G) mobile phones, social concerns have arisen concerning the possible health effects of radio frequency-electromagnetic fields (RF-EMFs) emitted by wideband code division multiple access (WCDMA) mobile phones in humans [1]. On the basis of limited evidence from both human and animal studies, the World Health Organization (WHO) has classified RF-EMFs as possibly carcinogenic to humans (Group 2B) [2]. WHO considered the RF-EMFs provocation studies on children of different ages to be a high-priority research in the 2010 Research Agenda [3]. Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP) announced that absorption of EMF in a child's brain was greater than in an adult's brain because larger brain areas including those responsible for intellectual development were exposed in a child's brain in their resolution [4]. As a child's brain is also undergoing development and its intellectual functions are maturing, it is more susceptible to environmental hazards than an adult's brain.

Lindholm et al. [5] monitored local cerebral blood flow during exposure to Global System for Mobile Communication (GSM) mobile phone radiation in a teenager group (14 – 15 years old). They also measured electrocardiogram (ECG), blood pressure, and temperature simultaneously. They concluded that there were no significant changes during the short-term RF-EMFs exposure. Kramarenko

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and Tan recorded electroencephalogram (EEG) changes during the exposure of ten adults and ten children (12 years old) to a GSM phone. They suggested that cellular phones may reversibly influence the human brain [6]. Preece et al. [7] examined whether a standard mobile phone exposure at 902 MHz had a significant effect on cognitive function in 18 children (10 – 12 years old). There was a tendency for reaction time to be shorter during exposure to radiation than in the sham condition, but no effects reached statistical significance after the Bonferroni correction. Haarala et al. [8] investigated the potential effects of a standard 902 MHz GSM mobile phone on 10 – 14 year old children's cognitive function, and found that the mobile phone had no effect on children's cognitive function. Kwon et al. [9] investigated the effects of GSM mobile phone use on the auditory sensory memory in 17 children (11 – 12 years old). They found that a short exposure to mobile phone EMF had no statistically significant effects on the neural change-detection profile measured with mismatch negativity. Although such studies as mentioned above have examined the effects of GSM mobile phone on teenagers or children, there are a few studies investigating about the effects of WCDMA mobile phone radiation on children or teenagers.

The autonomic nervous system (ANS) plays an important role not only in physiological situations, but also in various pathological settings. Among the different available noninvasive techniques for assessing the ANS, heart rate variability (HRV), which is obtained from heart rate, has emerged as a simple and noninvasive method to evaluate the sympathovagal balance at the sinoartrial level [10]. Respiration rate is also closely associated with HRV [11]. Therefore, we selected the three physiological variables including heart rate, respiration rate, and HRV to assess ANS activity.

In this double-blind study, two volunteer groups of 26 adults and 26 teenagers who were mostly middle school students were simultaneously investigated by measuring physiological changes in heart rate, respiration rate, and HRV for ANS, eight subjective symptoms, and perception of RF-EMFs during sham and real exposure sessions. In contrast to many other studies that have examined certain aspects of physiological changes, subjective symptoms, or perception respectively, this study investigated simultaneously these three factors to more reliably examine the bio-effects of WCDMA mobile phone radiation on two groups, especially teenagers. The aim of this study was to test whether RF-EMFs affected heart rate, respiration rate, and HRV, or gave rise to subjective symptoms in adults and teenagers. We also compared the ability of adults and teenagers to perceive exposure to RF radiation. We tested the null hypothesis that adult and teenager groups would have no differences in ANS, subjective symptoms, or perception between sham and real exposures.

Methods

Participants

The experiment was performed as a double-blind study with a total of 52 subjects: 26 adults and 26 teenagers. Only healthy subjects without any diseases and not on medications were chosen for the two groups, and 14 – 17 year old subjects were selected for teenager group because the experiment was demanding and potentially stressful, we did not recruit children younger than 14 years old. We used the electromagnetic hypersensitivity (EHS) screening tool developed by Eltiti et al. [12] to exclude EHS subjects. We excluded electromagnetic hypersensitive individuals, because their conditions were more psychological than physiochemical, resulting in some possible bias in our results [13]. Moreover, we already investigated effects of WCDMA mobile phone radiation on electromagnetic hypersensitive subjects [14].

As shown in Table 1, there were no significant differences in male-to-female ratio, height, weight, body-mass index, smoking status, TV viewing time per day (hr), or mobile phone usage time per day (hr) between the two groups. Because of the different characteristics of two groups, there were significant differences in age, computer usage time per day (hr), and mobile phone usage periods (yr).

The participants were advised not to consume caffeine, smoke or exercise before the day of the experiment to minimize confounding factors. All subjects, who were recruited by advertisements at the Yonsei University Health System, in Seoul, Korea, were informed of the purpose and procedure of the experiment, and were required to give written consent to participate. The Institutional Review Board of the Yonsei University Health System approved the protocol of this study (project no: 1-2010-0030).

Experimental setup

The laboratory was used exclusively for this experiment, and all other electrical devices were unplugged except for our instruments to minimize background field levels.

Table 1 Demographics of participants

	Adult	Teenager	*P*-value
No. of subjects (n)	26	26	-
Male, female	13: 13	13: 13	0.999
Age (yr)	28.4 ± 5.1	15.3 ± 0.7	< 0.001
Height (cm)	167.1 ± 8.0	164.4 ± 7.3	0.207
Weight (kg)	59.4 ± 11.1	57.8 ± 10.4	0.590
Body mass index (kg/m^2)	21.1 ± 2.3	21.3 ± 2.8	0.796
Non-smoker: smoker	24 : 2	25 : 1	0.999
Computer usage time (hr/day)	5.3 ± 3.7	2.2 ± 2.0	0.002
TV viewing time (hr/day)	1.7 ± 1.1	1.9 ± 1.6	0.783
Mobile phone usage time (hr/day)	0.6 ± 0.5	1.3 ± 1.3	0.116
Mobile phone usage periods (yr)	11.8 ± 2.4	5.7 ± 1.9	< 0.001

Background extremely low frequency (ELF) fields at the head level in the laboratory were measured to ensure that they did not influence the subjects. The average ELF electric and magnetic fields were 1.8 ± 0.0 V/m and 0.02 ± 0.01 μT, respectively, measured using an electric and magnetic field analyzer (EHP-50C; NARDA-STS, Milan, Italy). The average RF field was 0.05 ± 0.00 V/m with a microwave frequency range from 1920 to 1980 MHz, measured using a radiation meter (SRM 3000; NARDA-STS, Pfullingen, Germany). Both the average background ELF and RF-EMFs were negligible.

To achieve better control over exposure, we used a WCDMA module with Qualcomm chipsets (baseband: MSM6290, RF: RFR6285, power management: PM6658, San Diego, CA) to generate WCDMA RF-EMFs instead of a regular smart phone. The WCDMA module continuously transmitted at a mean output power of 250 mW (24 dBm) at 1950 MHz, which was measured using a wireless communication test set (E5515C, Agilent, Santa Clara, CA). The module was inserted into a dummy phone [15], and the location of the module was varied to meet the recommended restriction in specific absorption rate $(SAR)_{1g}$ of 1.6 W/kg for general public, according to the Institute of Electrical and Electronics Engineers (IEEE) Standard [16]. The SAR measurements were made with a DASY 4 measurement system (SPEAG, Zurich, Switzerland), and a Twin SAM (specific anthropomorphic mannequin) phantom was filled with head tissue-equivalent liquid (mass density, 1000 kg/m^3) as specified by the Federal Communications Commission (FCC). The measured dielectric properties of the liquid were $\sigma = 1.41$ S/m and $\varepsilon_r = 39.7$ for the WCDMA frequency range. When the antenna of the module was positioned 67.5 mm from the ear reference point (ERP) of the dummy phone, the averaged peak spatial SAR_{1g} was determined to be 1.57 W/kg at 1950 MHz at the left cheek position [17]. The electric field and power drift at the ERP were 6.9 V/m and −0.001 dB, respectively. The measured SAR distribution is shown in Figure 1.

The module was connected via a 5 m USB cable and a USB type ammeter to a portable laptop computer (X-Note R500, LG Electronics, Seoul, Korea), which controlled the module and monitored electrical current to check exposure conditions (Figure 2). The laptop computer was remotely controlled from another outside desktop computer to satisfy the double-blind study design. The dummy phone was attached to the subject's head using an earplug and headset to fix it at the ERP next to the cheek [18]. The phone was held at a distance of 3 mm from the ear using a piece of wood for insulation to prevent battery-generated heat from providing subjects with an indication that the phone was working. The apparatus was constructed from only plastic and rubber without any metal [18,19].

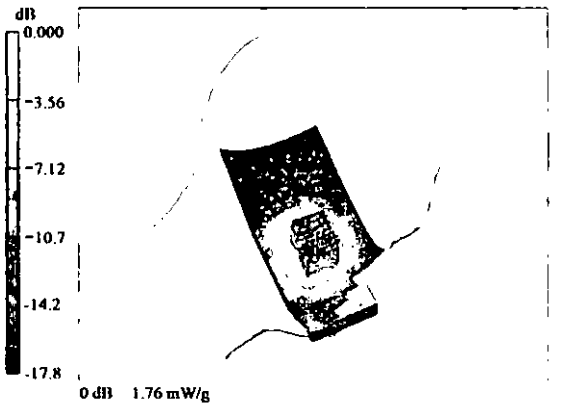

Figure 1 The measured specific absorption rate (SAR) distribution of the wideband code division multiple access (WCDMA) module on the left side.

Experimental procedures

No information was given to the subjects except that they would be asked about symptoms and RF-EMFs perception at the beginning of the first experimental day. Sham and real sessions were conducted as a double-blind test to minimize any test bias resulting from a subject and an experimenter recognizing the operational state of the WCDMA module. The experiment was performed for two days, one day for a real session and a second day for a sham session (or vice versa). No matter which came first, sham or real exposure, the second session was always conducted at approximately the same time of the day as the first session in order to maintain the subjects' physiological rhythm. The order of sham and real sessions for each subject was randomly assigned by our automatic exposure control program using MATLAB 2012b (Mathworks Inc. Natick, MA) to minimize experimental bias. The sham exposure was the first session for 14 teenagers and 15 adults. Time duration between the sessions was a minimum of one day and a maximum of 10 days.

Room temperature and relative humidity, which could considerably affect outcomes, were recorded and maintained as shown in Table 2. For the adult group, room temperature and humidity showed no significant differences between real and sham sessions. For the teenager group, room temperature and humidity showed no significant differences between real and sham sessions. For the sham sessions, room temperature and humidity showed no significant differences between adult and teenager groups. For the real sessions, room temperature and humidity showed no significant differences between adult and teenager groups.

Physiological measurements

The duration of each exposure session was 64 min, as shown in Figure 3. Before the experiments, subjects were

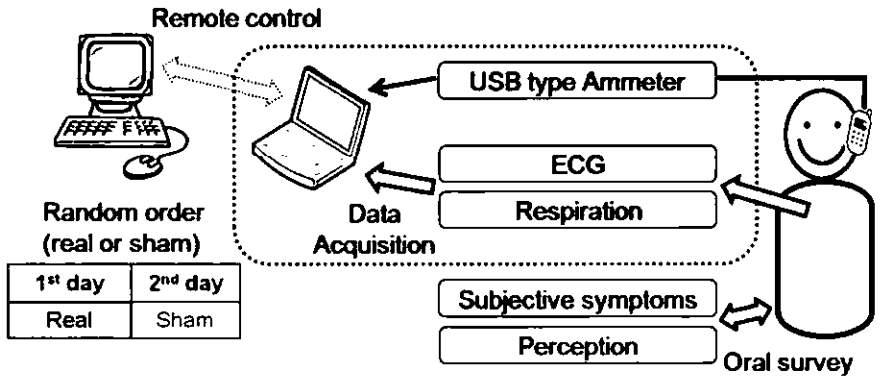

Figure 2 Block diagram of exposure setups.

instructed to rest in a sitting position for at least 10 min. Physiological data were collected for 5 min each for four different stages: pre-exposure (stage I), after 11 min of exposure (stage II), after 27 min of exposure (stage III), and post-exposure (stage IV) [14]. At each stage, ECG and respiration were simultaneously measured for 5 min because of the minimum data requirement for HRV [20]. Heart rate, respiration rate, and HRV were obtained with a computerized polygraph (PolyG-I, Laxtha, Daejeon, Korea) with a sampling frequency of 512 Hz. The data were transferred to a laptop computer (X-note R500, LG Electronics, Seoul, Korea) and analyzed using data acquisition software (Telescan 0.9, Laxtha) and analysis software (Complexity software, Laxtha). The PolyG-I recorded ECG through Ag-AgCl electrodes (2223, 3 M, St. Paul, MN) placed on both arms and the right leg of participants.

We first obtained heart rate from ECGs and then acquired HRV and the power spectrum of HRV. High-frequency power (HFP) reflects effects on respiratory sinus arrhythmia, an index of parasympathetic nerve activity, whereas low-frequency power (LFP) reflects effects on both sympathetic and parasympathetic nerves [21]. In this study, the LFP/HFP ratio was used as an index of autonomic nerve activity balance. Respiratory inductance plethysmography, with an excitation frequency of 3 MHz, was used to measure respiration rate.

Table 2 Room temperature (°C) and relative humidity (%) in the real and sham sessions for the adult and teenager groups (mean ± SD (min-max))

	Group	Real	Sham	*P*-value
Temperature	Adult	24.5 ± 0.9 (23–26)	24.5 ± 0.7 (23–26)	0.770
	Teenager	24.7 ± 0.9 (23–27)	24.6 ± 0.9 (23–27)	0.731
	P-value	0.430	0.724	
Humidity	Adult	40.5 ± 1.9 (37–45)	40.3 ± 3.2 (35–52)	0.823
	Teenager	41.8 ± 2.9 (38–50)	41.5 ± 2.9 (38–50)	0.319
	P-value	0.055	0.186	

Subjects wore a coiled band around their upper abdomen for measurement of inductance changes resulting from cross-sectional change.

Subjective symptoms and perception of RF-EMFs

The four shaded areas in Figure 3 denote periods during which subjects were questioned about eight symptoms, with each period lasting approximately 1 min. The eight subjective symptoms of throbbing, itching, warmth, fatigue, headache, dizziness, nausea, and palpitation were evaluated through verbal surveys, which were graded on a 4-point scale ranging from 1 (no sensation) to 4 (strong sensation) as suggested by Koivisto et al. [22]. In addition, perception of EMF exposure was investigated every 5 min throughout the entire session, denoted by an "o" in Figure 3. Subjects were asked to answer the question "Do you believe that you are exposed right now?" nine times during each session. Percentages of those who believed they were being exposed were calculated for pre-exposure, exposure, and post-exposure periods. The total number of inquiries was 260 (5 × 52) during real exposure and 676 (13 × 52) during non-exposure; the total number of subjects was 52 (26 + 26).

Data analysis

A repeated two-way analysis of variance (ANOVA) was performed using SPSS software (SPSS 20, SPSS, Chicago, IL) to investigate differences in heart rate, respiration rate, and LFP/HFP ratio with exposure and stage for adult and teenager groups. A P-value < 0.05 was considered statistically significant. Subjective symptoms, which are ordered paired data, were analyzed using a non-parametric Wilcoxon signed-rank test. A total of 64 P-values (4 stages × 8 symptoms × 2 groups) were obtained for the real and sham exposure sessions for the eight symptoms at four stages in both groups. The significance level was adjusted to 0.0125 (0.05/4) because testing was performed in four stages.

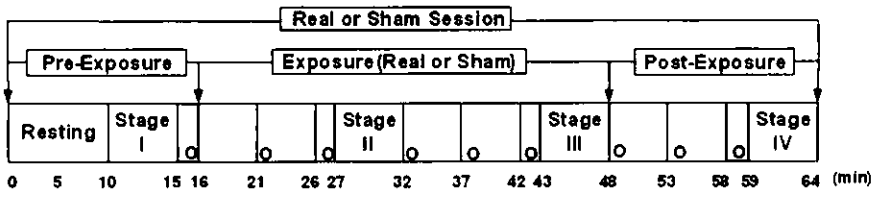

Figure 3 Experimental procedures for measuring physiological changes and investigating symptoms and perception. ECG and respiration were measured for 5 min each for four stages. The four shaded areas are periods in which the subjects were questioned about the eight symptoms "o" indicates timing of the inquiries about RF-EMF perception during each session.

There were two exposure sessions for each participant, and nine perception inquiries for each session, as shown in Figure 3. For each session, there was one inquiry during pre-exposure, five inquiries during sham or real exposure, and three inquiries during post-exposure. In both groups, the percentages of those who believed they were being exposed were obtained and evaluated for significant differences between real and sham sessions using McNemar's test. The pre-exposure period (first inquiry) of the sham sessions was compared with that of the real sessions to test whether conditions before sham and real exposures of subjects were the same. The sham exposure period was compared with the real exposure period to test whether the subjects could detect the fields (second through sixth inquiries). The post-exposure period after sham exposure was compared with the post-exposure period after real exposure to test whether the real exposure influenced the perception of exposure in the post-exposure period (seventh through ninth inquiries).

The significance level of the exposure period was adjusted to 0.01 (0.05/5), and that of the post-exposure period was adjusted to 0.017 (0.05/3) because testing was for five and three inquiries. Fisher's exact test was applied to evaluate differences in the percentages of those who answered "yes", which were nominal data, between the adult and teenager groups for sham and real exposure sessions. Fisher's exact test was used because the expected values in any cells in the contingency table were below 5.

Results

Physiological variables

Heart rate, respiration rate, and LFP/HFP ratios of the adult and teenager groups during real and sham exposures are shown in the top section of Table 3. A repeated two-way ANOVA showed no significant differences in heart rate or respiration rate for stage or exposure in either group. However, LFP/HFP ratios showed significant differences by stage in both groups, as shown in the bottom of Table 3. Therefore, a Bonferroni post hoc test was done after two-way ANOVA to investigate any differences in LFP/HFP ratios between stages for each group. For the adult group, LFP/HFP showed no significant difference between real and sham exposures ($P = 0.307$), but did show a significant difference among stages ($P = 0.033$). For the teenager group, LFP/HFP was not significantly different between real and sham exposures ($P = 0.661$), but was significantly different among stages ($P = 0.002$).

Subjective symptoms and perception percentages

Neither the adult nor the teenager group showed significant differences in any of the eight subjective symptoms surveyed (throbbing, itching, warmth, fatigue, headache, dizziness, nausea, and palpitation) between sham and real sessions in any of the four stages (Additional file 1: Table S1 and S2).

Table 4 shows the percentages of subjects who believed they were being exposed during exposure (real or sham) in the adult and teenager groups. We compared the percentages of those perceiving exposure during real and sham exposure period (second through sixth inquiries) using McNemar's test and found no significant difference between real and sham exposure period in the adult or teenager groups. To test for delayed effects of real exposure on post-exposure perception (seventh through ninth inquiries), we applied the same test and found no significant difference in the percentages of those who believed they were being exposed following real and sham exposures in the adult ($P = 0.999$ at all three inquiries) or teenager ($P = 0.500$, $P = 0.999$, $P = 0.999$) groups. Also, no significant difference was seen during pre-exposure period (first inquiry) between real and sham exposures in teenager ($P = 0.999$) group, indicating that the conditions experienced by subjects before real and sham exposures were the same. For adult group, we could not perform McNemar's test because no one answered "yes" in pre-exposure period. Similarly, a chi-square test for trend showed that the percentages of those who believed they were being exposed during pre-exposure, sham exposure, and post-exposure were not significantly different in the adult ($P = 0.440$) or teenager ($P = 0.195$) groups. This demonstrated that conditions could not be distinguished for participants throughout sham-exposure sessions.

Figure 4 shows the percentages of participants in the adult and teenager groups for each inquiry number who believed they were being exposed in sham (Figure 4A) and real (Figure 4B) exposure sessions. No significant

Table 3 Descriptive and statistical tests for heart rate, respiration rate, and LFP/HFP ratio among stage, exposure, and interaction

	Heart rate (bpm)				Respiration rate (bpm)				LFP/HFP ratio			
	Adult		Teenager		Adult		Teenager		Adult		Teenager	
	Sham	Real	Sham	Real	Sham	Real	Sham	Real	Sham	Real	Sham	Real
Stage	Mean (standard error)											
I	76.6 (2.1)	79.1 (1.9)	79.3 (2.1)	80.9 (1.7)	18.0 (0.5)	18.3 (0.5)	19.3 (0.5)	19.2 (0.4)	1.9 (0.3)	2.3 (0.4)	1.6 (0.3)	1.5 (0.3)
II	76.6 (2.1)	77.9 (1.7)	79.8 (1.8)	80.4 (1.6)	18.2 (0.4)	18.1 (0.6)	19.3 (0.5)	19.3 (0.6)	2.6 (0.4)	3.1 (0.7)	1.7 (0.3)	2.0 (0.4)
III	75.4 (2.0)	77.5 (1.7)	80.7 (1.8)	80.7 (1.7)	18.4 (0.5)	18.2 (0.5)	19.2 (0.5)	19.8 (0.5)	2.3 (0.3)	3.6 (1.0)	2.6 (0.5)	1.9 (0.3)
IV	76.5 (2.1)	77.1 (1.7)	81.2 (1.6)	81.0 (1.7)	18.2 (0.5)	17.9 (0.6)	19.7 (0.5)	20.3 (0.5)	3.2 (0.7)	2.9 (0.6)	2.3 (0.5)	2.3 (0.4)
Factor	*P*-value (F - statistic)											
Exposure	0.328 (0.997)		0.671 (0.184)		0.843 (0.040)		0.433 (0.635)		0.307 (1.088)		0.661 (0.197)	
Stage	0.211 (1.644)		0.323 (1.180)		0.677 (0.510)		0.067 (2.481)		0.033* (3.723)		0.002* (5.492)	
Interaction (exposure and stage)	0.324 (1.168)		0.209 (1.600)		0.633 (0.575)		0.444 (0.903)		0.267 (1.350)		0.222 (1.562)	

*$P < 0.05$, bpm; beats per min.
LFP/HFP ratio; low-frequency power/high-frequency power (power spectrum of heart rate variability).
Stage I; pre-exposure, Stage II; after 11 min of exposure, Stage III; after 27 min of exposure, Stage IV; post-exposure.

differences were seen between the adult and teenager groups in all inquiries during sham or real exposure session. Even though both groups showed low percentages of belief of being exposed during the sham exposure period (Figure 4A), they also showed low percentages during the real exposure period (Figure 4B). In summary, Table 4 shows no significant difference in perception percentages between real and sham exposure period in the adult or teenager groups. Figure 4 also shows no significant difference between the adult and teenager groups in sham or real exposure period. Therefore, we concluded that neither the adult nor the teenager group correctly perceived the RF-EMFs considering Table 4 and Figure 4.

Discussion

Neither the adults nor the teenagers showed significant differences in heart or respiration rate between real and sham exposures or among stages. For LFP/HFP, however, significant differences were seen between some stages during both real and sham exposure sessions in both groups. One disadvantage of the LFP/HFP analysis is that it is considerably influenced by stress, which can increase or decrease LFP/HFP [23]. Hjortskov et al. [24] reported that psychological stress could result in an increased LFP/HFP. Nam et al. [25] reported that LFP/HFP monotonically increased at each stage during 30 min of sham exposure in both EHS and non-EHS groups. In this experiment, one of the potential sources of stress was the requirement that the subjects not move during the 64-min experiment. In fact, the "no-movement" requirement was the factor that drew the most complaints from the participants, especially the teenagers. Therefore, the significant increase in LFP/HFP with time in the real and sham exposure sessions of both groups must have resulted from factors other than field exposure such as psychological stress, anxiety, or environmental factors.

For the eight subjective symptoms attributed to WCDMA mobile phone radiation, neither the adult group nor the teenager group showed significant differences between sham and real exposures in any of the four stages. Cinel et al. [26] found no evidence suggesting that exposure to mobile phone RF-EMFs affected subjective symptoms. Koivisto et al. [22] also reported that the RF-EMFs exposure did not produce any consistent subjective symptoms

Table 4 percentages of those who believed they were being exposed during sham and real exposure period, and *P*-values for sham and real exposures in adult and teenager groups

Group	Session	Exposure									
		2nd		3rd		4th		5th		6th	
		Mean (%)	*P*-value	Mean (%)	*P*-value	Mean (%)	*P*-value	Mean (%)	*P*-value	Mean (%)	*P*-value
Adult (n = 26)	Sham	7.7	0.999	7.7	0.999	3.8	0.999	7.7	0.999	3.8	0.999
	Real	3.8		3.8		7.7		7.7		7.7	
Teenager (n = 26)	Sham	7.7	0.999	0.0	0.250	3.8	0.250	11.5	0.999	7.7	0.999
	Real	7.7		11.5		15.4		11.5		11.5	

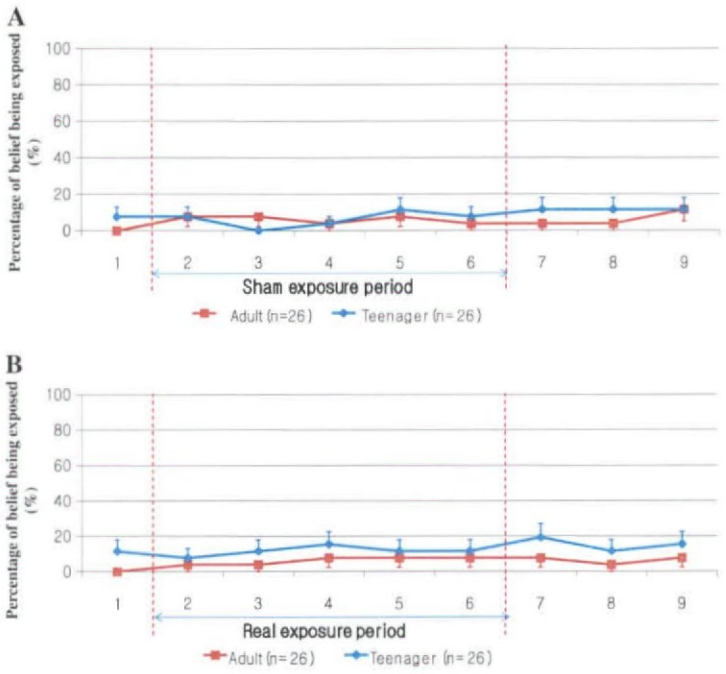

Figure 4 Percentages who believed they were being exposed at nine inquiry points in adult and teenager groups for sham (A) and real (B) exposure sessions. Bars indicate standard errors.

or sensations such as headache, dizziness, and fatigue in the non-EHS subjects. In conclusion, RF-EMFs did not give rise to subjective symptoms in adults or teenagers in this study.

No significant differences were seen in the percentages of participants who believed they were being exposed between the real and sham exposures in either the adult or the teenager group. Kwon et al. [27] reported that they found no evidence that their 84 participants perceived GSM mobile phone EMFs. All participants, even including six subjects with high self-rated sensibility, were not able to perceive mobile phone EMFs. No significant differences in percentages of perception were seen for either group among participants who believed they were being exposed during either pre-exposure or post-exposure periods between real and sham exposures. Also, no significant differences were observed in the percentages of perception for either the adult or teenager groups during sham exposure sessions (pre-exposure, sham exposure, post-exposure). Therefore, our experimental protocol appeared to be minimally biased since we confirmed no delayed effects, no differences in pre-exposure condition, and no difference in the percentages of those who believed they were being exposed during the pre-exposure, sham exposure, and post-exposure periods. In this study, the subjects had only two choices, "yes" or "no", to the perception inquiry of RF-EMFs. However, it could have been biased against subjects who were not sure. For future study, it is recommended to give subjects another choice, "unsure", and to exclude the answer in calculating the perception accuracy.

Children are more preferable to teenagers as participants in this study because the former are more vulnerable than the latter [28]. However, it is difficult for children due to stress to participate in our experiment, which needs a "no-movement" requirement for approximately one hour. It is also difficult to recruit children because of difficulty in obtaining parents' approval. We finally recruited teenagers as the second best. Those are the reasons why there are only a few provocation studies with children. Croft et al. [15] measured alpha activity for both GSM and WCDMA exposure among adolescents, young adults, and elderly groups. They reported an effect of GSM exposure in young adults, but observed no effect in adolescents or the elderly, or in any age group, as a function of WCDMA exposure. This result for WCDMA exposure is consistent with ours, even though they examined brain activity and we did heart rate.

There are three limitations in this study. The first limitation is the small number of participants. The number of 26 adults and 26 teenagers may not be to conclude that there are no effects of radiation emitted by WCDMA in both adults and teenagers. Moreover, any effect of WCDMA mobile phone radiation on the autonomic system might be quite limited and difficult to detect. Therefore, to draw some more definitive conclusions on this, a much larger sample will be needed. Secondly, in our study,

more subjects received sham exposure for the first session. Ideally, the same number for each session would be better. However, the skewness is small and probably makes no difference. Lastly, we did not investigate the effects of the repetitive and daily regular exposure to RF radiation emitted by WCDMA mobile phones, which could be hazardous to teenagers, as well as adults. Therefore, further study on repetitive and daily regular exposure is necessary to examine the long-term effects, especially on teenagers.

Conclusions

In both adults and teenagers, there were no significant differences in heart rate, respiration rate, or LFP/HFP, which are all related to ANS, between sham and real exposure to a WCDMA module (average power, 24 dBm at 1950 MHz; specific absorption rate, 1.57 W/kg) for 32 min. There was no association between eight subjective symptoms and short-term RF-EMFs exposure in either group. We could not find evidences of the hypothesis that the self-perception of the exposure between two groups was different. Therefore, based on our physiological data, survey of subjective symptoms, and percentages of participants who believed they were being exposed, no effects were observed in teenagers or adults as a result of 32 min exposure to RF radiation emitted by WCDMA mobile phones.

Additional file

Additional file 1: Table S1. Eight subjective symptoms of the each stage for the real and sham sessions in the adult group. **Table S2.** Eight subjective symptoms of the each stage for the real and sham sessions in the teenager group

Abbreviations

ANOVA: Analysis of variance; ANS: Autonomic nervous system; ECG: Electrocardiogram; EEG: Electroencephalograms; EHS: Electromagnetic hypersensitivity; ELF: Extremely low frequency; EMF: Electromagnetic field; ERP: Ear reference point; FCC: Federal Communications Commission; GSM: Global System for Mobile Communications; HFP: High-frequency power; HRV: Heart rate variability; IEEE: Institute of Electrical and Electronics Engineers; LFP: Low-frequency power; max: Maximum; min: Minimum; n: Number; RF-EMFs: Radio frequency-electromagnetic fields; RNCNIRP: Russian National Committee on Non-Ionizing Radiation Protection; SAR: Specific absorption rate; SD: Standard deviation; WCDMA: Wideband code division multiple access; WHO: World Health Organization; yr: Year; 3 G: Third generation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SBC set up the WCDMA module and collected experimental data. MKK performed statistical analyses, and JWC, JSP and KSC recruited the subjects and collected experimental data. DWK contributed to the development of the study protocol and editing of the manuscript. All authors read and approved the final manuscript.

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References

1. Heinrich S, Thomas S, Heumann C, von Kries R, Radon K: **Association between exposure to radiofrequency electromagnetic fields assessed by dosimetry and acute symptoms in children and adolescents: a population based cross-sectional study.** *Environ Health* 2010, 9:75.
2. Baan R, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Islami F, Galichet L, Straif K: **Carcinogenicity of radiofrequency electromagnetic fields.** *Lancet Oncol* 2011, 12:624–626.
3. World Health Organization: *WHO research agenda for radio frequency fields*; 2010 [http://www.who.int/emf] Accessed August 29, 2013.
4. Grigoriev Y, Nikitina V, Grigoriev O: *Resolution 'Electromagnetic fields from mobile phones. Health effect on children and teenagers'* Moscow: Russian National Committee on Non-Ionizing Radiation Protection; 2011.
5. Lncholm H, Alanko T, Rintamaki H, Kännälä S, Toivonen T, Sistonen H, Tikkaja M, Halonen J, Mäkinen T, Hietanen M: **Thermal effects of mobile phone RF fields on children: a provocation study.** *Prog Biophys Mol Biol* 2011, 107:399–403.
6. Kramarenko AV, Tan U: **Effects of high-frequency electromagnetic fields on human EEG: a brain mapping study.** *Int J Neurosci* 2003, 113:1007–1019.
7. Preece AW, Goodfellow S, Wright MG, Butler SR, Dunn EJ, Johnson Y, Manktelow TC, Wesnes K: **Effect of 902 MHz mobile phone transmission on cognitive function in children.** *Bioelectromagnetics* 2005, 26(Suppl 7):138–143.
8. Haarala C, Bergman M, Laine M, Revonsuo A, Koivisto M, Hämäläinen H: **Electromagnetic field emitted by 902 MHz mobile phones shows no effects on children's cognitive function.** *Bioelectromagnetics* 2005, 26(Suppl 7):144–150.
9. Kwon MS, Huotilainen M, Shestakova A, Kujala T, Näätanen R, Hämäläinen H: **No effects of mobile phone use on cortical auditory change-detection in children: an ERP study.** *Bioelectromagnetics* 2010, 31:191–199.
10. Szajzel J: **Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system.** *Swiss Med Wkly* 2004, 134:514–522.
11. Poyhonen M, Syvaoja S, Hartikainen J, Ruokonen E, Takala J: **The effect of carbon dioxide, respiratory rate and tidal volume on human heart rate variability.** *Acta Anaesthesiol Scand* 2004, 48:93–101.
12. Eltiti S, Wallace D, Zougkou K, Russo R, Joseph S, Rasor P, Fox E: **Development and evaluation of the electromagnetic hypersensitivity questionnaire.** *Bioelectromagnetics* 2007, 28:137–151.
13. Rubin GJ, Das Munshi J, Wessely S: **Electromagnetic hypersensitivity: a systematic review of provocation studies.** *Psychosom Med* 2005, 67:224–232.
14. Kwon MK, Choi JY, Kim SK, Yoo TK, Kim DW: **Effects of radiation emitted by WCDMA mobile phones on electromagnetic hypersensitive subjects.** *Environ Health* 2012, 11:69.
15. Croft RJ, Leung S, McKenzie RJ, Loughran SP, Iskra S, Hamblin DL, Cooper NR: **Effects of 2 G and 3 G mobile phones on human alpha rhythms: Resting EEG in adolescents, young adults, and the elderly.** *Bioelectromagnetics* 2010, 31:434–444.
16. IEEE Standard 1528–2003: *Recommended Practice for Determining the Peak Spatial-Average Specific Absorption Rate (SAR) in the Human Head From Wireless Communications Devices: Measurement Techniques.* IEEE Standard Coordinating Committee 34; New York; 2003 [https://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=1259064]
17. Beard BB, Kainz W, Onishi T, Iyama T, Watanabe S, Fujiwara O, Wang J, Bit-Babik G, Faraone A, Wiart J, Christ A, Kuster N, Ae-kyoung L, Kroeze H, Siegbahn M, Keshvari J, Abrishamkar H, Simon W, Monteuffel D, Nikoloski N: **Comparisons of computed mobile phone induced SAR in the SAM phantom to that in anatomically correct models of the human head.** *IEEE Trans Electromagn Compat* 2006, 48:397–407.

18. Haarala C, Takio F, Rintee T, Laine M, Koivisto M, Revonsuo A, Hamalainen H: **Pulsed and continuous wave mobile phone exposure over left versus right hemisphere: effects on human cognitive function.** *Bioelectromagnetics* 2007, **28**:289–295.
19. Unterlechner M, Sauter C, Schmid G, Zeitlhofer J: **No effect of an UMTS mobile phone-like electromagnetic field of 1.97 GHz on human attention and reaction time.** *Bioelectromagnetics* 2008, **29**:145–153.
20. Marek MJ, Thomas BA, John C, Robert LK, Alberto M, Arthu JM, Peter JS: **Heart rate variability: standards of measurement, physiological interpretation, and clinical use.** *Eur Heart J* 1996, 17:354–381.
21. Parazzini M, Ravazzani P, Tognola G, Thuroczy G, Molnar FB, Sacchettini A, Ardesi G, Mainardi LT: **Electromagnetic fields produced by GSM cellular phones and heart rate variability.** *Bioelectromagnetics* 2007, **28**:122–129.
22. Koivisto M, Haarala C, Krause CM, Revonsuo A, Laine M, Hamalainen H: **GSM phone signal does not produce subjective symptoms.** *Bioelectromagnetics* 2001, **22**:212–215.
23. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ: **Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control.** *Science* 1981, **213**:220–222.
24. Hjortskov N, Rissen D, Blangsted AK, Fallentin N, Lundberg U, Sogaard K: **The effect of mental stress on heart rate variability and blood pressure during computer work.** *Eur J Appl Physiol* 2004, **92**:84–89.
25. Nam KC, Kim SW, Kim SC, Kim DW: **Effects of RF exposure of teenagers and adults by CDMA cellular phones.** *Bioelectromagnetics* 2006, **27**:509–514.
26. Cinel C, Russo R, Boldini A, Fox E: **Exposure to mobile phone electromagnetic fields and subjective symptoms: a double-blind study.** *Psychosom Med* 2008, **70**:345–348.
27. Kwon MS, Koivisto M, Laine M, Hamalainen H: **Perception of the electromagnetic field emitted by a mobile phone.** *Bioelectromagnetics* 2008, **29**:154–159.
28. Kheifets L, Repacholi M, Saunders R, Van Deventer E: **The sensitivity of children to electromagnetic fields.** *Pediatrics* 2005, **116**:e303–e313.

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The Contribution of In Vivo Mammalian Studies to the Knowledge of Adverse Effects of Radiofrequency Radiation on Human Health

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Abstract

The proliferation of cellular antennas and other radiofrequency radiation (RFR) generating devices of the last decades has led to more and more concerns about the potential health effects from RFR exposure. Since the 2011 classification as a possible carcinogen by the International Agency for Research on Cancer (IARC), more experimental studies have been published that support a causal association between RFR exposure and health hazards. As regard cancer risk, two long-term experimental studies have been recently published by the US National Toxicology Program (NTP) and the Italian Ramazzini Institute (RI). Despite important experimental differences, both studies found statistically significant increases in the development of the same type of very rare glial malignant tumors. In addition to carcinogenicity, reproductive organs might be particularly exposed, as well as sensitive to RFR. In this work, we reviewed the currently available evidence from in vivo studies on carcinogenicity and reproductive toxicity studies in order to summarize the contribution of experimental research to the prevention of the adverse effects of RFR on human health.

Keywords: radiofrequency radiation, in vivo experimental studies, carcinogenicity, reproductive/developmental toxicity

1. Introduction

Since mobile phone usage has become an integral part of everyday life for the vast majority of the population, unprecedented human exposure to radiofrequency radiation (RFR) from conception until death has been occurring in the last two decades. Consequently, there is an increasing public interest in the possible health risks derived from mobile phone use and base station-related exposure.

RFR, which includes radio waves and microwaves, correspond to 30 kHz–300 GHz the electromagnetic spectrum. RFR has enough energy to move atoms in a molecule around or cause them to vibrate, but not enough to ionize (to detach electrons from atoms or molecules), which is, therefore,

known as non-ionizing radiation. The important properties of non-ionizing radiation include the frequency at which it is generated, measured in megahertz (MHz) or gigahertz (GHz), and the intensity of the waves, or the specific absorption rate (SAR), which is the rate of energy absorption per unit mass of biological tissue [1]. RFR can cause tissue heating when having sufficient intensity, which is the principle of the microwave oven functioning. Given the ability of RFR to heat tissues, the toxic effects of RFR are often pointed to thermal effects only.

Among the effects induced by RFR, tissue heating is a well-established and biologically plausible mechanism: When the RFR exposure occur at levels high enough, the absorption of energy by a biological system could overcome its capability to regulate the body temperature. Assuming the phones are not emitting more than permitted, typical human exposures to RFR occur at intensities that are not capable of inducing significant tissue heating if devices are used according to the manufacturers' recommendations for use.

Those biological changes occurring when body temperature increase is below 1 °C are referred to as nonthermal RFR effects. Temperature variations within the range of 1 °C are considered as thermal noise [2]. There is an ongoing debate regarding whether nonthermal biological effects can occur as a result of exposures to low-intensity RFR. Even though some authors have suggested that exposure to low-intensity RFR would not be able to induce significant biological effects through a plausible non-thermal mechanism [3,4,5], numerous are the studies that associate specific biological effects to RFR exposures at levels considered below those expected to result in a measurable amount of tissue heating. The mechanisms of interaction between living organisms and RFR have not yet been well characterized, but several mechanisms have been proposed, besides tissue heating; among these are included the induction of ferromagnetic resonance, the alteration of ligand binding to hydrophobic sites in receptor proteins, and above all the most plausible is the forced-oscillation of free ions in all biological cells resulting in irregular gating of voltage-gated ion channels in cell membranes [6]. Moreover, exposure to low levels of RFR can cause small temperature changes in localized areas of exposed tissues leading to conformational changes in temperature-sensitive proteins and inducing the expression of heat-shock or stress-response proteins [2].

In 2011 the International Agency for Research on Cancer (IARC), part of the WHO, declared RFR to be "possibly carcinogenic to humans", group 2B [2,7], basing primarily on limited evidence in humans that long-term users of mobile phones held to the head resulted in an elevated risk of developing brain cancer, and limited evidence from animal studies that RFR exposure lead to cancer.

One of the biggest concerns is that RFR might have reproductive and developmental adverse effects, in particular, disturbing testicular function and altering sperm parameters. It is indeed probable that the penetration of RFR into the testis may be more pronounced than other tissues, given the lower protection of this organ by tissue, in comparison to others. It is well known that the temperature of the testicles is 2 °C to 3 °C lower than the rectal one, and the right temperature for spermatogenesis is considered to be 35 °C [8]. The habit of keeping the cellphone in the trouser pocket or the prolonged use thereof may have an impact in generating hyperthermia of the scrotum, as well as oxidative stress, which represent the main damage generation mechanisms [9], besides non-thermal effects.

This review aims to address the current knowledge of both the carcinogenic and the reproductive/developmental hazards of RFR emerged from in vivo experimental studies. Firstly, cancer bioassays have been reviewed. Based on the animal model, reviewed articles were separated by paragraphs in studies on rats, mice and other models (including trans-genic models). Those experimental studies in which wild type or transgenic/tumor-prone strains of rats and mice were subjected to RFR long-term exposure of at least one year were taken into consideration. Those experiments with an exposure duration beneath the 12 months were deliberately excluded, since they can not be considered reliable carcinogenicity studies [10].

Secondly, apical endpoints investigated in reproductive and developmental toxicity peer-reviewed studies were used for PubMed selection of relevant in vivo animal studies. Hence, those experiments in which the authors evaluated the effect of RFR exposure towards reproductive system health were reviewed. Based on the animal model, reviewed articles were separated by paragraphs in studies on rats, mice and other mammalian models.

2. Cancer-Related In Vivo Investigations

Since billions of people are exposed to the potential carcinogenic risks of RFR, studies in laboratory animals must be as sensitive as possible for really being informative. The Organization for Economic Co-operation and Development (OECD) and the NTP have drawn up specific guidelines for the conduction of carcinogenicity studies [11,12]. Among the specifications for design and conduct of experimental studies to evaluate carcinogenic potential of xenobiotics, such as the physical agent RFR, are in example the following: (1) Each dose group and concurrent control group should contain at least 50 animals of each sex, (2) at least three dose levels should be used (in addition to the concurrent control group), and (3) the period of dosing and duration of the study should be of at least 24 months [10].

2.1. Rats

Among rat studies, La Regina et al. (2003), using a carousel system, tube-exposed Fischer 344 (F344) rats for 4 h/day, 5 days/week, for 24 months. Two different types of RFR (835.62 MHz FDMA, 847.74 MHz CDMA) at one brain SAR level of 1.3 ± 0.5 W/kg each were applied to the animals. Each group (2 RFR and 1 sham) consisted of 160 rats (80/80). No significant differences between treated and sham-exposed animals were found in the incidence of any spontaneous tumors [13].

Anderson et al. in 2004 exposed F344 rats to 1.6 GHz RFR. Animals were divided into three groups of treatment: One group was sham exposed, and two groups were subjected to a far-field RFR Iridium signal. Exposures started prenatally at levels resulting in fetal brain SAR of 0.16 W/kg. After parturition, 90 restrained animals per sex and per group underwent 2 h/day, head-first, near-field exposures for 5 days/week until the rats were two years old, with calculated levels of brain SAR corresponding to 0.16 W/kg and 1.6 W/kg and near-field sham controls. It was not observed any statistically significant difference among the three experimental groups as for the incidence of neoplastic lesions [14].

In 2007, Smith et al. divided 1170 Han Wistar rats among 65 male and 65 female per group, exposing the animals for 2 h/day, 5 days/week for up to 24 months at three nominal SARs of 0.44, 1.33, and 4.0 W/kg to Global System for Mobile Communications (GSM) or Digital Cellular System (DCS) wireless communication signals. No adverse reaction was observed following exposure to different levels of both the signals. Particularly, except for those results that the authors reputed as isolated, trivial observations not related to the treatment, this study did not report any statistically significant finding in the incidence, multiplicity, latency, or type of any primary cancers that can be attributable to RFR neither in male nor in female rats [15].

Also, five two-years promotional cancer studies involved promotion of N-Ethylnitrosourea (ENU)-induced cancer, four in F344 rats and one in SD rats [16,17,18,19,20]. RFR carrier frequencies ranged from 836 to 1950 MHz with different modulations. Nevertheless, none showed an increase nor in ENU-initiated brain cancer promotion nor any other statistically significant observation. It should be noted that in all the five studies, a carousel system to restrain rats was used, and this likely have presented a stress factor, complicating the interpretation of the results.

Among the studies conducted in Sprague-Dawley (SD) rats, in 1992 Chou et al. exposed 200 SD rats for 25 months, 21.5 h per day, to pulse modulated 2450-MHz RFR at 0.144–0.4 W/kg of whole body SARs. Exposed animals showed a statistically significant increase in the incidence of primary malignant tumors, compared to the same number (n = 200) of sham-exposed rats. Among those neoplasms found in the exposed rats were thyroid cancer and malignant lymphoma. The importance of these results is given by the fact that the thyroid gland is one of the most RFR-exposed organs during mobile phone usage, especially during a call [21].

The study by the US National Toxicology Program (NTP) was the largest rodent bioassay carried out by this U.S. government Institution. SD rats were exposed to RFR in special chambers for up to two years, with exposure began in the womb. The RFR exposure was intermittent, 10 min on and 10 min off, for a total of about 9 h a day, with exposure levels of 1.5, 3, and 6 W/Kg/bw. Rats were exposed at a frequency of 1900 MHz to total body RFR from two technologies, CDMA and GSM [22].

The Ramazzini Institute (RI) study was the largest long-term bioassay ever performed exploring the health effects of RFR, comprising 2448 rats. The whole-body exposure for 19 h/day of male and female SD rats to a 1.8 GHz GSM far field of 0, 5, 25, 50 V/m, started prenatally and lasted until natural death [23].

NTP doses were established to mimic the localized exposure on body tissues from a cell phone placed near the body—and were, therefore, particularly higher than those used by the RI, which were, instead, similar to those found in our living and working environment to mimic the full-body human exposure generated by mobile telephony base antennas. Despite these differences, recently, both the studies found statistically significant increases in the development of the same type of very rare glial malignant tumors [22,23].

During the use of cordless and handheld mobile phones, the brain is the main target of RFR. An increase in gliomas of the brain, the same tumor found in people after long-term cell phone use, was observed in both NTP and RI studies, although a statistically significant increase was observed only by NTP. The results published by the RI highlighted a statistically significant increased incidence of a very rare glial tumor of the heart, the Schwannoma, in male rats treated at the highest dose (50 V/m). This is the same type of tumors found to be increased by NTP using far higher exposure levels, tumor involving the same hystotype of the acustic nerve (vestibular) neurinoma observed in humans after intensive mobile phone use in epidemiological studies [2]. Both in the NTP and in the RI studies, the increase in the risk of Schwannomas was low.

In the NTP study, an increased number of male rats bearing adrenal gland tumors was also considered to be related to exposure. The RI publication only documents brain and heart findings. Data from the other organs are about to be published.

The studies discussed in this section are summarized in Table 1.

Table 1

Studies of carcinogenicity in rats exposed at least two years to radiofrequency radiation (RFR).

Strain, Species (Sex) Duration Reference	RFR Exposure Levelfrequencies, Intensities (Any Other Co-Exposure)	Exposure Time No. of Animals	Increased Tumor Incidence (Significance)
Fischer 344 rats (M, F) 24 months La Regina et al., (2003)	835.62 MHz FDMA, 847.74 MHz CDMA 1.3 ± 0.5 W/kg	4 h/day, 5 days/week 80/sex/group	Not any increased tumor incidence (NS)
Fischer 344 rats (M, F) 24 months Anderson et al., (2004)	1600 MHz iridium signal Prenatal brain SAR (fetuses): 0.16 W/kg Brain SAR: 0.16 W/kg, 1.6 W/kg	2 h/day, 5 days/week 90/sex/group	Not any increased tumor incidence (NS)
Fischer 344 rats (M, F) 24 months Smith et al., (2007)	900, 1800 MHz (GSM, CDS) 0.44, 1.33, and 4.0 W/kg	2 h/day, 5 days/week 65/sex/group	Not any increased tumor incidence (NS)
Sprague-Dawley rats (M) 25 months Chou et al., (1992)	2450 MHz pulse modulated 0.144–0.4 W/kg	21.5 h/day, 7 days/week 200/group	Total primary cancers malignant lymphoma thyroid cancer ($p < 0.05$)
Sprague-Dawley rats (M, F) Before birth trough 24 months Wyde et al., (2016)	900 MHz (GSM, CDMA) 1.5, 3, 5 W/kg	9 h/day, 7 days/week 105/sex/group	Male brain glioma and heart Schwannoma ($p < 0.05$)
Sprague-Dawley rats (M, F) Before birth trough spontaneous death Falcioni et al., (2018)	1800 MHz (GSM) 0.1 W/Kg, 0.03 W/Kg, 0.001 W/Kg	19 h/day, 7 days/week Groups I,II: 400/sex/group Groups III, IV: 200/sex/group	Male heart Schwannoma ($p < 0.05$) and female brain glioma (NS)

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M, males; F, female; h, hour(s); NS, not significant.

2.2. Mice

In addition to the study conducted in SD rats, NTP also conducted a carcinogenesis study in B6C3F1/N mice. Animals were located in special chambers and treated with RFR for up to two years, with exposure began at 5–6 weeks of age. The RFR exposure was intermittent, 10 min on and 10 min off, for a total of about 9 h a day, with exposure levels of 2.5, 5 m and 10 W/Kg. The whole body of the mice were exposed to RFR at a frequency of 1900 MHz, from two technologies, CDMA and GSM. In male or female mice, exposure to cell phone RFR did not significantly increase the incidence of any neoplastic lesions [24].

Using B6C3F1 mice, Tillmann et al. (2007) experimentally evaluated the possible carcinogenic effects of RFR at 902 and 1747 MHz, respectively of the GSM and of the DCS standards. Mice were restrained and exposed over a period of 2 years for 2 h/day, 5 days/week to three different whole-body averaged SAR levels of 0.4, 1.3, 4.0 W/Kg body weight, or were sham exposed. No statistically significant increase in the incidence of any particular tumor type was observed in the RF exposed groups as compared to the sham exposed group [25].

RFR cocarcinogenic effect was explored in the 2010 tumor promotion study by the same authors. Mice were exposed for two years to Universal Mobile Telecommunications System (UMTS) fields at intensities of 0 (sham), 4.8, and 48 W/m^2. The low-dose group (4.8 W/m^2) was subjected to additional prenatal ethylnitrosourea (ENU) treatment of 40 mg/kg body weight, and showed an increase in the rate of lung tumor and in the incidence of lung carcinomas in comparison with the control group treated only with ENU [26].

Similar results were obtained from a follow-up study in which mice were treated with RFR at SAR levels of 0 (sham), 0.04, 0.4, and 2 W/kg [27]. The incidence of lung and liver tumors and of malignant lymphomas was significantly higher in RFR exposed animals, compared to control (sham-exposed) rats.

The studies discussed in this section are summarized in Table 2.

Table 2

Studies of carcinogenicity in mice exposed at least two years to RFR.

Strain, Species, (Sex) Duration Reference	RFR Exposure Level: Frequencies Intensities (Any Other Co-Exposure)	Exposure time No. of Animals	Increased Tumor Incidence (Significance)
B6C3F1 mice (M, F) Tillmann et al., (2007)	902 MHz (GSM) 1747 MHz (DCS) 0.4, 1.3, 4.0 W/Kg	2 h/day, 5 days/week 50/sex/group	Not any increased tumor incidence (NS)
B6C3F1/N mice (M, F) Before birth trough 24 months Wyde et al., (2016)	1900 MHz (GSM, CDMA) 2.5, 5, and 10 W/Kg	9 h/day, 7 days/week 105/sex/group	Not any increased tumor incidence (NS)
B6C3F1 mice (F) 24 months Tillmann et al., (2010)	UMTS fields 48 W/m^2 and 4.8 W/m^2 + prenatal ENU treatment of 40 mg/kg/b.w.	23.5 h/day, 7 days/week 60/group	Female lung carcinoma and lung tumor rate in ENU-pretreated group (tumor promotion) ($p < 0.05$)
B6C3F1 mice (F) 24 months Lerchl et al., (2015)	UMTS fields 0.04, 0.4, and 2 W/kg + prenatal ENU treatment of 40 mg/kg/b.w.	23.5 h/day, 7 days/week 96/group	Female lymphoma, lung adenoma and carcinoma, liver carcinoma (tumor promotion) ($p < 0.05$)

M, males; F, female; h, hour(s); NS, not significant.

2.3. Other Models

Among the studies conducted on transgenic/tumor-prone animal strains, in 1997, Repacholi et al. observed an increase in β-cell lymphoma in transgenic mice exposed to pulsed digital RFR. Such increase was observed for female pim1 (carrying a lymphomagenic oncogene) mice after 18 months of exposure to a modulated 900 MHz GSM signal of two 30-min periods per day, at SAR exposure levels ranging from 0.008 to 4.2 W/kg, averaging 0.13–1.4 W/kg [28]. This result was not replicated by the studies of Utteridge et al. (2002) and Oberto et al. (2007) that had similar designs [29,30].

In particular, in 2002 Utteridge and collaborators used a modulated 898.4 MHz GSM signal for exposing female heterozygous pim1 transgenic (lymphoma-prone) mice (n = 120 per group) in a "ferris-wheel" system. Tube-restrained animals were RFR-radiated for 1 h/day and 5 days/week within two years at 0, 0.25, 1.0, 2.0, and 4.0 W/kg as whole body SAR levels. In addition, the study included a non-restrained cage control group, and a positive control, treated only with 50 mg/kg ENU. All in all, contrary to what observed by Repacholi, no lymphoma increase was observed in the RFR exposed groups [29].

In the study by Oberto et al. (2007) that the authors defined as "an extension of a previously published study conducted by Repacholi et al.", female Pim1 transgenic mice (n = 50/group/sex) were tube-restrained and treated for 1 h/day, 7 days/week, up to 18 months, at whole body SAR levels of 0, 0.5, 1.4, 4.0 W/kg. The results of this bioassay do not suggest any effect due to pulsed 900 MHz RFR exposure on the onset of tumors, thus, disproving once again, the results by Repacholi et al., under the conditions used [30].

The possible co-carcinogenic effect of 2450 MHz RFR exposure and 3, 4-benzopyrene was explored by Szmigielski et al. in 1982 in two different types of transgenic mice. Balb/c and C3H/HeA mice were exposed to RFR from 6 weeks of age for 2 h a day, 6 days a week, up to 1 year of age, either before (over 1 or 3 months) or concurrently to the treatment with benzopyrene (over five months). RFR at the frequency of 2450 MHz showed to induce cancer promotion at both 50 and 150 W/m^2. The findings of this study revealed an increase in chemically-induced and spontaneous tumors [31].

The studies discussed in this section are summarized in Table 3.

Table 3

Studies of carcinogenicity in other models exposed at least two years to RFR.

Strain, Species, (Sex) Duration Reference	RFR Exposure Level: Frequencies Intensities (Any Other Co-Exposure)	Exposure Time No. of Animals	Increased Tumor Incidence (Significance)
E mu-Pim1 mice (lymphoma-prone) (M, F) 24 months Utteridge et al. (2002)	898.4 MHz GSM 0.25, 1.0, 2.0, and 4.0 W/kg	1 h/day, 5 days/week 120/sex/group	Not any increased tumor incidence (NS)
E mu-Pim1 mice (lymphoma-prone) (M, F) 18 months Oberto et al. (2007)	900 MHz pulse modulated 0.5, 1.4, 4.0 W/kg	1 h/day, 7 days/week 50/sex/group	Not any increased tumor incidence (NS)
E mu-Pim1 mice (lymphoma-prone) (F) 18 months Repacholi et al. (1997)	900 MHz GSM 0.008–4.2 W/kg, averaging 0.13–1.4 W/kg	1 h/day, 7 days/week 100/group	B-cell lymphoma ($p < 0.01$)
C3H/HeA (breast cancer-prone) and Balb/c mice 12 months (M, F) Szmigielski et al. (1982)	2450 MHz 50, 150 W/m^2 Balb/c mice also treated with 3, 4-benzopyrene (BP)	2 h/day, 6 days/week NR	Acceleration of breast tumor developed in C$_3$H/HeA mice Acceleration of BP-induced skin cancer in Balb/c mice ($p < 0.05$)

M, males; F, female; h, hour(s); NS, not significant; NR, not reported.

3. Reproductive/Developmental Toxicity In Vivo Investigations

Both OECD and NTP recently updated their study guidelines for reproductive and developmental toxicity adding various functional endpoints for assessing how an agent can affect the reproductive and endocrine status of animals [32,33]. Here, we used those endpoints for articles selection by Pubmed to assess the state of art of the literature about RFR toxicity potential, distinguishing for studies on the male and female reproductive system, and other reproductive/developmental endpoints, discriminating by strains of experimental animals. In particular, the apical endpoints used for literature search on Pubmed were the following: Assessment of sperm quality, viable litter size/live birth index, neonatal growth, neonatal survival indices, prenatal mortality, weight and morphology of reproductive organs, estrous ciclicity, precoital interval, mating and fertility indices and reproductive outcome, duration of gestation, parturition, landmarks of sexual maturity (vaginal opening, urogenital distance, balano-preputial separation), functional toxicities and CNS maturation, qualitative and quantitative physiologic endpoints revealing unique toxicities of pregnancy and lactation, nesting and nursing behavior, sexual behavior, sex ratio in progeny, oocyte quantification.

3.1. Male Reproductive System

3.1.1. Rats

One of the first studies investigating cellphone RFR was conducted in SD rats and examined just the effects, due to exposure on testicular and sperm function. Rats were restrained in cages built ad hoc in Plexiglas, and mobile phones were placed 0.5 cm under the cages. Cellular phones with frequencies between 890 and 915 MHz were activated at a SAR level of 0.52 W/kg for 20 min/day for up to one month. No statistically significant difference between treated and control animals was reported in this study for any of the analyzed parameters [34].

Testicular histological changes in rats exposed to 848.5 MHz RFR for 12 weeks were explored by Lee et al. (2010). Male SD rats underwent two daily exposure periods of 45-min, with an interval period of 15 min. The authors investigated the concentrations of MDA in the testis and epididymis, the sperm count in the cauda epididymis, the frequency of the stages of spermatogenesis, the germ cell count, and the appearance of apoptotic cells in the testis. According to the results of this study, rat spermatogenesis was not influenced by any detectable adverse effect [35].

Two years later, the same researchers examined the effects of combined exposure to 848.5 MHz CDMA and 1950 MHz WCDMA RFR on most of the same parameters analyzed in their previous study. SD rats underwent RFR exposure for 45 min a day, 5 days a week for a total of 3 months, at an average whole-body SAR of 4.0 W/kg for both the frequencies. Based on the findings, they concluded that not even such simultaneous exposure resulted in any observable adverse effect on rat testicular function [36].

In 2011, Imai et al. performed a study on adolescent SD rats, exposing the animals to 1.95 GHz RFR at a whole-body SAR level of 0.4 W/kg for 5 h/day, 7 days/week, for a total of 5 weeks. The exposure period corresponded with the reproductive maturation of the rats. No difference in weights of the epididymis, seminal vesicles, testis or prostate was observed between exposed and control rats. Sperm count in the epididymis and testis was not influenced by the treatment, and no alterations in the sperm motility, morphology, or in the histological appearance of seminiferous tubules, including the spermatogenic cycle stage, was observed between treated and untreated animals [37].

Among "non-influential" studies conducted in Wistar rats, in 2007 Ribeiro et al. investigated the effects of subchronic RFR exposure emitted by GSM cellular phone (1.835–1.850 GHz) for 1 h/day for 11 weeks on the testicular function. Epididymal sperm count, epididymal and testicular weight, total testosterone in the serum and lipid peroxidation levels in these organs, such as various qualitative testicular histopathological end points were analyzed. No statistically significant difference was found between treated and control animals for all the considered endpoints [38].

A study by Trošić et al. was aimed to establish the possible negative impact of RFR exposure on Wistar rat male reproductive health. The research group evaluated the count, motility and form of spermatozoa from the cauda epididymis, as well as the histology of the testis. Animals were total body irradiated for 1 h/day over two weeks to 915 MHz RFR, at the average SAR value of 0.6 W/kg. An haemocytometer was used for microscopically determining the quality, quantity and structure of free sperm cells taken from the epididymis. This study revealed no statistically significant changes in any of the evaluated endpoints [39].

In 2007, the study by Yan et al. reported a statistically significant decreased motility of the epididymal sperm of SD rats treated with 1.9 GHz RFR, in comparison to controls. Furthermore, treated rats, unlike the unexposed, showed abnormal clumping of spermatozoa. Compared to the previous study by Dasdag et al., the apparent opposition in the results obtained by Yan et al. may be likely due to the longer treatment period to which rats from the same strain were subjected in this experiment. In fact,

the experimental rats, restrained in special plastic holding tubes, were daily exposed to cell phone RFR for three hours, followed by 30 min of non-exposure outside the tubes, and, to follow, by another exposure period of 3 h more [40].

In 2014, Qin et al. used adult male SD rats to explore the circadian effects of the exposure to RFR on reproductive functional markers. Animals in circadian rhythm (based on melatonin measurements) underwent RFR exposure at 1.8 GHz, at a SAR level of 0.0405 W/kg, for 2 hours a day, for 32 days in total. Circadian rhythms were found disrupted in animals exposed to RFR, as well as testosterone levels, daily sperm production and sperm motility were found decreased, the activity of γ-GT and ACP down-regulated, and the mRNA expression of cytochrome P450 and steroidogenic acute regulatory protein were altered in comparison to sham exposed rats. These results show that RFR exposure can negatively impact male reproductive functional markers, both in terms of total daily levels and in terms of circadian rhythmicity [41].

Finally, in 2019, Guo et al. explored the effects of 30 days exposure to pulsed modulated RFR at 220 MHz on the sperm quality in male adult SD rats. Calculated average whole body and testis SAR values were 0.030 W/kg and 0.014 W/kg, respectively. Compared to controls, the sperm quality in the treated group decreased significantly. Sperm cells quality was assessed by measuring the survival rate, the number and the abnormalities of spermatozoa. After the treatment, the level of Leydig cells secreting factor assessed by ELISA decreased significantly, whereas the Western blotting-assessed levels of caspase 3, cleaved caspase 3, and the BAX/BCL2 ratio in the testis markedly increased. Moreover, the levels of secreted factors of Sertoli cells assessed by ELISA and the testis morphology by HE staining, showed an evident change following RFR treatment [42].

Different outcomes emerged from most of the studies conducted in Wistar rats, for example when rats of the same strain were exposed to RFR emitted by an active cell phone at the GSM frequencies of 0.9 and 1.8 GHz for 1 h a day for 1 month, in comparison to a group of animals exposed for the same period to a no battery cell phone. Total sperm count was not affected, but sperm motility of experimental rats was significantly reduced by the exposure. The average percent of motile sperm reduced of about 40%, being 72.0% ± 8.7% in control rats and 43.1% ± 10.0% in RFR-exposed rats. Moreover, epididymis and testis of RFR exposed animals showed a marked increase in lipid peroxidation and a significant decrease in GSH content [43].

Kesari et al. (2010) exposed adult rats for 2 h/day up to 5 weeks to 900 MHz RFR with a level of SAR estimated to be 0.9 W/kg. Treated animals showed a significantly decreased total sperm count and level of protein kinase C (PKC), as well as an increase in apoptosis of spermatozoa. The enzyme PKC is commonly located in head, neck, and tail of human sperm, and plays an important role in the acrosomal reaction and sperm motility. The authors associated the reduction in PKC activity to the RFR-dependent possible overproduction of ROS in the sperm of exposed animals [44].

To investigate the hypothesis of an increased production of free radicals and other effects on fertility, the same researchers exposed the same strain of male rats to the same type and duration of RFR exposure. They found the antioxidant enzymes glutathione peroxidase and superoxide dismutase significantly lowered, and the catalase and malondialdehyde significantly increased in the exposed group, compared to the unexposed. Moreover, testicular sperm of exposed rats showed an increased content in micronuclei and a significantly changed cell cycle of G(0)–G(1) and G(2)/M. Generation of free radicals (ROS) was significantly increased in sperm [45].

In the same year, Meo and collaborators exposed male Wistar albino rats to GSM cell phone RFR for half an hour a day or for 1 h a day, for a 3 months period in total. In comparison to control rats, 18.75% of longer exposed rats showed the arrest of sperm maturation and hypospermatogenesis. The exposure to cell phone RFR for half an hour a day did not result in any abnormal findings in the animals [46].

In a study of 2012, Kesari and Behari exposed Wistar rats to cell phone RFR for 2 h a day for 45 days. Exposed rats showed a significant decrease in testosterone levels and an increased activity of caspase-3 protein, compared to controls. Transmission Electron Microscope observations also revealed sperm head and midpiece of sperm mitochondrial sheath distortions. Furthermore, this study revealed a reduction in litter size and weight of the progeny deriving from RFR-exposed male rats mated with unexposed females, compared to controls. The authors attributed these observations to ROS overproduction in rats exposed to cell phone RFR [47].

A long-term study analyzed the effects of exposure to cell phone emitting at 900 MHz on the reproductive organs of male rats. Specific levels of SAR for testis and prostate ranged from 0.0373 to 0.0623 W/kg. Exposed rats underwent RFR 3 h daily for one year. Once the experiment ended, the authors claimed that, under the condition used, RFR alter some reproductive parameters. In particular, the morphologically abnormal spermatozoa rates of treated rats were found significantly higher, in comparison to control rats. Moreover, at the histological examination of the seminiferous tubules, the Johnsen testicular biopsy score and the tunica albuginea thickness were found significantly decreased in the exposed animals [48].

In the same year, a study by Meena et al. aimed to evaluate the protective effect of the well-known antioxidant melatonin (MEL) in male Wistar rats, demonstrated that the prolonged RFR exposure could generate oxidative stress-mediated damage of the testes. The animals were divided into four groups: Controls (sham exposure); 2 mg/kg MEL treated; 2.45 GHz RFR exposed; and co-treated with RFR + MEL. Exposure was of 2 h a day for 45 days, and SAR was estimated at 0.14 W/Kg. Experimental observations showed that RFR biochemically induced oxidative damage by significantly decreasing testicular LDH levels and by increasing testicular MDA and ROS levels. Furthermore, RFR significantly affected the sperm count, the levels of testosterone, the fragmentation of DNA in testicular cells, the content of xanthine oxidase and carbonylated proteins [49].

Exposure effects on testes were also evaluated in another long-term study, in which rats underwent Wi-Fi-emitted 2.4 GHz RFR for 24 h a day during 12 months. Among the different parameters analyzed, the weight of the seminal vesicles and epididymis, the diameter of the seminiferous tubules and the thickness of tunica albuginea were found significantly lowered, compared to controls, while head defects and mitochondrial distribution alterations in the mid-piece of sperms significantly increased in the exposure group [50].

Adult male Wistar-Albino rats were long-term treated with 2.4 GHz RFR reproducing Wi-Fi exposure to evaluate the potential DNA damage on a series of different tissues. The detection of possible DNA damage was realized through the method of the single cell gel electrophoresis assay (comet). In exposed rats, the % tail DNA values of the liver, brain, kidney, and skin tissues were higher than that of the controls, but the increase resulted statistically significant only in testes tissue [51].

In a study conducted in 2018, Narayanan et al. investigated the possible adverse effects on blood biochemical and reproductive parameters of adolescent male albino Wistar rats exposed to 1h/day 900 MHz RFR from a mobile phone for 28 days. The treatment caused a slight reduction in sperm motility and a statistically significant increase in abnormal sperm percentage, in comparison to unexposed rats. Moreover, the testes of 900 MHz-exposed animals showed a loss of germ cells, in particular, spermatids and spermatocytes. Furthermore, the activity of testes caspase-3 was slightly increased, and MDA concentration was found increased in exposed rats [52].

The most recent study performed in Wistar rats for evaluating the effects of RFR on the male reproductive system was that of Gautam et al. in 2019. Rats underwent cell phone RFR exposure from 3G technology for 2 h/day for 45 days in specially designed exposure structures. A spermatogenic cells decrease was detected through histopathological examination, as well as sperm membrane and sperm

tail morphology alterations. Among the various biochemical and physiological parameters analyzed, significant increases in lipid peroxidation and ROS levels with concomitant sperm count decrease and alteration in the mitochondrial activity of spermatozoa were observed [53].

The studies discussed in this section are summarized in Table 4.

Table 4

Male reproductive studies in rats exposed to RFR.

Strain, Species Reference	RFR Exposure Level Frequencies, Intensities (Any Other Co-Exposure)	Exposure Time No. of Animals	Endpoint(s) Impacted by RFR (Significance)
Sprague-Dawley rats Dasdag et al., (2003)	890–915 MHz (GSM) 0.52 W/kg	20 min/day, 7 days/week, 1 month 8/group	Not any statistically significant alteration (NS)
Sprague-Dawley rats Lee et al., (2010)	848.5 MHz 2.0 W/kg (CDMA)	90 min/day, 5 days/week, 12 weeks 20/group	Not any statistically significant alteration (NS)
Sprague-Dawley rats Lee et al., (2012)	848.5 MHz (CDMA), 1950 MHz (WCDMA) 4.0 W/kg	45 min/day, 5 days/week, 12 weeks 20/group (cage control group: 5)	Not any statistically significant alteration (NS)
Sprague-Dawley rats Imai et al., (2011)	1950 MHz (CDMA) 0.4 W/kg, 0.08 W/kg	5 h/day, 7 days/week, 5 weeks 24/group	Not any statistically significant alteration (NS)
Wistar rats Ribeiro et al., (2007)	1.835–1.850 GHz (GSM) 1.4 mW/cm^2, 0.04 mW/cm^2	1 h/day, 7 days/week, 11 weeks 8/group	Not any statistically significant alteration (NS)
Wistar rats Trošić et al., (2013)	915 MHz 0.6 W/kg	1 h/day, 7 days/week, 2 weeks 9/group	Not any statistically significant alteration (NS)
	1900 MHz		

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h, hour(s); NS, not significant; NR, not reported; ROS, reactive oxygen species; GSH, glutathione; PKC, protein kinase C; HI, histone kinase; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase; XO, xanthine oxidase; MDA, malondialdehyde; HOS test, Hypo-Osmotic Swelling test; StAR, steroidogenic acute regulatory protein; γ-GT, γ-glutamyltransferase; ACP, acid phosphatase; T, testosterone; BAX, bcl-2-like protein 4; BCL2, B-cell lymphoma 2.

3.1.2. Mice

In the first study reported on mice, the animals were subjected to 0.09 W/kg RFR exposure, at 900 MHz. Polycarbonate cages were accommodated within an ad hoc built waveguide where mice were irradiated for 12 h/day for a week. The mitochondrial genome and the nuclear β-globin locus resulted significantly damaged when the DNA integrity was detailed analyzed through qPCR. Apart from this genotoxic effect in epididymal spermatozoa, this study did not document any other detrimental effect by RFR exposure on the development of male germ cell [54]. Nonetheless, it should be pointed out that in this study it was used a SAR value about 10 times lower than that used in the 2010 study by Kesari et al. conducted in Wistar rats [44]. The contrasting outcomes from these two studies may be partly explained by both the different experimental exposure conditions, and the different strain used (mice are much smaller than rats).

A 2010 study by Otitoloju et al. exposed male mice at residential quarters and a workplace complex to RFR from radio base antenna at 900 to 1800 MHz for six months, and compared to unexposed animals observed a statistically significant increase (39.78 and 46.03%, versus 2%, respectively) in the occurrence of sperm head defects. Such abnormalities were found to be dose-dependent, and mainly consisted of pin-head, banana-shaped and knobbed hook sperm head [55].

The study conducted by Pandey et al. in 2017 using Swiss albino mice investigated the effects of RFR exposure on male germ cell transformation kinetics, and evaluated the possible recovery. Animals were exposed to 900 MHz RFR for 4 or 8 h a day for a total of 35 days. Some animals were sacrificed after those 35 days of exposure, while others were given the opportunity to recover from exposure for further 35 days. The damage index of germ cells and the sperm head abnormalities were significantly increased in exposed mice. Flow cytometric estimation of germ cell subtypes in mice testis revealed 2.5-fold increases in spermatogonial populations with significant decreases in spermatids. A reduction of almost three times in primary spermatocyte to spermatid turnover, and a fourfold reduction in spermatogonia to spermatid turnover were found, to indicate a spermatogenesis arrest in the premeiotic stage. As a consequence, post-meiotic germ cells markedly decreased at the histological observation of the testes, as well as the sperm count lowered in mice exposed to RFR. Furthermore, histological alterations, such as epithelium depletion, maturation arrest and loss of immature germ cells into the seminiferous tubule lumen were also observed. Nevertheless, all the observed effects showed varying degrees of recovery when animals underwent to a post-treatment recovery period [56].

In a very recent experimental study, the same authors investigated the impact of GSM RFR at 900 MHz on germ cells development during spermatogenesis of Swiss albino mice. Animals were divided into four groups, one of which underwent RFR exposure 3 h a day twice, for 35 days, another received the same exposure with 5 mg/kg bw/day MEL supplementation, a third group received only MEL, and the last one remained unexposed. As consistent with the previous experiment, RFR exposure caused extensive DNA damage in germ cells, arrest in pre-meiotic stages of spermatogenesis, eventually leading to sperm head defects and low sperm count. Moreover, excess free radical generation was revealed through biochemical assays, thus, leading to histological and morphological changes, respectively in testis and germ cells morphology. These effects were either diminished or absent in RFR-exposed animals supplemented with MEL [57]. This result confirms the findings by Meena et al. in Wistar rats [49].

The studies discussed in this section are summarized in Table 5.

Table 5

Male reproductive studies in mice exposed to RFR.

Strain, Species Reference	RFR Exposure level Frequencies, Intensities (Any Other Co-exposure)	Exposure Time No. of Animals	Endpoint(s) Impacted by RFR (Significance)
CD1 Swiss mice Aitken et al. (2005)	900 MHz (GSM) 0.09 W/kg	12 h/day, 7 days/week, 1 week5/group	Damage in the mitochondrial genome and in the nuclear β-globin locus by DNA integrity analysis using qPCR
Albino mice, *mus musculus* Otitoloju et al. (2010)	900 to 1800 MHz (GSM) NR	24 h/day, 7 days/week, 6 months5/group	Increased sperm head abnormalities (knobbed hook, pin-head and banana-shaped sperm head) ($p < 0.05$)
Swiss albino mice, Pandey et al. (2017)	900 MHz (GSM) 0.0054 – 0.0516 W/kg	4 or 8 h/day, 7 days/week, 35 days15/group	Increased damage index in germ cells, sperm head defects, decreased sperm count, arrest in pre-meiotic stage of spermatogenesis, loss of immature germ cells into the seminiferous tubule lumen, epithelium depletion and maturation arrest ($p < 0.05$)
Swiss albino mice, Pandey et al. (2018)	900 MHz (GSM) (Melatonin 5 mg/kg bw/day) 0.0054 – 0.0516 W/kg	6 h/day, 7 days/week, 35 days 15/group	Decreased sperm count, sperm head abnormalities, extensive DNA damage in germ cells, arrest in pre-meiotic stages of spermatogenesis, excess free radical generation resulting in histological and morphological changes in testis and germ cells morphology ($p < 0.05$)

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h, hour(s); NS, not significant; NR, not reported.

3.1.3. Other Models

Two studies were performed by the same research group using adult male rabbits to evaluate the effect of mobile phone-emitted RFR on weekly collected semen samples. The two lagomorph studies were conducted with the identical experimental design and protocol of exposure and differed only in the frequencies used, which were of 900 MHz [58] and 800 MHz [59]. The whole-body average SAR was 0.43 W/kg in both studies. Mobile phone in standby mode was positioned close to the genitalia of the animal for 8 h/day over 12 weeks in order to assess testicular function. Salama et al. evaluated fructose and citrate levels, sperm motility and viability, serum testosterone levels, histological sections from the

prostatic complex, ampulla, and vesicular gland. Compared to the unexposed group, there was a significant decrease in both fructose levels and number of motile sperms in the exposed group at the 10th week of exposure. There were no changes in citrate levels. A significant reduction in the sperm concentration in the exposed group at week 8 and a drop in sperm motility at week 10, accompanied by a significant decrease in the diameter of seminiferous tubules were also observed. The studies discussed in this section are summarized in Table 6.

Table 6

Male reproductive studies in other models exposed to RFR.

Strain, Species Reference	RFR Exposure Level Frequencies, Intensities (Any Other Co-Exposure)	Exposure Time No. of Animals	Endpoint(s) Impacted by RFR (Significance)
Rabbits Salama et al. (2009)	900 MHz (mobile phone in standby mode) 0.43 W/kg	8 h/day, 7 days/ week, 12 weeks 10/sex/group	Decreased fructose levels in sperm and sperm motility ($p < 0.05$)
Rabbits Salama et al. (2010)	800 MHz (mobile phone in standby mode) 0.43 W/kg	8 h/day, 7 days/ week, 12 weeks 11/sex/group	Decreased sperm concentration and motility, decrease in the diameter of seminiferous tubules ($p < 0.05$)

h, hour(s); NS, not significant; NR, not reported.

3.2. Female Reproductive System and Other Reproductive/Developmental Endpoints

3.2.1. Rats

Lary et al. exposed pregnant SD rats to 100 MHz RFR at a SAR level of 0.4 W/kg, for 6 h and 40 min/day. The exposure period was from gestation day 6 to 11, for a total of 40 h. The SAR value used corresponded to the maximum permissible level as indicated by the 1982 American National Standards Institute (ANSI) standard for RFR exposure. Irradiated rats did not differ in the percentage number of malformed fetuses, implantations per litter, percentage of implantations dead or resorbed, fetal weight, fetal crown-rump length, or fetal sex ratio, compared to untreated (sham-exposed) rats. The results showed a lack of embryotoxic or teratogenic effect in rats at the maximum permissible RFR exposure level following the 1982 ANSI recommendations [60].

A 2009 study by Ogawa et al. investigated the effect of 90 min/day 1.95 GHz CDMA emission on SD rats embryogenesis, exposing the dams from gestation day 7 to 17. All the animals were killed at gestational day 20, and the fetuses were extracted by cesarean section. The authors compared treated and untreated rats for placental weights, number of live, dead or resorbed embryos, sex ratios, weights, or visceral, external, or skeletal abnormalities of live fetuses Neither differences in maternal body weight gain, nor adverse effects of RFR exposure on any considered embryotoxic and reproductive parameters analyzed were observed [61].

In a study of 2010, Takahashi et al. evaluated the effects of 2.14 GHz RFR reproducing the emission of radio base antennas of mobile telephony. Pregnant SD rats were long-term whole-body exposed at two different exposure levels and compared to untreated animals. The calculated average SAR at the high exposure level ranged from 0.066 to 0.093 W/kg for the dams, and from 0.068 to 0.146 W/kg for the fetuses and the F(1) progeny. At the low exposure level, the SARs were estimated to be around 43% of those calculated for the high level. Treated rats underwent 20 h/day RFR exposure during gestation and lactation period. Parameters evaluated in dams were gestational conditions, organ weights and growth, while the F(1) generation was evaluated at 10 weeks of age for growth, development, survival rates, hormonal status, physical and functional development, memory function and reproductive ability. The F(2) offspring were analyzed for possible effects of teratogenicity and embryotoxicity. Both the RFR exposed dams and F(1) progeny showed no treatment-related effect for any of the parameters evaluated. The same was observed for the F(2) generation. The RFR exposure conditions used in this study did not determine any adverse effect on rat pregnancy or development [62].

Among the "non-influential" studies conducted in Wistar rats, two were conducted by Poulletier de Gannes and collaborators. Animals were exposed over two generations to a 2450 MHz Wi-Fi emission signal during gestation and lactation periods. In 2012, these researchers evaluated the possible adverse effects on pregnant rats and their F(1) generation of a Wi-Fi signal prenatal exposure of 2 h a day, 6 days a week, for a total of 18 days. The whole-body SARs used corresponded to 0.08, 0.4, and 4 W/kg. No statistically significant effects were noted at each dose tested in both dams and their pups, respectively in terms of observed abnormalities and pre- and postnatal development signs of toxicity [63].

The following year, another similar study from the same authors was conducted in rats of the same strain for investigating the exposure effects to a Wi-Fi signal with the same frequency on the male and female reproductive system. Adolescent animals were exposed for 1 h a day and 6 days a week to RFR, for three weeks in the case of males and for two weeks in the case of females. Afterwards, the rats were mated, and the couples treated for three more weeks. The day before parturition, clinical signs, abnormalities and mortality were evaluated in the fetuses. Under the condition used, the exposure to the Wi-Fi RFR signal did not determine any detrimental effect on fertility and reproductive organs either in male or in female rats. Likewise, the fetuses did not show any macroscopic abnormality [64].

Aït-Aïssa assessed immunological biomarkers in the sera of young Wistar rats RFR-exposed in utero and postnatally. Pregnant rats were located in a reverberation chamber and exposed to a Wi-Fi signal at the frequency of 2.45 GHz for 2 h a day and 5 days a week. Dams were whole-body exposed at average SAR values of 0, 0.08, 0.4, and 4 W/kg from gestation day 6 to 21. Furthermore, three newborns per litter were exposed for an additional 35 days. Antibodies directed against 15 different antigens related to damage and/or pathological markers were analyzed using the biochemical assay ELISA performed on sera of the experimental rats. The humoral response of the newborns showed no changes among different groups, for all the SAR levels used and the biomarker types considered. Some data on gestational outcome following in utero exposure to Wi-Fi signals were also provided by the present study; in particular, mass evaluation of dams and pups and the number of pups per litter were monitored, as well as the genital tracts of young rats were analyzed for abnormalities by measuring anogenital distance. Under the conditions used, the author's findings indicate the absence of adverse effects, due to Wi-Fi signal exposure on general conditions and delivery in of Wistar rats [65].

One of the first evidence of teratogenicity, due to RFR in SD rats was that showed by Lary et al. in the early 1980s using low, non-thermal exposure level, with results in contrast with other findings from the same authors [60]. For investigating possible embryotoxic effects, in this study, pregnant SD rats were irradiated with 27.12 MHz RFR on gestation day 9 at an approximately SAR value of 11 W/kg. Treatment determined a relatively quick rise in the temperature of rat colony. As the duration of the exposure increased, embryotoxic and teratogenic effects of the RFR-induced hyperthermia also

increased. Both the temperature of the dams and the amount of time the temperature remains high in the dams were responsible for embryotoxic and teratogenic effects, due to treatment-related hyperthermia [66,67].

A series of experimental studies conducted by Nelson and his research group investigated in depth the combinatory developmental toxicity effects of RFR at 10MHz and the organic compound 2-methoxyethanol (2ME). For this purpose, SD rats were exposed to the two agents individually or concurrently, in comparison to sham-exposed animals. The authors used highly diverse doses and timing of exposure of pregnant rats; after the sacrifice of the animals on gestation day 20, external malformations were evaluated in the examined progeny. Results showed that the adverse effects produced by both the treatments administered alone were enhanced when the agents were co-administered. In addition to a significant, dose-related, increased frequency of malformations, the combination treatment enhanced the severity of malformations too. This extended project demonstrated that the co-administration of RFR and 2ME synergistically induced teratogenic effects, but only at hyperthermic levels of RFR [68,69,70,71].

The same research group administered with methanol SD rat dams concomitantly exposed to RFR, in order to evaluate whether the interactive effects observed for RFR and 2ME were limited to this agent, or if similar synergies could be observed with other chemicals. After the sacrifice of the animals on gestation day 20, external malformations were evaluated in the examined progeny. When RFR or methanol was administered alone, the authors observed a statistically significant increase in the number of resorbed fetuses. The same was not observed when the agents were co-administered to experimental rats [72]. Further studies in the field of developmental toxicology would be needed in order to clarify these contradictory results about the complex role of the different agents in interacting with RFR.

To evaluate the impact of the prenatal exposure to 1800 MHz RFR on bone development, pregnant SD rats were exposed for 6, 12, and 24 h/daily for a total of 20 days. The rats were inspected at the end of the day 60 after birth, and compared to the progeny of untreated dams. The increase in prenatal RFR exposure time caused a significant decrease in the levels of cartilage at rest and a significantly increased number of apoptotic myocytes and chondrocytes. Importantly, Erkut et al. demonstrated that an exposure of 24 h per day to RFR impaired tibia, ulna and femur development. Furthermore, a calcineurin activity decrease was observed in both muscle and bone tissues. This study indicated that the development of muscle and bone tissues was negatively impaired by prenatal exposure to 1800 MHz RFR [73].

The study by Oral et al., in 2006, investigated the potential adverse effects of RFR on the endometrial tissue of Wistar rat, with particular reference to RFR-induced oxidative stress and apoptosis. Animals were exposed to 900 MHz cell phone RFR for 30 min a day, for one month in total. In their study, the authors evaluated lipid peroxidation trough MDA as a marker of endometrial impairment induced by oxidative stress, and Bax, Bcl-2, caspase-3, and caspase-8 to assess apoptosis immunohistochemically. Based on the findings from this study, the electromagnetic fields emitted from cell phones may cause oxidative stress and endometrial apoptosis [74].

The studies discussed in this section are summarized in Table 7.

Table 7

Female reproductive studies and other reproductive/developmental endpoints in rats exposed to RFR.

Strain, Species (Sex) Duration Reference	RFR Exposure Level Frequencies, Intensities (Any Other Co-Exposure)	Exposure Time No. of Animals	Evaluated Endpoint(s)	Endpoint(s) Impacted by RFR (Significance)
Sprague-Dawley rats (F) Lary et al., (1983)	100 MHz 0.4 W/kg	6 h 40 min/day, 6 days 3-10/group	Viable litter size/live birth index, neonatal growth, neonatal survival indices, prenatal mortality	Not any statistically significant alteration (NS)
Sprague-Dawley rats (F) Ogawa et al., (2009)	1950 MHz CDMA 0.4 W/kg	90 min/day, 7 days/week, 10 days 20/group	Landmarks of sexual maturity, viable litter size/live birth index, neonatal growth, neonatal survival indices, sex ratio in progeny, physiologic endpoints revealing unique toxicities of pregnancy and lactation	Not any statistically significant alteration (NS)
Sprague-Dawley rats (M, F) Takahashi et al., (2010)	2140 MHz (CDMA) Dams:0.066–0.093 W/kg Pups: 0.068–0.146 W/kg	20 h/day, 7 days/week, Gestation (3 weeks) + lactation (3 weeks) 4/group	Landmarks of sexual maturity, viable litter size/live birth index, neonatal growth, neonatal survival indices, sex ratio in progeny, physiologic endpoints revealing unique toxicities of pregnancy and lactation	Not any statistically significant alteration (NS)
Wistar rats (F) Poulletier de Gannes et al., (2012)	2450 MHz (CDMA Wi-Fi signal) 0.08, 0.4, 4 W/kg	2 h/day, 6 days/week, 18 days Prenatal study: 5 dams/group, and their pups Postnatal study: 15 dams/group	Prenatal study: Number of live and dead fetuses per uterine horn, number and location in each uterine horn of early and late resorption sites, distribution of implantation sites on each uterine horn. Postnatal study: Landmarks of sexual maturity, viable litter size/live birth index, neonatal growth, neonatal survival indices, sex ratio in progeny, physiologic endpoints revealing unique toxicities of	Not any statistically significant alteration (NS)

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h, hour(s); NS, not significant; NR, not reported.

3.2.2. Mice

Two studies regarding the potential RFR impact on mating were conducted in mice. In 2009, a large study performed in both female and male C57BL mice living over four generations under constant (life-long, 24 h/day) exposure to three doses of UMTS RFR at 1966 MHz investigated histological, physiological, behavioral and also reproductive parameters. Adult mice underwent whole-body RFR exposure at the average SARs of 0.08, 0.4, and 1.3 W/kg (means calculated at mating time), and were compared to control (sham-exposed) animals. This study did not report any RFR-induced significant effect on both male and female reproductive system, particularly for what concerns the offspring development, the appearance of reproductive organs, and the fertility potential [75].

In the study by Zhu et al. (2015), mice were treated for 15 days with continuous 900 MHz RFR for 4 h/day at average SAR of 0.731 W/kg. Ended the treatment, all the exposed mice were individually mated with three mature virgin females. After 7 days, each male mouse was moved to a new cage for mating with three other female mice. This process was repeated for four consecutive weeks in total. At gestation day 18, all female mice were sacrificed for examining uterine content and evaluating the putative mating. All the observations conducted during the one month-long mating period showed no statistically significant differences in untreated female mice mated with treated male mice as regards total and live/dead uterine implants and percentage of pregnancies, in comparison to females mated with control male mice. As consistent with the previous study, this study showed no RFR mutagenic potential on male germ line [76].

In three different works, Finnie and collaborators explored the impact of cell phone RFR exposure on fetal mouse brain development, investigating different outcomes. The common design contemplated the use of a specifically built 900 MHz exposure system, through which pregnant mice received for 1 h/day a far-field, whole body exposure at a SAR level of 4 W/kg from gestation day 1 to 19. In the first experiment, the authors explored the effect of cell phone exposure on blood-brain barrier (BBB) permeability in the immature mouse brain. On the 19th day of gestation, just before delivery, heads were collected from fetuses. The integrity of BBB was evaluated by immunohistochemistry using endogenous albumin as a vascular tracer in the cerebral cortex, thalamus, hippocampus, midbrain, cerebellum, medulla and basal ganglia. No albumin extravasation was found in brains from control or exposed mice, indicating no increase in vascular permeability of the considered regions of the fetal brain [77].

To study the expression of immediate early gene c-fos as a marker of neural stress, the authors collected fetal heads on gestational day 19 as in the previous experiment. No statistically significant change in the expression of c-fos was immunohistochemically observed in the same brain regions evaluated in the previous experiment. The lack of difference in c-fos immunoreactivity between control and exposed brains indicated no stress response in the brain of the fetuses following cell phone RFR exposure during gestation period [78].

In their third study, the same authors collected from each animal three coronal sections of the brain, including various anatomical regions to investigate a possible stress response given by heat shock proteins (HSPs) induction. The immunostaining of HSP25, 32 and 70 revealed no stress response. Exposure of mouse fetal brains to cell phone RFR during the gestation period did not cause any stress response by immunohistochemically evaluating HSPs [79].

Magras et al. in 1997 studied the possible adverse effects of RFR exposure on the prenatal development of mice. Mice were located in sites of different power densities around an "antenna park" and were mated five times in a row, until 118 pups were generated. Thus, newborns were weighed, measured, and macro- and microscopically examined. Authors observed a progressive decrease in the number of generated newborns per dam, leading to irreversible infertility [80].

The evaluation of RFR-induced possible toxic effect on mouse ovaries was the aim of the 2009 study by Gul et al. using 21 days old mouse female pups. Pregnant mice underwent cell phone RFR by placing mobile devices under the cages for the entire pregnancy period. Cell phones in standby position were turned on to speech position for 15 min every 12 h. Female newborns were sacrificed on day 21 after delivery, and the right ovaries were collected for determining the number of follicles. Findings from this study indicated a significantly decreased number of follicles in exposed pups compared to controls, indicating a toxic intrauterine effect of RFR on pup ovaries [81].

The studies discussed in this section are summarized in Table 8.

Table 8

Female reproductive studies and other reproductive/developmental endpoints in mice exposed to RFR.

BALB/c mice (F)	Co-Exposure)		(a) Blood-brain barrier permeability in the immature brain of fetal heads; (b) immediate early gene c-fos expression as a marker of neural stress; (c) stress response by induction of heat shock proteins	Not any statistically significant alteration (NS)
Finnie et al. (a) (2006). Finnie et al. (b) (2006) Finnie et al.. (c) (2009)	900 MHz 4 W/kg	1 h/day. 7 days/week. 19 days 10/group		
BALB/c mice (M. F) Magras et al.. (1997)	80–900 MHz (different power densities around an "antenna park")	24 h/day. 7 days/week. 6 months (multi-generation study) 6/sex/group over five generations (118 newborns analyzed)	Infertility for dams and males. lethality for embryos. teratogenicity or the reduction in deformity for fetuses	Decreased number of newborns per dam, ending in irreversible infertility (NR)
Swiss mice	NR (mobile phone in standby position for	12 h/day. 7	Oocyte quantification in F pups	Decreased number of follicles in

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h. hour(s); NS. not significant; NR. not reported.

4. Discussion

Some long-term studies published up to the 2011 RFR IARC evaluation failed to demonstrate the carcinogenicity of RFR in experimental animals. RFR exposure has not been considerably linked to an increased incidence of tumors at any site both in rats or mice exposed for 24 months at different RFR intensities [13,14,15,25], and in transgenic and tumor-prone mouse strains [29,30]. If these studies have contributed to an increase in knowledge about potential RFR toxicity, critical limitations in the design of the majority of these studies seriously limit the usefulness of the information for properly evaluating the carcinogenic potential of RFR. Among the limitations are very short daily exposure durations (less than two hours a day) in strongly restrained experimental animals or exposed to radiations levels too low for properly evaluating the carcinogenicity of RFR [14,15,25] or had one exposure level only [13]. Moreover, most of these long-term studies did not justify the selected dose(s), and was characterized by poor dosimetry which did not consider the growth of the animals. Those studies using tumor-susceptible and genetically altered animals were not focused on evaluating RFR carcinogenic potential, but rather on investigating the effects in the specific target sites in that particular model [29,30]. Furthermore, some of the studies made with tumor-prone laboratory animals did not expose the animals to RFR for sufficient time (< 1 year), and for this reason, were not included in this review [82,83,84]. Lastly, additional points of criticism are the limited number of organs assessed for histopathology and inadequate group sizes (<50 animals/sex/group).

As reported in the present review, more in vivo bioassays on RFR have been conducted since the 2011 IARC review, most of which adopted improved exposure systems and more accurate measures of RFR dosimetry. In particular, the two long-term experimental studies by NTP and RI were performed for evaluating the effects of everyday human exposure to RFR electromagnetic fields. NTP doses have been established to mimic the localized exposure on body tissues from a cell phone placed near the body, and, are therefore, particularly higher than those used by the RI, that is instead similar to those found in our living and working environment to mimic the full-body human exposure generated by mobile telephony base antennas. Despite the differences, recently, both studies reported a statistically significant increased incidence of the same type of very rare glial malignant tumors of heart (schwannoma) and brain (glioma). These tumors involve the same cells of the acoustic nerve vestibular neurinoma observed in humans in certain epidemiological studies. Another fact supporting this consistency is that the Schwann cells are glial cells of the peripheral nervous system whose role is to form myelin, and are analogous to oligodendrocytes of the central nervous system. The NTP defined as clear evidence the carcinogenic activity of GSM-modulated 900 MHz RFR of mobile phones, particularly based on this statistically significant increase in the development of malignant heart Schwannoma in male SD rats [22]. Nonetheless, it must be reported that both NTP and RI used simulated mobile telephony signals emitted by generators, rather than real-life signals from cell phones and mobile telephony base antennas, respectively, and this represents a limit shared with many other studies discussed within this review. In fact, these simulated signals employ fixed parameters and no variability, thus, resulting in very different from the corresponding real emissions that instead vary constantly and unpredictably. This makes real-life signals more bioactive, and living organisms seem to have much less defense against highly variable environmental stressors [85,86]. Therefore, the use of simulated signals might also lead to an underestimation of the potential harmful effects.

As indicated by animal studies focusing on female reproductive system outcomes, the main targets of the potential adverse effect, due to RFR exposure are endometrial tissue, ovarian follicle numbers, granulosa cells, quality of oocytes and embryos during pregnancy. However, to date studies conducted in mammals regarding both female reproductive system and other reproductive/developmental endpoints are highly diverse, very inconsistently conducted and, most of all, report different specific outcomes, making it difficult to come to a conclusion about the different specific subjects.

On the other side, the evidence from studies on male reproductive system suggest that RFR exposure might negatively affect male fertility. The increased sperm cell death rate accompanied by reduced sperm quality and motility seems to be the more recurring effects, due to RFR exposure in SD rats [38,39,40]. Guo et al., based on their findings from the most recent study conducted in SD rats suggested that the RFR-induced impaired sperm quality in SD rats might be accounted for by the increased apoptosis of testicular cells and the disruption of the secreting function of Leydig cells. Based on the available literature on male Wistar rats, most of the studies show that RFR exposure causes the decrease of sperm motility and number, and the increase of oxidative stress [43,44,45,47,49,52,53]. Sperm head abnormalities accompanied by an altered mitochondrial distribution, are also often reported [47,48,50,52,53]. Since an appropriate distribution of mitochondria plays a key role in sperm motility, the alterations found by some studies may explain the reported reduced sperm motility [47,50]. Most of the available mouse studies are in agreement that the oxidative stress induced by RFR exposure can cause DNA damage in germ cells. This genotoxic effect can alter cell cycle progression, causing decreased sperm count and motility, and abnormalities of the sperm head in mice [54,55,56,57]. Interestingly the two recent works by Pandey proved the MEL inhibitory effect, as well as the reversibility of such harmful effects in case of suspension of RFR exposure. The same authors also hypothesized that exposure to RFR led to mitochondrial membrane depolarization in mice germ cells which in turn results in altered cellular redox homeostasis [56,57].

Seminiferous tubules, spermatozoa and Leydig cells are the main targets of this damage, and sperm count, motility and morphology represent the more frequently affected parameters. The abnormalities highlighted in many studies are likely related in a direct manner to the duration of mobile phone use and/or to the proximity to the RFR source. Several studies support the hypothesis that RFR exposure causes an increase in oxidative stress, leading to DNA and sperm membrane lipid damage which eventually cause the aforementioned effects. Therefore, it is essential to conduct mechanistic studies for elucidating the manner in which RFR impairs biological function, thus, providing a solid rational cause. Moreover, more studies are needed to supply stronger evidence that RFR emitted from mobile telephony base antennas and the use of the cell phone alter sperm and gonads functions given the many limitations characterizing the existing literature. Nevertheless, based on the in vivo animal studies conducted so far, it is likely that RFR could negatively impact sperm damaging male human fertility, especially when the mobile phone is kept in active mode in an area close to testicles.

5. Conclusions

In conclusion, according to NTP, there is now clear evidence that RFR causes cancer in experimental animals. RFR re-evaluation has also been listed as a priority by IARC [87]. There is also stronger evidence that RFR exposure is responsible for causing alteration of various sperm parameters, thus, affecting male fertility. Although a clear quantification of the carcinogenic and reproductive risk is still lacking, these animal findings suggest that a precautionary approach should be promoted by regulatory and health agencies, especially for children and pregnant women. Caution should also be considered in the development and spread of the upcoming 5G technology, particularly in light of the proposed higher frequencies and intensities of the signal. Long-term animal studies are urgently necessary to verify the possible health effects of 5G technology.

Author Contributions

Conceptualization: A.V., D.M., F.B.; Methodology: A.V., D.M.; Literature analysis/search: A.V.; Writing—original draft preparation: A.V.; Writing—review and editing: A.V., D.M., F.B., L.F., L.B.

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Conflicts of Interest

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References

1. Durney C.H., Massoudi H., Iskander M.F. Radiofrequency Radiation Dosimetry Handbook. 4th ed. USAF School of Aerospace Medicine; San Antonio. TX. USA: 1986. Report No. TR-85-73. [Google Scholar]

2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans Non-ionizing radiation. Part 2: Radiofrequency electromagnetic fields. Pt 2IARC Monogr. Eval. Carcinog. Risks Hum. 2013;102:1–460. [PMC free article] [PubMed] [Google Scholar]

3. Adair R.K. Biophysical limits on athermal effects of RF and microwave radiation. Bioelectromagnetics. 2003;24:39–48. doi: 10.1002/bem.10061. [PubMed] [CrossRef] [Google Scholar]

4. Prohofsky E.W. RF absorption involving biological macromolecules. Bioelectromagnetics. 2004;25:441–451. doi: 10.1002/bem.20013. [PubMed] [CrossRef] [Google Scholar]

5. Sheppard A.R., Swicord M.L., Balzano Q. Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes. Health Phys. 2008;95:365–396. doi: 10.1097/01.HP.0000319903.20660.37. [PubMed] [CrossRef] [Google Scholar]

6. Panagopoulos D.J., Karabarbounis A., Margaritis L.H. Mechanism for Action of Electromagnetic Fields on Cells. Biochem. Biophys. Res. Commun. 2002;298:95–102. doi: 10.1016/S0006-291X(02)02393-8. [PubMed] [CrossRef] [Google Scholar]

7. Baan R., Grosse Y., Lauby-Secretan B., El Ghissassi F., Bouvard V., Benbrahim-Tallaa L., Guha N., Islami F., Galichet L., Straif K. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncol. 2011;12:624–626. doi: 10.1016/S1470-2045(11)70147-4. [PubMed] [CrossRef] [Google Scholar]

8. Saikhun J., Kitiyanant Y., Vanadurongwan V., Pavasuthipaisit K. Effects of sauna on sperm movement characteristics of normal men measured by computer-assisted sperm analysis. Int. J. Androl. 1998;21:358–362. doi: 10.1046/j.1365-2605.1998.00138.x. [PubMed] [CrossRef] [Google Scholar]

9. Deepinder F., Makker K., Agarwal A. Cell phones and male infertility: Dissecting the relationship. Reprod. Biomed. Online. 2007;15:266–270. doi: 10.1016/S1472-6483(10)60338-0. [PubMed] [CrossRef] [Google Scholar]

10. Huff J., Jacobson M.F., Davis D.L. The limits of two-year bioassay exposure regimens for identifying chemical carcinogens. Environ. Health Perspect. 2008;116:1439–1442. doi: 10.1289/ehp.10716. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

11. OECD (Organisation for Economic Co-operation and Development) Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4. OECD; Paris, France: 2018. [Google Scholar]

12. Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP) [(accessed on 10 July 2019)];2011 Available online: https://ntp.niehs.nih.gov/ntp/test_info/finalntp_toxcarspecsjan2011.pdf.

13. La Regina M., Moros E.G., Pickard W.F., Straube W.L., Baty J., Roti Roti J.L. The effect of chronic exposure to 835.62 MHz FDMA or 847.74 MHz CDMA radiofrequency radiation on the incidence of spontaneous tumors in rats. Radiat. Res. 2003;160:143–151. doi: 10.1667/RR3028. [PubMed]

[CrossRef] [Google Scholar]

14. Anderson L.E., Sheen D.M., Wilson B.W., Grumbein S.L., Creim J.A., Sasser L.B. Two-year chronic bioassay study of rats exposed to a 1.6 GHz radiofrequency signal. Radiat. Res. 2004;162:201–210. doi: 10.1667/RR3208. [PubMed] [CrossRef] [Google Scholar]

15. Smith P., Kuster N., Ebert S., Chevalier H.J. GSM and DCS wireless communication signals: Combined chronic toxicity/carcinogenicity study in the Wistar rat. Radiat. Res. 2007;168:480–492. doi: 10.1667/RR0680.1. [PubMed] [CrossRef] [Google Scholar]

16. Adey W.R., Byus C.V., Cain C.D., Higgins R.J., Jones R.A., Kean C.J., Kuster N., MacMurray A., Stagg R.B., Zimmerman G., et al. Spontaneous and nitrosourea-induced primary tumors of the central nervous system in Fischer 344 rats chronically exposed to 836 MHz modulated microwaves. Radiat. Res. 1999;152:293–302. doi: 10.2307/3580329. [PubMed] [CrossRef] [Google Scholar]

17. Adey W.R., Byus C.V., Cain C.D., Higgins R.J., Jones R.A., Kean C.J., Kuster N., MacMurray A., Stagg R.B., Zimmerman G. Spontaneous and nitrosourea-induced primary tumors of the central nervous system in Fischer 344 rats exposed to frequency-modulated microwave fields. Cancer Res. 2000;60:1857–1863. [PubMed] [Google Scholar]

18. Shirai T., Kawabe M., Ichihara T., Fujiwara O., Taki M., Watanabe S., Wake K., Yamanaka Y., Imaida K., Asamoto M., et al. Chronic exposure to a 1.439 GHz electromagnetic field used for cellular phones does not promote N-ethylnitrosourea induced central nervous system tumors in F344 rats. Bioelectromagnetics. 2005;26:59–68. doi: 10.1002/bem.20079. [PubMed] [CrossRef] [Google Scholar]

19. Shirai T., Ichihara T., Wake K., Watanabe S., Yamanaka Y., Kawabe M., Taki M., Fujiwara O., Wang J., Takahashi S., et al. Lack of promoting effects of chronic exposure to 1.95-GHz W-CDMA signals for IMT-2000 cellular system on development of N-ethylnitrosourea-induced central nervous system tumors in F344 rats. Bioelectromagnetics. 2007;28:562–572. doi: 10.1002/bem.20324. [PubMed] [CrossRef] [Google Scholar]

20. Zook B.C., Simmens S.J. The effects of 860 MHz radiofrequency radiation on the induction or promotion of brain tumors and other neoplasms in rats. Radiat. Res. 2001;155:572–583. doi: 10.1667/0033-7587(2001)155[0572:TEOMRR]2.0.CO;2. [PubMed] [CrossRef] [Google Scholar]

21. Chou C.K., Guy A.W., Kunz L.L., Johnson R.B., Crowley J.J., Krupp J.H. Long term, low-level microwave irradiation of rats. Bioelectromagnetics. 1992;13:469–496. doi: 10.1002/bem.2250130605. [PubMed] [CrossRef] [Google Scholar]

22. NTP TR 595: NTP Technical Report on the Toxicology and Carcinogenesis Studies in Hsd: Sprague Dawley SD Rats Exposed to Whole-Body Radio Frequency Radiation at a Frequency (900 MHz) and Modulations (GSM and CDMA) Used by Cell Phones. National Toxicology Program, US Department of Health and Human Services. [(accessed on 8 November 2018)]:2018 Available online: https://ntp.niehs.nih.gov/results/areas/cellphones/index.html.

23. Falcioni L., Bua L., Tibaldi E., Lauriola M., De Angelis L., Gnudi F., Mandrioli D., Manservigi M., Manservisi F., Manzoli I., et al. Report of final results regarding brain and heart tumors in Sprague-Dawley rats exposed from prenatal life until natural death to mobile phone radiofrequency field representative of a 1.8 GHz GSM base station environmental emission. Environ. Res. 2018;165:496–503. doi: 10.1016/j.envres.2018.01.037. [PubMed] [CrossRef] [Google Scholar]

24. NTP TR 596: NTP Technical Report on the Toxicology and Carcinogenesis Studies in B6C3F1/N Mice Exposed to Whole-Body Radio Frequency Radiation at a Frequency (1,900 MHz) and Modulations (GSM and CDMA) Used by Cell Phones. National Toxicology Program, US Department

of Health and Human Services. [(accessed on 8 November 2018)];2018 Available online: https://ntp.niehs.nih.gov/results/areas/cellphones/index. html.

25. Tillmann T., Ernst H., Ebert S., Kuster N., Behnke W., Rittinghausen S., Dasenbrock C. Carcinogenicity study of GSM and DCS wireless communication signals in B6C3F1 mice. Bioelectromagnetics. 2007;28:173–187. doi: 10.1002/bem.20283. [PubMed] [CrossRef] [Google Scholar]

26. Tillmann T., Ernst H., Streckert J., Zhou Y., Taugner F., Hansen V., Dasenbrock C. Indication of cocarcinogenic potential of chronic UMTS-modulated radiofrequency exposure in an ethyl-nitrosourea mouse model. Int. J. Radiat. Biol. 2010;86:529–541. doi: 10.3109/09553001003734501. [PubMed] [CrossRef] [Google Scholar]

27. Lerchl A., Klose M., Grote K., Wilhelm A.F., Spathmann O., Fiedler T., Streckert J., Hansen V., Clemens M. Tumor promotion by exposure to radiofrequency electromagnetic fields below exposure limits for humans. Biochem. Biophys. Res. Commun. 2015;459:585–590. doi: 10.1016/j.bbrc.2015.02.151. [PubMed] [CrossRef] [Google Scholar]

28. Repacholi M.H., Basten A., Gebski V., Noonan D., Finnie J., Harris A.W. Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields. Radiat. Res. 1997;147:631–640. doi: 10.2307/3579630. [PubMed] [CrossRef] [Google Scholar]

29. Utteridge T.D., Gebski V., Finnie J.W., Vernon-Roberts B., Kuchel T.R. Long-term exposure of E-mu-Pim1 transgenic mice to 898.4 MHz microwaves does not increase lymphoma incidence. Radiat. Res. 2002;158:357–364. doi: 10.1667/0033-7587(2002)158[0357:LTEOEP]2.0.CO;2. [PubMed] [CrossRef] [Google Scholar]

30. Oberto G., Rolfo K., Yu P., Carbonatto M., Peano S., Kuster N., Ebert S., Tofani S. Carcinogenicity study of 217 Hz pulsed 900 MHz electromagnetic fields in Pim1 transgenic mice. Radiat. Res. 2007;168:316–326. doi: 10.1667/rr0425.1. [PubMed] [CrossRef] [Google Scholar]

31. Szmigielski S., Szudzinski A., Pietraszek A., Bielec M., Janiak M., Wrembel J.K. Accelerated development of spontaneous and benzopyrene-induced skin cancer in mice exposed to 2450-MHz microwave radiation. Bioelectromagnetics. 1982;3:179–191. doi: 10.1002/bem.2250030202. [PubMed] [CrossRef] [Google Scholar]

32. Organisation for Economic Co-operation and Development (OECD) Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4. OECD; Paris, France: 2018. [Google Scholar]

33. Guidance Document for the Developmental Neurotoxicity Testing Arm of the Modified One-Generation Study, NTP Modified One-Generation Study, MOG. [(accessed on 10 July 2019)];2015 Available online: https://ntp.niehs.nih.gov/ntp/test_info/mog_guidance_508.pdf.

34. Dasdag S., Zulkuf Akdag M., Aksen F., Yilmaz F., Bashan M., Mutlu Dasdag M., Salih Celik M. Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. Bioelectromagnetics. 2003;24:182–188. doi: 10.1002/bem.10083. [PubMed] [CrossRef] [Google Scholar]

35. Lee H.J., Pack J.K., Kim T.H., Kim N., Choi S.Y., Lee J.S., Kim S.H., Lee Y.S. The lack of histological changes of CDMA cellular phone-based radio frequency on rat testis. Bioelectromagnetics. 2010;31:528–534. doi: 10.1002/bem.20589. [PubMed] [CrossRef] [Google Scholar]

36. Lee H.J., Jin Y.B., Kim T.H., Pack J.K., Kim N., Choi H.D., Lee J.S., Lee Y.S. The effects of simultaneous combined exposure to CDMA and WCDMA electromagnetic fields on rat testicular function. Bioelectromagnetics. 2012;33:356–364. doi: 10.1002/bem.20715. [PubMed] [CrossRef]

[Google Scholar]

37. Imai N., Kawabe M., Hikage T., Nojima T., Takahashi S., Shirai T. Effects on rat testis of 1.95-GHz W-CDMA for IMT-2000 cellular phones. Syst. Biol. Reprod. Med. 2011;57:204–209. doi: 10.3109/19396368.2010.544839. [PubMed] [CrossRef] [Google Scholar]

38. Ribeiro E.P., Rhoden E.L., Horn M.M., Rhoden C., Lima L.P., Toniolo L. Effects of subchronic exposure to radio frequency from a conventional cellular telephone on testicular function in adult rats. J. Urol. 2007;177:395–399. doi: 10.1016/j.juro.2006.08.083. [PubMed] [CrossRef] [Google Scholar]

39. Trošić I., Mataušić-Pišl M., Pavičić I., Marjanović A.M. Histological and cytological examination of rat reproductive tissue after short-time intermittent radiofrequency exposure. Arh. Hig. Rada. Toksikol. 2013;64:513–519. doi: 10.2478/10004-1254-64-2013-2394. [PubMed] [CrossRef] [Google Scholar]

40. Yan J.G., Agresti M., Bruce T., Yan Y.H., Granlund A., Matloub H.S. Effects of cellular phone emissions on sperm motility in rats. Fertil. Steril. 2007;88:957–964. doi: 10.1016/j.fertnstert.2006.12.022. [PubMed] [CrossRef] [Google Scholar]

41. Qin F., Zhang J., Cao H., Guo W., Chen L., Shen Q., Sun J., Yi C., Li J., Wang J., et al. Circadian alterations of reproductive functional markers in male rats exposed to 1800 MHz radiofrequency field. Chronobiol. Int. 2014;31:123–133. doi: 10.3109/07420528.2013.830622. [PubMed] [CrossRef] [Google Scholar]

42. Guo L., Lin J.J., Xue Y.Z., An G.Z., Zhang J.P., Zhang K.Y., He W., Wang H., Li W., Ding G.R. Effects of 220 MHz pulsed modulated radiofrequency field on the sperm quality in rats. Int. J. Environ. Res. Public Health. 2019;16:1286. doi: 10.3390/ijerph16071286. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

43. Mailankot M., Kunnath A.P., Jayalekshmi H., Koduru B., Valsalan R. Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8 GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. Clinics (Sao Paulo) 2009;64:561–565. doi: 10.1590/S1807-59322009000600011. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

44. Kesari K.K., Kumar S., Behari J. Mobile phone usage and male infertility in Wistar rats. Indian J. Exp. Biol. 2010;48:987–992. [PubMed] [Google Scholar]

45. Kesari K.K., Kumar S., Behari J. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. Appl. Biochem. Biotechnol. 2011;164:546–559. doi: 10.1007/s12010-010-9156-0. [PubMed] [CrossRef] [Google Scholar]

46. Meo S.A., Arif M., Rashied S., Khan M.M., Vohra M.S., Usmani A.M., Imran M.B., Al-Drees A.M. Hypospermatogenesis and spermatozoa maturation arrest in rats induced by mobile phone radiation. J. Coll. Physicians Surg. Pak. 2011;21:262–265. [PubMed] [Google Scholar]

47. Kesari K.K., Behari J. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: Role of ROS. Electromagn. Biol. Med. 2012;31:213–222. doi: 10.3109/15368378.2012.700292. [PubMed] [CrossRef] [Google Scholar]

48. Tas M., Dasdag S., Akdag M.Z., Cirit U., Yegin K., Seker U., Ozmen M.F., Eren L.B. Long-term effects of 900 MHz radiofrequency radiation emitted from mobile phone on testicular tissue and epididymal semen quality. Electromagn. Biol. Med. 2014;33:216–222. doi: 10.3109/15368378.2013.801850. [PubMed] [CrossRef] [Google Scholar]

49. Meena R., Kumari K., Kumar J., Rajamani P., Verma H.N., Kesari K.K. Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats. Electromagn. Biol. Med. 2014;33:81–91. doi: 10.3109/15368378.2013.781035. [PubMed] [CrossRef] [Google Scholar]

50. Dasdag S., Taş M., Akdag M.Z., Yegin K. Effect of long-term exposure of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on testes functions. Electromagn. Biol. Med. 2015;34:37–42. doi: 10.3109/15368378.2013.869752. [PubMed] [CrossRef] [Google Scholar]

51. Akdag M.Z., Dasdag S., Canturk F., Karabulut D., Caner Y., Adalier N. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J. Chem. Neuroanat. 2016;75:116–122. doi: 10.1016/j.jchemneu.2016.01.003. [PubMed] [CrossRef] [Google Scholar]

52. Narayanan S.N., Lukose S.T., Arun G., Mohapatra N., Pamala J., Concessao P.L., Jetti R., Kedage V., Nalini K., Bhat P.G. Modulatory effect of 900 MHz radiation on biochemical and reproductive parameters in rats. Bratisl. Lek. Listy. 2018;119:581–587. doi: 10.4149/BLL_2018_105. [PubMed] [CrossRef] [Google Scholar]

53. Gautam R., Singh K.V., Nirala J., Murmu N.N., Meena R.M., Rajamani P. Oxidative stress-mediated alterations on sperm parameters in male Wistar rats exposed to 3G mobile phone radiation. Andrologia. 2019;51:e13201. doi: 10.1111/and.13201. [PubMed] [CrossRef] [Google Scholar]

54. Aitken R.J., Bennetts L.E., Sawyer D., Wiklendt A.M., King B.V. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. Int. J. Androl. 2005;28:171–179. doi: 10.1111/j.1365-2605.2005.00531.x. [PubMed] [CrossRef] [Google Scholar]

55. Otitoloju A.A., Obe I.A., Adewale O.A., Otubanjo O.A., Osunkalu V.O. Preliminary study on the induction of sperm head abnormalities in mice, Mus musculus, exposed to radiofrequency radiations from global system for mobile communication base stations. Bull. Environ. Contam. Toxicol. 2010;84:51–54. doi: 10.1007/s00128-009-9894-2. [PubMed] [CrossRef] [Google Scholar]

56. Pandey N., Giri S., Das S., Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. Toxicol. Ind. Health. 2017;33:373–384. doi: 10.1177/0748233716671206. [PubMed] [CrossRef] [Google Scholar]

57. Pandey N., Giri S. Melatonin attenuates radiofrequency radiation (900 MHz)-induced oxidative stress, DNA damage and cell cycle arrest in germ cells of male Swiss albino mice. Toxicol. Ind. Health. 2018;34:315–327. doi: 10.1177/0748233718758092. [PubMed] [CrossRef] [Google Scholar]

58. Salama N., Kishimoto T., Kanayama H.O., Kagawa S. The mobile phone decreases fructose but not citrate in rabbit semen: A longitudinal study. Syst. Biol. Reprod. Med. 2009;55:181–187. doi: 10.3109/19396360903013126. [PubMed] [CrossRef] [Google Scholar]

59. Salama N., Kishimoto T., Kanayama H.O. Effects of exposure to a mobile phone on testicular function and structure in adult rabbit. Int. J. Androl. 2010;33:88–94. doi: 10.1111/j.1365-2605.2008.00940.x. [PubMed] [CrossRef] [Google Scholar]

60. Lary J.M., Conover D.L., Johnson P.H. Absence of embryotoxic effects from low-level (nonthermal) exposure of rats to 100 MHz radiofrequency radiation. Scand. J. Work Environ. Health. 1983;9:120–127. doi: 10.5271/sjweh.2434. [PubMed] [CrossRef] [Google Scholar]

61. Ogawa K., Nabae K., Wang J., Wake K., Watanabe S., Kawabe M., Fujiwara O., Takahashi S., Ichihara T., Tamano S., et al. Effects of gestational exposure to 1.95-GHz W-CDMA signals for IMT-2000 cellular phones: Lack of embryotoxicity and teratogenicity in rats. Bioelectromagnetics. 2009;30:205–212. doi: 10.1002/bem.20456. [PubMed] [CrossRef] [Google Scholar]

62. Takahashi S., Imai N., Nabae K., Wake K., Kawai H., Wang J., Watanabe S., Kawabe M., Fujiwara O., Ogawa K., et al. Lack of adverse effects of whole-body exposure to a mobile telecommunication electromagnetic field on the rat fetus. Radiat. Res. 2010;173:362–372. doi: 10.1667/RR1615.1. [PubMed] [CrossRef] [Google Scholar]

63. Poulletier de Gannes F., Haro E., Hurtier A., Taxile M., Athane A., Ait-Aissa S., Masuda H., Percherncier Y., Ruffié G., Billaudel B., et al. Effect of in utero wi-fi exposure on the pre- and postnatal development of rats. Birth Defects Res. B Dev. Reprod. Toxicol. 2012;95:130–136. doi: 10.1002/bdrb.20346. [PubMed] [CrossRef] [Google Scholar]

64. Poulletier de Gannes F., Billaudel B., Haro E., Taxile M., Le Montagner L., Hurtier A., Ait Aissa S., Masuda H., Percherancier Y., Ruffié G., et al. Rat fertility and embryo fetal development: Influence of exposure to the Wi-Fi signal. Reprod. Toxicol. 2013;36:1–5. doi: 10.1016/j.reprotox.2012.11.003. [PubMed] [CrossRef] [Google Scholar]

65. Aït-Aïssa S., Billaudel B., Poulletier de Gannes F., Ruffié G., Duleu S., Hurtier A., Haro E., Taxile M., Athané A., Geffard M., et al. In utero and early-life exposure of rats to a Wi-Fi signal: Screening of immune markers in sera and gestational outcome. Bioelectromagnetics. 2012;33:410–420. doi: 10.1002/bem.21699. [PubMed] [CrossRef] [Google Scholar]

66. Lary J.M., Conover D.L., Foley E.D., Hanser P.L. Teratogenic effects of 27.12 MHz radiofrequency radiation in rats. Teratology. 1982;26:299–309. doi: 10.1002/tera.1420260312. [PubMed] [CrossRef] [Google Scholar]

67. Lary J.M., Conover D.L., Johnson P.H., Burg J.R. Teratogenicity of 27.12-MHz radiation in rats is related to duration of hyperthermic exposure. Bioelectromagnetics. 1983;4:249–255. doi: 10.1002/bem.2250040305. [PubMed] [CrossRef] [Google Scholar]

68. Nelson B.K., Conover D.L., Brightwell W.S., Shaw P.B., Werren D., Edwards R.M., Lary J.M. Marked increase in the teratogenicity of the combined administration of the industrial solvent 2-methoxyethanol and radiofrequency radiation in rats. Teratology. 1991;43:621–634. doi: 10.1002/tera.1420430618. [PubMed] [CrossRef] [Google Scholar]

69. Nelson B.K., Conover D.L., Shaw P.B., Werren D.M., Edwards R.M., Hoberman A.M. Interactive developmental toxicity of radiofrequency radiation and 2-methoxyethanol in rats. Teratology. 1994;50:275–293. doi: 10.1002/tera.1420500403. [PubMed] [CrossRef] [Google Scholar]

70. Nelson B.K., Conover D.L., Krieg E.F., Jr., Snyder D.L., Edwards R.M. Interactions of radiofrequency radiation-induced hyperthermia and 2-methoxyethanol teratogenicity in rats. Bioelectromagnetics. 1997;18:349–359. doi: 10.1002/(SICI)1521-186X(1997)18:5<349::AID-BEM2>3.0.CO;2-2. [PubMed] [CrossRef] [Google Scholar]

71. Nelson B.K., Conover D.L., Shaw P.B., Snyder D.L., Edwards R.M. Interactions of radiofrequency radiation on 2-methoxyethanol teratogenicity in rats. J. Appl. Toxicol. 1997;17:31–39. doi: 10.1002/(SICI)1099-1263(199701)17:1<31::AID-JAT384>3.0.CO;2-1. [PubMed] [CrossRef] [Google Scholar]

72. Nelson B.K., Snyder D.L., Shaw P.B. Developmental toxicity interactions of methanol and radiofrequency radiation or 2-methoxyethanol in rats. Int. J. Toxicol. 2001;20:89–100. [PubMed] [Google Scholar]

73. Erkut A., Tumkaya L., Balik M.S., Kalkan Y., Guvercin Y., Yilmaz A., Yuce S., Cure E., Sehitoglu I. The effect of prenatal exposure to 1800 MHz electromagnetic field on calcineurin and bone development in rats. Acta Cir. Bras. 2016;31:74–83. doi: 10.1590/S0102-865020160020000001. [PubMed] [CrossRef] [Google Scholar]

74. Oral B., Guney M., Ozguner F., Karahan N., Mungan T., Comlekci S., Cesur G. Endometrial apoptosis induced by a 900-MHz mobile phone: Preventive effects of vitamins E and C. Adv. Ther. 2006:23:957–973. doi: 10.1007/BF02850217. [PubMed] [CrossRef] [Google Scholar]

75. Sommer A.M., Grote K., Reinhardt T., Streckert J., Hansen V., Lerchl A. Effects of radiofrequency electromagnetic fields (UMTS) on reproduction and development of mice: A multi-generation study. Radiat. Res. 2009:171:89–95. doi: 10.1667/RR1460.1. [PubMed] [CrossRef] [Google Scholar]

76. Zhu S., Zhang J., Liu C., He Q., Vijayalaxmi P.T.J., Tong J., Cao Y. Dominant lethal mutation test in male mice exposed to 900MHz radiofrequency fields. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 2015:792:53–57. doi: 10.1016/j.mrgentox.2015.07.004. [PubMed] [CrossRef] [Google Scholar]

77. Finnie J.W., Blumbergs P.C., Cai Z., Manavis J., Kuchel T.R. Effect of mobile telephony on blood-brain barrier permeability in the fetal mouse brain. Pathology. 2006:38:63–65. doi: 10.1080/00313020500459607. [PubMed] [CrossRef] [Google Scholar]

78. Finnie J.W., Cai Z., Blumbergs P.C., Manavis J., Kuchel T.R. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 2006:38:333–335. doi: 10.1080/00313020600820864. [PubMed] [CrossRef] [Google Scholar]

79. Finnie J.W., Chidlow G., Blumbergs P.C., Manavis J., Cai Z. Heat shock protein induction in fetal mouse brain as a measure of stress after whole of gestation exposure to mobile telephony radiofrequency fields. Pathology. 2009;41:276–279. doi: 10.1080/00313020902756261. [PubMed] [CrossRef] [Google Scholar]

80. Magras I.N., Xenos T.D. RF radiation-induced changes in the prenatal development of mice. Bioelectromagnetics. 1997;18:455–461. doi: 10.1002/(SICI)1521-186X(1997)18:6<455::AID-BEM8>3.0.CO;2-1. [PubMed] [CrossRef] [Google Scholar]

81. Gul A., Celebi H., Ugras S. The effects of microwave emitted by cellular phones on ovarian follicles in rats. Arch. Gynecol. Obstet. 2009;280:729–733. doi: 10.1007/s00404-009-0972-9. [PubMed] [CrossRef] [Google Scholar]

82. Sommer A.M., Streckert J., Bitz A.K., Hansen V.W., Lerchl A. No effects of GSM-modulated 900 MHz electromagnetic fields on survival rate and spontaneous development of lymphoma in female AKR/J mice. BMC Cancer. 2004;4:77. doi: 10.1186/1471-2407-4-77. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

83. Sommer A.M., Bitz A.K., Streckert J., Hansen V.W., Lerchl A. Lymphoma development in mice chronically exposed to UMTS-modulated radiofrequency electromagnetic fields. Radiat. Res. 2007;168:72–80. doi: 10.1667/RR0857.1. [PubMed] [CrossRef] [Google Scholar]

84. Lee H.J., Jin Y.B., Lee J.S., Choi S.Y., Kim T.H., Pack J.K., Choi H.D., Kim N., Lee Y.S. Lymphoma development of simultaneously combined exposure to two radiofrequency signals in AKR/J mice. Bioelectromagnetics. 2011;32:485–492. doi: 10.1002/bem.20655. [PubMed] [CrossRef] [Google Scholar]

85. Panagopoulos D.J., Johansson O., Carlo G.L. Real versus simulated mobile phone exposures in experimental studies. BioMed Res. Int. 2015;2015:607053. doi: 10.1155/2015/607053. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

86. Panagopoulos D.J. Comparing DNA Damage Induced by Mobile Telephony and Other Types of Man-Made Electromagnetic Fields. Mut. Res. Rev. 2019;781:53–62. doi: 10.1016/j.mrrev.2019.03.003. [PubMed] [CrossRef] [Google Scholar]

87. IARC Monographs Priorities Group Advisory Group recommendations on priorities for the IARC Monographs. Lancet Oncol. 2019;20:763–764. doi: 10.1016/S1470-2045(19)30246-3. [PubMed] [CrossRef] [Google Scholar]

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Research Article

Evaluation of the Genotoxicity of Cell Phone Radiofrequency Radiation in Male and Female Rats and Mice Following Subchronic Exposure

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The National Toxicology Program tested two common radiofrequency radiation (RFR) modulations emitted by cellular telephones in a 2-year rodent cancer bioassay that included interim assessments of additional animals for genotoxicity endpoints. Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice were exposed from Gestation day 5 or Postnatal day 35, respectively, to code division multiple access (CDMA) or global system for mobile modulations over 18 hr/day, at 10-min intervals, in reverberation chambers at specific absorption rates of 1.5, 3, or 6 W/kg (rats, 900 MHz) or 2.5, 5, or 10 W/kg (mice, 1,900 MHz). After 19 (rats) or 14 (mice) weeks of exposure, animals were examined for evidence of RFR-associated genotoxicity using two different measures. Using the alkaline (pH > 13) comet assay, DNA damage was assessed in cells from three brain regions, liver cells, and peripheral blood leukocytes; using the micronucleus assay, chromosomal damage was assessed in immature and mature peripheral blood erythrocytes. Results of the comet assay showed significant increases in DNA damage in the frontal cortex of male mice (both modulations), leukocytes of female mice (CDMA only), and hippocampus of male rats (CDMA only). Increases in DNA damage judged to be equivocal were observed in several other tissues of rats and mice. No significant increases in micronucleated red blood cells were observed in rats or mice. In conclusion, these results suggest that exposure to RFR is associated with an increase in DNA damage. Environ. Mol. Mutagen. 00:000–000, 2019.

Key words: DNA damage; micronucleus assay; comet assay; brain; Sprague Dawley; glioma

INTRODUCTION

Over the past two decades, cellular telephone use has become nearly ubiquitous worldwide; cell phone subscriptions numbered ~7.68 billion in 2017 according to the International Telecommunication Union (2017) with ~5.12 billion unique subscribers (GSMA Intelligence 2019). Radiofrequency radiation (RFR) is a form of electromagnetic radiation that ranges from 3 kHz to 300 GHz. Most cell phones transmit RFR signals within the 800–900 and 1,800–2,200 MHz ranges (International Agency for Research on Cancer [IARC] Working Group on the Evaluation of Carcinogenic Risks to Humans 2013).

Concern exists as to whether cell phone RFR frequencies are capable of adversely affecting human health. Although some epidemiological studies suggest that cell phone use might increase the risk for certain brain

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cancers, such as gliomas and acoustic neuromas (a,k,a, vestibular schwannomas), the odds ratios for these increased risks are quite low (INTERPHONE Study Group 2010; Cardis et al. 2011; Hardell et al. 2011; Larjavaara et al. 2011; Sato et al. 2011; Hardell and Carlberg 2015). Conclusions drawn from these observations may be premature, as cell phone use has become commonplace only within the past two decades, a period of time that may be insufficient to accurately assess cancer-related outcomes. Results of previous rodent cancer studies conducted with a variety of RFR exposures and durations are inconsistent and inconclusive, and many of these studies used experimental protocols with important limitations, indicating a need for a more definitive study (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013).

Additionally, extensive reviews of the literature on the genotoxicity of various frequencies and modulations of RFR have concluded that evidence for RFR-associated genotoxicity is inconsistent and weak (Brusick et al. 1998; Ruediger 2009; Verschaeve et al. 2010), and some key studies reporting RFR-associated genotoxicity in human cell lines could not be replicated (Speit et al. 2013). As with the cancer studies, interpretations of the genotoxicity studies, particularly those performed *in vivo*, have also been limited by issues of experimental design. In 2013, after reviewing the available data, the IARC classified radiofrequency electromagnetic fields (RF-EMF), which include the RFR wavelength range, as "possibly carcinogenic to humans (Group 2B)," based on limited evidence in experimental animals and limited evidence in humans on the association between RF-EMF and cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013).

To help inform human health risk assessments, the National Toxicology Program (NTP) designed and conducted a 2-year rodent cancer study of cell phone RFR, using code division multiple access (CDMA) or global system for mobile (GSM) modulations, the principal modulations used in the United States (CDMA and GSM) and in the rest of the world (GSM). GSM and CDMA are second-generation (2G) and third-generation (3G) technologies, respectively, and they differ in the method in which information is incorporated and transmitted within frequency bands. The previous inconsistent genotoxicity and tumorigenicity findings that have been reported following RFR exposure could be due in part to the immense and unique technical challenges inherent in studying the effects of nonionizing radiation, including RFR (Capstick et al. 2017; Gong et al. 2017). To address these challenges and provide data to clarify possible adverse biological effects of cell phone RFR exposure, the NTP took into account numerous variables and parameters in designing its rodent cancer bioassay. Key features included construction of custom-designed reverberation chambers that exposed animals to a clearly defined, statistically homogenous radiofrequency field, that shielded animals from all other sources of RFR, and eliminated the need for restraint, a method commonly employed by other researchers for point-source exposures (Capstick et al. 2017; Gong et al. 2017). Animals were housed inside the reverberation chambers and exposed to RFR for a total of 9 hr 10 min per day in 10-min on/off cycles (over the course of an ~18 hr period) at frequencies with modulations being used in cellular networks (Capstick et al. 2017). In addition, the exposure levels selected for this study were based on the results of previously conducted dosimetry studies and thermal pilot studies that demonstrated no measurable hyperthermia in rats and mice at the exposure levels chosen for this study (Gong et al. 2017; Wyde et al. 2018).

In the NTP study design, Sprague Dawley rats and B6C3F1/N mice of both sexes were whole-body exposed to RFR (CDMA or GSM modulations). Rats were exposed *in utero* beginning on Gestation day 5 (GD5), and mice were exposed beginning at 5 weeks of age. After a total of 19 weeks of exposure for rats and 14 weeks for mice, subsets of 5 rats and 5 mice of each sex from each exposure group were removed from the ongoing 2-year cancer bioassay after subchronic exposure and assessed for DNA damage using the comet assay, and for changes in chromosomal structure and/or number using the peripheral blood erythrocyte micronuclei (MN) assay. For the comet assay, cells from three functionally distinct structures of the brain (frontal cortex, hippocampus, and cerebellum), along with liver cells and peripheral blood leukocytes were assessed. Brain tissue was analyzed in the comet assay due to concerns that RFR may increase risk for brain cancer in humans, whereas liver cells and blood leukocytes were selected for analysis as these cells are part of typical analyses conducted at the NTP for DNA damage.

MATERIALS AND METHODS

Animal Husbandry

Time-mated Hsd:Sprague Dawley SD rats (11–14 weeks of age) (Harlan, Indianapolis, IN) were received on GD2 at the laboratory (Illinois Institute of Technology Research Institute; IITRI, Chicago, IL). After littering, male and female pups were housed with their dams until weaning on Postnatal day 28 (PND28). During the perinatal phase, rats were fed irradiated NIH-07 wafers; from weaning until study completion, rats were fed irradiated NTP-2000 rodent diet (Zeigler Brothers, Gardners, PA). Male and female B6C3F1/N mice (Taconic, Germantown, NY) were received at 3–4 weeks of age. Mice were quarantined for 10–14 days and were 5–6 weeks of age at the start of exposure. Mice were fed irradiated NTP-2000 rodent diet. All animals were provided food and tap water (city of Chicago, IL, municipal supply) *ad libitum*. During the studies, animal health was monitored according to the NTP sentinel animal program. Mice, and rats after weaning, were housed individually in solid polycarbonate cages with irradiated hardwood bedding (Sani-chips, P.J. Murphy, Montville, NJ) within custom designed, stainless steel reverberation chambers. Environmental conditions were set to maintain a 12-hr light/dark

cycle, a temperature of 72 ± 3°F, a humidity range of 50 ± 15%, and >10 air changes/hr.

Animal use was in accordance with the U.S. Public Health Service policy on humane care and use of laboratory animals and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). Animal housing facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care; all procedures were approved by the IITRI Institutional Animal Care and Use Committee. The RFR exposures performed at IITRI were in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21CFR, Part 58). Animals were euthanized by CO_2 asphyxiation.

Reverberation Chambers

Reverberation chambers were self-contained rooms that were designed to house unrestrained animals in cages and expose them to a uniform field of RFR (GSM or CDMA) and to shield animals from all outside RFR. Detailed descriptions of the design of the reverberation chambers and the RFR exposure system are provided in Capstick et al. (2017) and Gong et al. (2017). Uniformity of the RFR field was achieved by installing excitation antennas with rotating horizontal and vertical reflective surface paddles to ensure uniform distribution of statistically homogenous RFR fields within the volume of the chambers. Therefore, animals were exposed to all polarizations of RFR fields from all directions regardless of their posture or orientation to the antenna. Animals were housed one per cage to prevent interference in energy absorption. Cages, cage racks, and materials used to deliver food and water to the animals were designed to minimize interference with RFR exposure. Chamber design and animal housing were developed in collaboration with the National Institute of Standards and Technology (NIST) and the Foundation for Research on Information Technologies in Society (IT'IS). RFR field intensity, uniformity, quality of modulation, and numerous other parameters were validated by NIST, and consistency of exposure was monitored in real time by IT'IS. Further evaluation of the exposure systems is presented in NTP Technical Reports 595 (NTP 2018a) and 596 (NTP 2018b).

Dosimetry, Specific Absorption Rates, and Exposure Regimen

Briefly, in pilot studies, body temperatures were monitored using subcutaneously implanted temperature chips (Wyde et al. 2018). Both young and older animals were tested for the possibility of thermal effects from radiation. An upper limit of 1°C was set as an acceptable increase in body temperature. Models predicted that thermoregulation might not be maintained in rats exposed to an specific absorption rate (SAR) > 6.0 W/kg, delivered at a frequency of 900 MHz, and in mice exposed to an SAR > 10.0 W/kg, delivered at a frequency of 1,900 MHz (Gong et al. 2017; Wyde et al. 2018). Thus, these were selected as the highest exposure levels in the current study, and the two lower exposures were each reduced by half. Due to technical constraints, body temperatures could not be monitored in the current study.

Rats were exposed to SARs of 0, 1.5, 3.0, or 6.0 W/kg (CDMA or GSM) RFR (900 MHz) beginning *in utero* at GD5 and continuing through gestation (~2 weeks) until weaning at PND28. Exposures continued for 14 weeks after weaning. Mice were exposed to SARs of 0, 2.5, 5.0, or 10.0 W/kg (CDMA or GSM) RFR (1,900 MHz) for 14 weeks beginning at 5–6 weeks of age. Rat exposures were initiated at the time of implantation (GD5) to simulate whole-life exposures in humans, but because B6C3F1/N mice are poor and unpredictable breeders, this animal model is not suitable for whole-life exposure assessments. Exposures ran daily from 11:00 A.M. to 2:00 P.M. and from 3:40 P.M. to 7:00 A.M., with RFR cycling on and off every 10 min, resulting in a total duration of exposure of 9 hr 10 min per 24-hr period. This exposure schedule accommodated two daily intervals for animal care. Animals were housed individually in a total of 21 reverberation chambers, 7 for the mice and 14 for the rats. Each reverberation chamber emitted one power level for one modulation. Male and female mice, due to similarity in weight and size, were exposed together in 7 reverberation chambers. In contrast, due to gender-related differences in weight and size, male and female rats were exposed in separate chambers, thus requiring 14 chambers. To control for possible positional differences in RFR field strength, cages were rotated in the racks weekly. Because SAR is dependent on body weight, the energy used to emit RFR was adjusted twice weekly for rats and once weekly for mice based on the average weight of all animals in an exposure chamber.

The sham control rats and mice were housed in reverberation chambers without activation of RFR. One group of five animals of each sex/species served as the sham control for both CDMA and GSM exposures.

Tissue Sample Collection

On the day of necropsy, RFR exposure ceased at 7 A.M. Necropsies were performed in two shifts. For each species, 35 male animals (5 controls, 15 exposed to CDMA, and 15 exposed to GSM) were necropsied 1.5–4 hr after cessation of exposure and 35 female animals (5 controls, 15 exposed to CDMA, and 15 exposed to GSM) were necropsied approximately 4.5–7 hr after cessation of exposure. Animals were necropsied in the following order: one animal from each dose group starting with the sham exposed group, moving through each dose group for each RFR modulation in turn, then rotating back to the sham control group; animals were necropsied in numerical order within each dose group. Five tissues were collected from each animal for the comet assay. One blood sample per animal collected by retro-orbital bleeding was divided into two aliquots: one for the comet assay and the other for the MN assay.

For the comet assay, 50 µL of blood were transferred to a tube containing 1 mL of freshly prepared cold mincing buffer (Mg^{+2}, Ca^{+2}, and phenol free Hank's Balanced Salt Solution [Life Technologies, Carlsbad, CA] with 20 mM ethylenediamine tetraacetic acid (EDTA) pH 10.0 and 10% vol/vol fresh dimethyl sulfoxide [DMSO]) pH 7.47. The liver and the hippocampus, cerebellum, and frontal cortex sections of the brain were removed, rinsed with cold mincing buffer, and held on ice (≤5 min) until processed. Small portions (3–4 mm^3) of each tissue were placed in tubes containing cold mincing solution and rapidly minced until finely dispersed. Blood and minced tissue samples were immediately flash frozen in liquid nitrogen and transferred to a −80°C freezer for a minimum of 1 week until shipment by overnight air courier on dry ice to the analytical laboratory (ILS, Research Triangle Park, NC).

For the MN assay, blood samples (~200 µL) were placed into EDTA tubes and immediately refrigerated. The samples were sent on the day of collection to ILS on cold packs via overnight air courier. Upon arrival, samples were diluted in anticoagulant (heparin) and fixed in ice-cold methanol (Sigma-Aldrich, St. Louis, MO) according to instructions provided with the MicroFlow^{PLUS} Kit (Litron Laboratories, Rochester, NY). Fixed samples were stored in a −80°C freezer for at least 3 days prior to analysis by flow cytometry.

Comet Assay

Slides were prepared and analyzed as described previously (Hobbs et al. 2012; Recio et al. 2012) with some modifications. In a laboratory with controlled humidity (≤60%), samples were thawed on ice and a portion of the cell suspension was diluted with 0.5% low melting point agarose (Lonza, Walkersville, MD) dissolved in Dulbecco's phosphate buffer (Ca^{+2}, Mg^{+2}, and phenol free) at 37°C and layered onto each well of a 2-well CometSlide™ (Trevigen, Gaithersburg, MD). Slides were prepared one tissue at a time, such that 35 slides were prepared at a time in 3 batches of 10 and 1 batch of 5, and each batch was immediately refrigerated to solidify the agarose and prevent deterioration of the samples. Once all slides per tissue had been prepared and refrigerated for at least 20 min (typically ≤2 hr for completion of an entire set), the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM tris

[hydroxymethyl]aminomethane, pH 10, containing freshly added 10% DMSO, and 1% Triton X-100) overnight with refrigeration. After rinsing in 0.4 M Trizma base (pH 7.5), slides were treated with cold alkali solution (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) for 20 min to allow DNA unwinding, electrophoresed at 4–9°C for 20 min at 25 V (0.7 V/cm), with a current of approximately 300 mA, neutralized with Trizma base, dehydrated in absolute ethanol (Pharmco-AAPER, Shelbyville, KY), and air-dried. Slides from the same species, sex, and tissue were run together during electrophoresis and were placed randomly into the electrophoresis tank by exposure level and modulation to control for any possible variations in electrical field. Slides were stored at room temperature in a desiccator (relative humidity ≤60%) until stained and scored. NaCl, Na_2EDTA, Triton X-100, DMSO, and Trizma base were purchased from Sigma-Aldrich; NaOH was purchased from Fisher Scientific (Pittsburgh, PA).

After staining with SYBR® Gold (Molecular Probes, Life Technologies, Grand Island, NY), slides, independently coded to mask treatment, were scored using Comet Assay IV Imaging Software, Version 4.3.1 (Perceptive Instruments, Suffolk, UK). DNA migration was quantified as % tail DNA (OECD 2016). Comets were classified as scorable, nonscorable, or "hedgehog." Comets were classified as hedgehogs if they had no easily defined head, that is, all DNA appeared to be in the tail, or the head and tail appeared separated. Initially, % tail DNA was determined for 100 scorable comet figures per animal/tissue, standard practice at the time the study was conducted (prior to OECD Guideline 489). In addition, the frequency of hedgehogs was determined by tabulating the number of hedgehogs per 100 cells per animal/tissue, but hedgehog frequencies were not analyzed for statistical significance, in accordance with OECD Guideline 489. Although it has been proposed that hedgehogs are apoptotic cells, some studies strongly suggest that hedgehogs represent cells with high levels of repairable DNA damage (Rundell et al. 2003; Lorenzo et al. 2013), and it remains uncertain in the field as to what hedgehogs represent.

In the initial scoring of the rat samples, we noted that the range of % tail DNA values appeared truncated at ~ 65%. To better understand this observation, we reanalyzed the rat slides, scoring 150 cells/tissue/animal, as recommended by the OECD guideline (OECD 2016). In this second scoring exercise, we included analysis of scorable comet images that, upon visual inspection, appeared to be hedgehogs to determine if this affected the capture of DNA damage levels between 65 and 100% tail DNA. For the 150-cell scoring method, because the % hedgehogs were not independently determined, the value was estimated by dividing the number of comets with ≥90% tail DNA by 150. Several mouse tissues were also

TABLE 1. DNA damage in Male Sprague Dawley Rats Exposed to CDMA-Modulated Cell Phone Radiofrequency Radiation (900 MHz) for 19 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value[c]	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value	% Hedgehogs (150 cells)[b,d]
Frontal cortex							
	0[e]	6.18 ± 0.72		2.00 ± 0.71	9.73 ± 0.81		0.27 ± 0.27
CDMA	1.5	6.00 ± 0.48	1.000	1.00 ± 0.77	8.24 ± 0.39	1.000	0.13 ± 0.13
	3.0	9.51 ± 1.17	0.081	10.60 ± 3.89	18.77 ± 3.27	0.043	2.53 ± 1.29
	6.0	12.78 ± 3.96	0.049	12.20 ± 6.84	23.62 ± 8.66	0.092	3.20 ± 1.72
		P = 0.004			*P* = 0.005[f]		
Hippocampus							
	0	5.88 ± 0.39		3.40 ± 1.21	8.99 ± 1.55		1.07 ± 0.45
CDMA	1.5	8.06 ± 1.20	0.135	3.80 ± 2.33	12.27 ± 2.21	0.244	0.40 ± 0.27
	3.0	8.16 ± 0.98	0.151	6.20 ± 2.56	15.46 ± 2.25	0.107	2.53 ± 0.90
	6.0	10.42 ± 2.18	0.019	4.40 ± 2.98	16.77 ± 5.44	0.069	2.40 ± 1.44
		P = 0.014			*P* = 0.043		
Cerebellum							
	0	5.57 ± 0.92		0.40 ± 0.24	4.90 ± 0.82		0 ± 0
CDMA	1.5	5.60 ± 0.71	1.000	1.80 ± 0.80	6.33 ± 1.00	0.681	0.27 ± 0.16
	3.0	10.70 ± 3.66	0.504	9.40 ± 6.81	13.75 ± 6.01	0.504	2.93 ± 2.20
	6.0	10.58 ± 3.52	0.731	8.00 ± 3.91	15.86 ± 5.91	0.163	2.40 ± 1.07
		P = 0.156			*P* = 0.061		
Liver							
	0	13.81 ± 2.88		33.60 ± 17.89	25.71 ± 8.71		1.73 ± 1.73
CDMA	1.5	22.99 ± 2.77	0.081	68.60 ± 15.70	55.41 ± 7.91	0.136	14.67 ± 5.57
	3.0	16.04 ± 2.14	0.098	7.80 ± 0.86	19.11 ± 2.28	0.164	0.80 ± 0.49
	6.0	20.79 ± 3.10	0.057	41.10 ± 14.80	40.01 ± 7.90	0.114	9.07 ± 7.10
		P = 0.154			*P* = 0.385		
Peripheral blood leukocytes							
	0	1.48 ± 0.29		0.20 ± 0.20	0.69 ± 0.20		0 ± 0
CDMA	1.5	1.22 ± 0.45	0.596	0.80 ± 0.80	1.16 ± 0.47	0.295	0 ± 0
	3.0	2.13 ± 0.34	0.156	0.40 ± 0.40	1.83 ± 0.74	0.121	0.13 ± 0.13
	6.0	2.08 ± 0.43	0.166	1.40 ± 1.17	2.57 ± 0.80	0.026	0 ± 0
		P = 0.071			*P* = 0.012		

[a]Exposure began *in utero* on GD5.

[b]Mean ± SE.

[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \leq 0.025$ by Williams' or Dunn's test.

[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.

[e]Sham control; no exposure to CDMA-modulated cell phone RFR.

[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \leq 0.025$.

reevaluated using the 150-cell method for comparison. Although there was no concurrent positive control group (as is standard for all NTP chronic and subchronic animal toxicity tests), slides made with human lymphoblastoid TK6 cells treated with ethyl methanesulfonate were processed in parallel with each tissue set as an internal technical control for slide preparation, staining, and electrophoresis.

Micronucleus Assay

Flow cytometric analysis of red blood cells was performed using MicroFlow^{PLUS} Kit reagents and a FACSCalibur^{TM} dual-laser bench top system (Becton Dickinson Biosciences, San Jose, CA) as described previously (Witt et al. 2008) and was consistent with OECD Test Guideline 474 (OECD 2014). Briefly, both immature erythrocytes (reticulocytes, RET) and mature erythrocytes were analyzed for the presence of MN. For each sample, 20,000 (±2,000) RET were analyzed and ~1 × 10^6 mature erythrocytes were enumerated concurrently during micronucleated-RET (MN-RET) analysis, allowing for calculation of the percentage of RET (%RET) among total erythrocytes as a measure of bone marrow toxicity.

Data analysis

Data from both the comet and the MN assays, presented as mean ± standard error (SE), were analyzed using the same statistical methods (Kissling et al. 2007). Mean % tail DNA was calculated for each tissue per animal; likewise, mean MN–RET and MN–erythrocytes per 1,000 cells, as well as %RET, were calculated for each animal. Levene's test was used to determine if variances among treatment groups were equal at significance level 0.05. When variances were equal, linear regression analysis was used to test for trend and Williams' test was used to evaluate pairwise differences between each treated group and the control. When variances were unequal, Jonckheere's test was used to evaluate linear trend and Dunn's test was used to assess the significance of pairwise differences of each treated group with the control group. To maintain the overall significance level at 0.05, the trend as well as the pairwise differences were declared statistically significant if $P < 0.025$. A result was considered positive if the trend test was significant and at least one dose group was significantly elevated over the control, or if two or more dose groups were significantly increased over the corresponding control. A response was considered equivocal if only

TABLE II. DNA Damage in Male Sprague Dawley Rats Exposed to GSM-Modulated Cell Phone Radiofrequency Radiation (900 MHz) for 19 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value[c]	% Hedgehogs (150 cells)[b,d]
Frontal cortex							
	0[e]	6.18 ± 0.72		2.00 ± 0.71	9.73 ± 0.81		0.27 ± 0.27
GSM	1.5	6.98 ± 0.42	0.465	1.40 ± 0.51	11.96 ± 1.65	0.634	0.40 ± 0.27
	3.0	8.66 ± 1.96	0.247	8.20 ± 2.69	17.98 ± 5.12	0.545	1.20 ± 0.57
	6.0	6.30 ± 0.32	1.000	3.00 ± 1.55	9.57 ± 1.57	1.000	1.30 ± 0.13
		$P = 0.343$			$P = 0.500$[f]		
Hippocampus							
	0	5.88 ± 0.39		3.40 ± 1.21	8.99 ± 1.55		1.07 ± 0.45
GSM	1.5	11.82 ± 2.68	0.092	4.80 ± 2.84	17.24 ± 4.09	0.186	0.27 ± 0.16
	3.0	9.64 ± 1.27	0.111	4.80 ± 1.53	14.77 ± 2.54	0.227	1.47 ± 0.57
	6.0	11.69 ± 3.92	0.072	10.20 ± 7.98	21.32 ± 9.55	0.080	3.60 ± 2.03
		$P = 0.103$			$P = 0.076$		
Cerebellum							
	0	5.57 ± 0.92		0.40 ± 0.24	4.90 ± 0.82		0 ± 0
GSM	1.5	7.36 ± 2.48	0.295	2.40 ± 1.91	9.43 ± 4.69	0.190	1.33 ± 1.17
	3.0	6.37 ± 0.77	0.354	3.40 ± 1.17	8.66 ± 2.17	0.232	1.47 ± 0.68
	6.0	8.48 ± 1.85	0.149	5.00 ± 2.86	12.11 ± 3.89	0.088	1.07 ± 1.07
		$P = 0.132$			$P = 0.076$		
Liver							
	0	13.81 ± 2.88		33.60 ± 17.89	25.71 ± 8.71		1.73 ± 1.73
GSM	1.5	13.26 ± 2.38	0.547	21.00 ± 12.30	23.27 ± 9.43	0.539	4.13 ± 3.64
	3.0	13.09 ± 2.32	0.634	28.40 ± 15.07	25.15 ± 8.43	0.604	0.40 ± 0.40
	6.0	14.49 ± 2.71	0.536	24.80 ± 16.13	28.25 ± 10.55	0.534	4.93 ± 3.94
		$P = 0.404$			$P = 0.390$		
Peripheral blood leukocytes							
	0	1.48 ± 0.29		0.20 ± 0.20	0.69 ± 0.20		0 ± 0
GSM	1.5	1.83 ± 0.63	0.352	3.20 ± 2.71	3.97 ± 2.75	0.146	0.27 ± 0.27
	3.0	1.78 ± 0.33	0.419	1.20 ± 0.49	1.97 ± 0.35	0.021	0 ± 0
	6.0	1.50 ± 0.27	0.446	0.40 ± 0.24	1.28 ± 0.23	0.272	0 ± 0
		$P = 0.550$			$P = 0.089$		

[a]Exposure began *in utero* on GD5.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \leq 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to GSM-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \leq 0.025$.

the trend test was significant or only a single dose group was significantly increased over the control. In the absence of either a significant trend or a significantly elevated dose group, the result was considered negative.

RESULTS

Comet Assay

Eight hundred tissue samples were analyzed for % tail DNA in the comet assay. The mean % tail DNA, SE, and statistical outcomes for pairwise and trend comparisons are shown for all 40 sets of tissues (5 tissues × 8 conditions of the study) in Tables 1–8. Results are reported based on the standard 100-cell scoring approach in use at the time that the data were collected. Data obtained using the 150-cell scoring approach (OECD 2016) are noted for the few instances where results differed between the two methods. In addition, results that were either positive or equivocal are presented in figures to illustrate interanimal variability in response, and to compare the 100- versus 150-cell scoring results (Figs. 1–3). Samples were not removed from analysis unless a technical issue was identified with acquisition of the sample, or if the result was considered to be biologically implausible, as apparent outliers or influential data points could represent true biological variability. Of the 800 tissue samples that were analyzed for % tail DNA, three samples were omitted from analysis. Two samples, female rat hippocampal tissue exposed to 1.5 W/kg GSM and female rat hippocampal tissue exposed to 3.0 W/kg, were omitted due to a labeling error that occurred during necropsy. A sample of hippocampal tissue from a sham-exposed female rat was

TABLE III. DNA Damage in Female Sprague Dawley Rats Exposed to CDMA-Modulated Cell Phone Radiofrequency Radiation (900 MHz) for 19 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value[c]	% Hedgehogs (150 cells)[b,d]
Frontal cortex							
	0[e]	7.03 ± 1.21		3.80 ± 1.46	12.23 ± 2.18		0.40 ± 0.16
CDMA	1.5	12.70 ± 5.15	0.205	19.00 ± 15.04	25.37 ± 12.96	0.782	8.67 ± 7.67
	3.0	9.50 ± 2.27	0.249	9.80 ± 5.12	18.70 ± 5.28	0.634	1.87 ± 0.88
	6.0	13.00 ± 3.63	0.150	25.40 ± 11.44	33.49 ± 11.14	0.092	7.20 ± 5.62
		P = 0.166[f]			*P* = 0.035		
Hippocampus							
	0[g]	13.14 ± 1.20		9.00 ± 2.58	18.08 ± 1.30		0.83 ± 0.32
CDMA	1.5	14.94 ± 0.70	0.346	8.40 ± 1.96	20.58 ± 2.06	0.531	1.07 ± 0.34
	3.0	15.24 ± 1.97	0.379	9.40 ± 2.89	20.63 ± 1.92	0.382	1.33 ± 0.21
	6.0	19.11 ± 5.27	0.126	21.20 ± 11.12	29.55 ± 9.44	0.218	6.53 ± 5.23
		P = 0.080			*P* = 0.068		
Cerebellum							
	0	5.94 ± 0.98		3.80 ± 1.07	4.93 ± 1.09		0 ± 0
CDMA	1.5	4.91 ± 0.58	0.671	2.00 ± 1.05	4.61 ± 1.61	0.621	0.53 ± 0.53
	3.0	5.46 ± 0.83	0.747	2.00 ± 0.63	3.89 ± 0.43	0.709	0.13 ± 0.13
	6.0	5.86 ± 0.84	0.650	1.20 ± 0.37	5.88 ± 0.63	0.342	0.27 ± 0.16
		P = 0.421			*P* = 0.249		
Liver							
	0	10.09 ± 0.87		7.00 ± 1.87	12.41 ± 1.64		0.13 ± 0.13
CDMA	1.5	15.26 ± 3.35	0.634	33.40 ± 15.11	26.15 ± 8.57	0.145	4.00 ± 3.67
	3.0	11.49 ± 2.05	1.000	12.40 ± 3.59	16.17 ± 2.17	0.176	0.67 ± 0.42
	6.0	18.35 ± 3.44	0.163	31.40 ± 12.33	26.65 ± 6.91	0.059	2.00 ± 1.17
		P = 0.113			*P* = 0.102		
Peripheral blood leukocytes							
	0	3.15 ± 0.40		0.20 ± 0.20	3.32 ± 0.09		0.13 ± 0.13
CDMA	1.5	3.77 ± 1.19	0.371	1.20 ± 0.80	4.45 ± 1.53	1.000	0.40 ± 0.27
	3.0	4.13 ± 0.54	0.361	0.40 ± 0.40	3.94 ± 0.40	0.465	0.13 ± 0.13
	6.0	6.06 ± 2.18	0.082	9.80 ± 8.81	12.76 ± 7.59	0.028	2.93 ± 2.77
		P = 0.048			*P* = 0.013		

[a]Exposure began *in utero* on GD5.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \le 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to CDMA-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \le 0.025$.
[g]$n = 4$.

omitted because it had a biologically implausible value of 56.1% tail DNA.

In rats, the only clear positive result was observed in hippocampus cells of male rats exposed to the CDMA modulation when evaluated using the 100-cell scoring approach (Table I; Fig. 1A,B). Although the levels of DNA damage in hippocampus cells were also increased in an exposure-related fashion using the 150-cell scoring approach, the increases did not meet our criteria for statistical significance (Table I). Equivocal results were obtained for the frontal cortex (CDMA) of male rats using both scoring approaches (Table I; Fig. 2A, B). For male rat blood leukocytes (both modulations), results from scoring 100 cells were negative; however, equivocal responses were seen with the 150-cell method based on a significant trend test ($P = 0.012$) or pairwise test ($P = 0.021$) for CDMA- and GSM-exposed rats, respectively (Tables I and II). No statistically significant increases in % tail DNA were observed in any of the samples from female rats exposed to either modulation (Tables III and IV). Although it would appear that an equivocal result was obtained for CMDA-exposed female rat blood leukocytes using the 150-cell scoring approach (Table III), this result was driven by a single animal in the high exposure (6 W/kg) group.

In mice, positive results were obtained with both scoring approaches in frontal cortex of male mice (CDMA and GSM) (Tables V and VI; Fig. 3A–D) and blood leukocytes of female mice (CDMA) (Table VII; Fig. 3E,F). Scoring 150 cells resulted in a positive response in liver of female mice exposed to CDMA; a similar pattern of response was seen with the 100-cell scoring method, but none of the increases met our criteria for significance (Table VII). No

TABLE IV. DNA Damage in Female Sprague Dawley Rats Exposed to GSM-Modulated Cell Phone Radiofrequency Radiation (900 MHz) for 19 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value[c]	% Hedgehogs (150 cells)[b,d]
Frontal cortex							
	0[e]	7.03 ± 1.21		3.80 ± 1.46	12.23 ± 2.18		0.40 ± 0.16
GSM	1.5	4.87 ± 0.47	0.820	2.20 ± 0.73	6.28 ± 1.00	0.856	0 ± 0
	3.0	6.18 ± 0.67	0.843	5.60 ± 2.36	9.83 ± 1.11	0.877	0.67 ± 0.21
	6.0	6.74 ± 0.74	0.723	6.40 ± 2.73	13.74 ± 2.79	0.376	0.13 ± 0.13
		$P = 0.386$			$P = 0.137$[f]		
Hippocampus							
	0[g]	13.14 ± 1.20		9.00 ± 2.58	18.08 ± 1.30		0.83 ± 0.32
GSM	1.5[g]	13.22 ± 1.56	0.936	7.25 ± 3.20	17.54 ± 3.59	1.000	1.50 ± 1.29
	3.0[g]	17.67 ± 3.64	0.351	19.50 ± 7.89	28.08 ± 7.00	0.662	3.66 ± 1.40
	6.0	13.21 ± 1.03	1.000	10.00 ± 3.81	18.19 ± 3.35	1.000	2.93 ± 1.53
		$P = 0.334$			$P = 0.534$		
Cerebellum							
	0	5.94 ± 0.98		3.80 ± 1.07	4.93 ± 1.09		0 ± 0
GSM	1.5	5.69 ± 0.75	0.662	2.00 ± 0.71	5.11 ± 0.63	0.731	0 ± 0
	3.0	4.62 ± 0.85	0.749	0.60 ± 0.24	3.51 ± 0.74	1.000	0 ± 0
	6.0	6.62 ± 0.96	0.381	2.40 ± 1.03	6.54 ± 2.33	1.000	0.27 ± 0.16
		$P = 0.302$			$P = 0.705$		
Liver							
	0	10.09 ± 0.87		7.00 ± 1.87	12.41 ± 1.64		0.13 ± 0.13
GSM	1.5	9.91 ± 2.60	1.000	13.20 ± 11.23	17.05 ± 7.24	1.000	0.93 ± 0.62
	3.0	9.46 ± 2.07	1.000	17.00 ± 14.76	14.06 ± 5.68	1.000	0.27 ± 0.16
	6.0	18.99 ± 6.20	1.000	35.20 ± 19.42	26.03 ± 10.69	1.000	4.00 ± 3.23
		$P = 0.394$			$P = 0.580$		
Peripheral blood leukocytes							
	0	3.15 ± 0.40		0.20 ± 0.20	3.32 ± 0.09		0.13 ± 0.13
GSM	1.5	2.80 ± 0.33	0.593	0.80 ± 0.49	3.07 ± 0.43	1.000	0.27 ± 0.16
	3.0	3.39 ± 0.68	0.447	0.60 ± 0.24	2.82 ± 0.52	1.000	0.13 ± 0.13
	6.0	3.93 ± 0.63	0.203	1.00 ± 0.32	3.86 ± 0.76	1.000	0.40 ± 0.16
		$P = 0.093$			$P = 0.580$		

[a]Exposure began in utero on GD5.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \le 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to GSM-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \le 0.025$.
[g]$n = 4$.

statistically significant increases in % tail DNA were observed in any of the samples from female mice exposed to the GSM modulation (Table VIII).

In general, for those data sets that were scored using both methods (100- and 150-cell scoring methods), similar conclusions were reached when considering positive or equivocal results (see Supporting Information Fig. S1A–D for examples) except for hippocampus from male rats (CDMA) (Table I), blood leukocytes from male rats (CDMA and GSM) (Tables I and II), and liver from female mice (CDMA) (Table VII). In summary, 8 of 40 tissue sets exhibited positive or equivocal results when assessed using the 100- or 150-cell scoring approaches.

In all instances, where both methods were used, the 150-cell method that included all scorable cells, even those that visually appeared to be hedgehogs before software analysis, revealed a much broader spectrum of DNA damage than the 100-cell method that excluded all apparent hedgehogs (Supporting Information Figs. S2A–D and S3A–D).

We noticed considerable interanimal variability in % tail DNA in both sexes of mice and rats. To rule out any influence from technical artifacts or protocol features, % tail DNA values for all tissues and % hedgehogs for the rat tissues were correlated to the position of slides in the electrophoresis chambers, the interval from exposure cessation to tissue collection, and the date of slide preparation. No patterns in the level of observed DNA damage emerged for any of these variables. To investigate the interanimal variability more closely, we plotted the % tail DNA response data for all tissues using the 100-cell data

TABLE V. DNA Damage in Male B6C3F1/N Mice Exposed to CDMA-Modulated Cell Phone Radiofrequency Radiation (1,900 MHz) for 14 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value[c]	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value	% Hedgehogs (150 cell)[d]
Frontal cortex							
	0[e]	0.63 ± 0.08		0.40 ± 0.24	1.32 ± 0.21		0 ± 0
CDMA	2.5	3.46 ± 0.65	0.014	0.60 ± 0.40	4.52 ± 0.57	0.131	0 ± 0
	5.0	5.88 ± 1.06	0.001	0.60 ± 0.24	6.06 ± 0.96	0.018	0 ± 0
	10.0	8.85 ± 1.09	0.001	4.40 ± 1.69	10.04 ± 2.08	0.001	0.53 ± 0.39
		$P = 0.001$[f]			$P = 0.001$		
Hippocampus							
	0	7.69 ± 2.00		1.20 ± 0.58			
CDMA	2.5	9.59 ± 4.33	0.521	5.40 ± 2.11			
	5.0	6.44 ± 1.21	0.606	2.80 ± 0.97			
	10.0	6.38 ± 0.93	0.641	4.40 ± 2.27			
		$P = 0.740$					
Cerebellum							
	0	5.48 ± 1.30		1.80 ± 0.80			
CDMA	2.5	7.35 ± 2.47	0.339	4.40 ± 2.06			
	5.0	7.87 ± 2.80	0.404	4.60 ± 2.34			
	10.0	5.43 ± 2.43	0.431	1.60 ± 0.93			
		$P = 0.554$					
Liver							
	0	16.30 ± 2.21		6.80 ± 2.82			
CDMA	2.5	20.27 ± 5.53	1.000	21.60 ± 16.88			
	5.0	16.15 ± 1.15	1.000	11.00 ± 3.77			
	10.0	16.43 ± 0.83	1.000	7.20 ± 1.11			
		$P = 0.368$					
Peripheral blood leukocytes		1.60 ± 0.68		0.40 ± 0.24			
	0						
		2.10 ± 0.50	0.449	1.20 ± 0.58			
CDMA	2.5	1.30 ± 0.28	0.527	0.40 ± 0.24			
	5.0	2.86 ± 0.26	0.046	1.40 ± 0.87			
	10.0						
		$P = 0.057$					

[a]Exposure began at ~5 weeks of age.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \le 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to CDMA-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \le 0.025$.

set. The median % tail DNA was included in each plot as a measure of central tendency in the distribution (see Supporting Information Fig. S4A–D). We found that % tail DNA values were relatively small (<5%) in blood leukocytes in both sexes and species, while the other four tissues exhibited a much greater interanimal variability in response with % tail DNA values that exceeded 30% in some cases. Female mice generally displayed less variability in response than male mice in the hippocampus, cerebellum, and liver. Female rats exposed to RFR also seemed to show less variability in response than male rats exposed to RFR in the cerebellum.

Micronucleus Assay

The MN assay data are reported in Supporting Information Tables S1 and S2. For male mice exposed to CDMA, although a significant trend was observed for MN–RET ($P = 0.013$), the absolute increase was quite small (the mean MN–RET for sham exposure was 2.55 vs. 2.93 for the 10 W/kg exposure) and within the laboratory's historical control range (1.66–3.06), and no corresponding increase was observed in the mature erythrocyte population that should be in steady-state equilibrium after continuous subchronic exposure. Thus, the overall MN assay result for male mice exposed to CDMA was considered to be negative. No other significant effects were seen in rats or mice exposed to either modulation of RFR.

RFR Exposure

The power levels for RFR exposure were adjusted based on the average weight of all animals in a chamber. Due to normal variations in animal weights, the actual SAR in individual animals differed slightly among animals in the

TABLE VI. DNA Damage in Male B6C3F1/N Mice Exposed to GSM-Modulated Cell Phone Radiofrequency Radiation (1,900 MHz) for 14 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value[c]	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value	% Hedgehogs (150 cell)[d]
Frontal cortex							
	0[e]	0.63 ± 0.08		0.40 ± 0.24	1.32 ± 0.21		0 ± 0
GSM	2.5	1.71 ± 0.46	0.081	1.80 ± 0.97	4.25 ± 1.20	0.063	0.13 ± 0.13
	5.0	1.39 ± 0.15	0.081	1.60 ± 0.81	3.69 ± 0.53	0.063	0 ± 0
	10.0	3.73 ± 0.65	0.001	1.00 ± 0.45	5.60 ± 1.28	0.006	0.13 ± 0.13
		$P = 0.001$[f]			$P = 0.004$		
Hippocampus							
	0	7.69 ± 2.00		1.20 ± 0.58			
GSM	2.5	8.74 ± 1.93	0.514	5.40 ± 2.11			
	5.0	7.17 ± 1.08	0.598	2.20 ± 0.97			
	10.0	6.90 ± 1.19	0.633	5.40 ± 2.54			
		$P = 0.720$					
Cerebellum							
	0	5.48 ± 1.30		1.80 ± 0.80			
GSM	2.5	3.66 ± 0.30	0.831	3.00 ± 1.38			
	5.0	3.90 ± 0.59	0.896	1.80 ± 0.92			
	10.0	3.85 ± 1.08	0.919	3.40 ± 1.50			
		$P = 0.838$					
Liver							
	0	16.30 ± 2.21		6.80 ± 2.82			
GSM	2.5	17.66 ± 1.89	0.469	8.20 ± 3.84			
	5.0	15.40 ± 1.20	0.549	6.60 ± 1.96			
	10.0	18.94 ± 2.00	0.213	12.80 ± 4.40			
		$P = 0.198$					
Peripheral blood leukocytes							
	0	1.60 ± 0.68		0.40 ± 0.24			
GSM	2.5	1.85 ± 0.96	0.416	1.20 ± 1.20			
	5.0	1.75 ± 0.37	0.491	1.00 ± 0.55			
	10.0	1.85 ± 0.24	0.494	0.80 ± 0.58			
		$P = 0.408$					

[a]Exposure began at ~5 weeks of age.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \le 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to GSM-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \le 0.025$.

same exposure chamber (Wyde et al. 2018). These minor deviations were considered to have negligible effect, as no correlations between actual individual animal SAR and comet assay outcomes were seen in any of several tissues, including brain, that were examined to evaluate possible associations (data not shown).

DISCUSSION

The two main RFR modulations used for cellular telephone communication worldwide, CDMA and GSM, were tested by the NTP in the 2-year rodent cancer bioassay. The reverberation chambers used to expose the animals for the bioassay were designed by physicists and engineers from NIST and ITIS in collaboration with the NTP to overcome confounding factors that have limited the interpretation of other RFR studies. As a component of the bioassay, we examined the potential for RFR to induce DNA damage as measured by the comet assay and chromosomal damage as measured by the peripheral blood erythrocyte MN assay. Although results of the MN assays were negative, significant increases in the levels of DNA damage measured by the comet assay were seen in several tissues from rats and mice, indicating that RFR may be capable of causing increases in DNA damage.

DNA damage was primarily observed in brain tissue from male rats and mice exposed to RFR. Using the 100-cell scoring approach, the hippocampus of CDMA-exposed male rats showed a significant, exposure-related increase in % tail DNA, while no tissues in exposed female rats were found to have significant increases in % tail DNA compared to controls. Male mice exhibited significant CDMA exposure-related increases in % tail DNA compared to controls at all exposure levels in the frontal cortex,

TABLE VII. DNA Damage in Female B6C3F1/N Mice Exposed to CDMA-Modulated Cell Phone Radiofrequency Radiation (1,900 MHz) for 14 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value[c]	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value	% Hedgehogs (150 cell)[d]
Frontal cortex							
	0[e]	8.11 ± 2.13		3.40 ± 1.47			
CDMA	2.5	4.88 ± 0.55	0.911	0.80 ± 0.49			
	5.0	4.89 ± 0.57	0.955	1.20 ± 0.49			
	10.0	4.80 ± 0.90	0.968	0.80 ± 0.58			
		P = 0.935[f]					
Hippocampus							
	0	8.15 ± 1.65		2.60 ± 1.69			
CDMA	2.5	5.76 ± 1.00	0.839	1.80 ± 0.80			
	5.0	5.22 ± 1.02	0.903	1.20 ± 0.58			
	10.0	5.34 ± 1.82	0.925	2.20 ± 0.97			
		P = 0.892					
Cerebellum							
	0	5.88 ± 0.85		0.20 ± 0.20			
CDMA	2.5	6.78 ± 1.67	0.296	1.75 ± 1.03			
	5.0	8.39 ± 1.13	0.194	0.20 ± 0.20			
	10.0	6.73 ± 0.77	0.207	0.40 ± 0.40			
		P = 0.298					
Liver							
	0	5.48 ± 0.60		0.60 ± 0.40	4.34 ± 0.60		0 ± 0
CDMA	2.5	7.54 ± 0.90	0.034	1.00 ± 0.45	6.20 ± 0.99	0.050	0 ± 0
	5.0	7.36 ± 0.72	0.041	4.40 ± 2.11	8.30 ± 0.92	0.009	0 ± 0
	10.0	7.63 ± 0.59	0.030	2.00 ± 0.77	6.14 ± 0.26	0.009	0 ± 0
		P = 0.050			*P* = 0.100		
Peripheral blood leukocytes							
	0	1.03 ± 0.13		0.20 ± 0.20	2.15 ± 0.08		0 ± 0
CDMA	2.5	2.52 ± 0.54	0.020	2.00 ± 1.14	3.62 ± 0.66	0.011	0 ± 0
	5.0	1.71 ± 0.37	0.024	0 ± 0	3.39 ± 0.45	0.015	0.13 ± 0.13
	10.0	2.20 ± 0.19	0.018	0.20 ± 0.20	2.45 ± 0.24	0.428	0 ± 0
		P = 0.085			*P* = 0.173		

[a]Exposure began at ~5 weeks of age.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \leq 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to CDMA-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \leq 0.025$.

and a GSM exposure-related increase in % tail DNA compared to controls at the highest exposure level in the frontal cortex. Female mice showed small, but statistically significant, increases in % tail DNA compared to controls at all exposure levels in blood. No other potentially exposure-related patterns were apparent based on visual inspection of the % tail DNA data (see Figs. 1–3). A larger number of animals per treatment group may have improved the ability to detect increases in DNA damage; however, the size of the reverberation chambers limited the number of animals that could be used for genetic toxicity testing to 5 per treatment group, which is the standard for comet assay studies conducted at the NTP and consistent with OECD recommendations (Hartmann et al. 2003; OECD 2016).

A limitation in this study is the absence of histopathological assessment for indications of inflammation and cytotoxicity. Although histopathology was not performed on the animals used for genetic toxicity studies, an additional set of animals was removed from the 2-year cancer bioassay for histopathological evaluation at the same time as the animals used for the genetic toxicity studies. No evidence of neoplastic lesions or nonneoplastic lesions, such as inflammation or necrosis was observed in the brains or livers of these animals, which could be attributable to RFR exposure (NTP 2018a; 2018b). Furthermore, RFR-induced inflammation and necrosis were not observed in the brains or livers of rats or mice at the end of the 2-year cancer bioassay (NTP 2018a; 2018b).

The NTP bioassay was designed to evaluate nonthermal effects of cell phone RFR exposure, which meant that body temperature could not change more than 1°C under our exposure conditions. To meet that requirement, pilot studies conducted to establish acceptable SARs for the bioassay indicated that no body temperature increases over 1°C would be expected in rats (including pregnant rats) or mice at exposures up to 6.0 or 10.0 W/kg, respectively (Wyde

TABLE VIII. DNA Damage in Female B6C3F1/N Mice Following Exposure to GSM-Modulated Cell Phone Radiofrequency Radiation (1,900 MHz) for 14 Weeks[a]

	Dose (W/kg)	% Tail DNA (100 cells)[b]	*P* value[c]	% Hedgehogs (100 cells)[b]	% Tail DNA (150 cells)[b]	*P* value	% Hedgehogs (150 cell)[d]
Frontal cortex							
	0[e]	8.11 ± 2.13		3.40 ± 1.47			
GSM	2.5	7.33 ± 0.90	0.657	1.00 ± 0.45			
	5.0	7.69 ± 1.98	0.744	2.00 ± 0.84			
	10.0	5.74 ± 0.62	0.779	1.00 ± 0.32			
		P = 0.861[f]					
Hippocampus							
	0	8.15 ± 1.65		2.60 ± 1.69			
GSM	2.5	6.23 ± 1.00	0.866	0.80 ± 0.58			
	5.0	4.54 ± 1.29	0.923	1.20 ± 0.58			
	10.0	5.22 ± 1.23	0.942	1.60 ± 1.36			
		P = 0.933					
Cerebellum							
	0	5.88 ± 0.85		0.20 ± 0.20			
GSM	2.5	6.56 ± 1.22	1.000	1.20 ± 0.73			
	5.0	5.26 ± 0.59	1.000	0.60 ± 0.40			
	10.0	6.54 ± 1.71	1.000	1.80 ± 0.73			
		P = 0.606					
Liver							
	0	5.48 ± 0.60		0.60 ± 0.40	4.34 ± 0.60		0 ± 0
GSM	2.5	7.06 ± 0.61	0.096	3.40 ± 1.17	7.44 ± 0.48	0.027	0 ± 0
	5.0	6.36 ± 0.25	0.117	1.20 ± 0.37	5.45 ± 0.96	0.032	0 ± 0
	10.0	6.47 ± 0.79	0.124	2.60 ± 1.33	6.52 ± 0.75	0.030	0 ± 0
		P = 0.249			*P* = 0.133		
Peripheral blood leukocytes							
	0	1.03 ± 0.13		0.20 ± 0.20	2.15 ± 0.08		0 ± 0
GSM	2.5	1.25 ± 0.44	0.335	0.20 ± 0.20	2.58 ± 0.35	0.504	0 ± 0
	5.0	1.17 ± 0.08	0.400	0 ± 0	2.23 ± 0.19	1.000	0 ± 0
	10.0	1.32 ± 0.34	0.316	0 ± 0	2.28 ± 0.51	1.000	0 ± 0
		P = 0.266			*P* = 0.657		

[a]Exposure began at ~5 weeks of age.
[b]Mean ± SE.
[c]Pairwise comparison with the sham control group; exposed group values are significant at $P \le 0.025$ by Williams' or Dunn's test.
[d]A comet figure was considered a hedgehog if ≥90% DNA was in the tail. % Hedgehogs = number of comets with ≥90% tail DNA/150.
[e]Sham control; no exposure to GSM-modulated cell phone RFR.
[f]Dose-related trend derived from one-tailed linear regression or Jonckheere's test; the trend is significant when $P \le 0.025$.

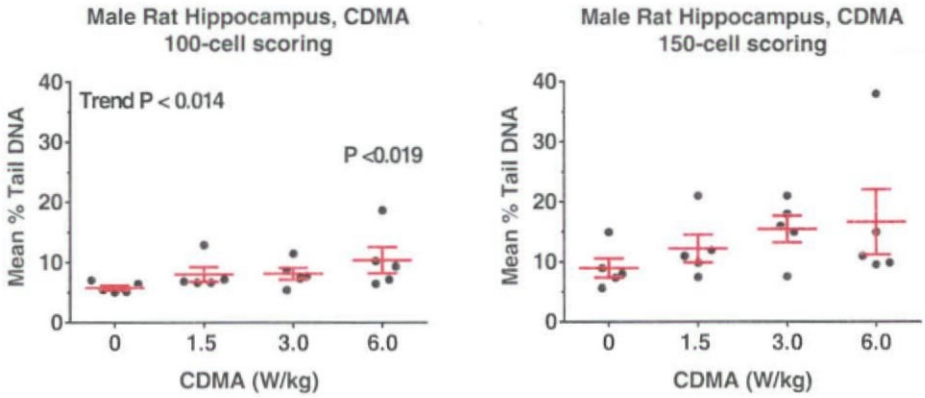

Fig. 1. (A,B) Male rat hippocampus, CDMA, was the only rat tissue judged to be positive in the comet assay when using the 100-cell scoring approach (A). Central horizontal bar indicates mean % tail DNA; upper and lower error bars indicate SE.

et al. 2018). Therefore, we consider it unlikely that thermal effects were a confounding factor for our genetic toxicity tests, although more work in general is needed to clarify the thermal effects of RFR on different tissues, and the degree to which increases in body or tissue temperature affect genomic integrity. Few studies have closely examined the relationship between increased body temperature and induction of DNA damage in mice, and there is almost no information on this relationship in rats. In one study in which the body temperatures of mice were closely monitored, an increase of ~2°C was required before increases in micronuclei were detected (Asanami and Shimono 1997).

Little is known about the mechanism by which RFR could induce DNA damage in the absence of heating. Unlike ionizing radiation or ultraviolet light, the radiation emitted by cell phones is not sufficiently energetic, by several orders of magnitude, to directly damage macromolecules (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013). Calculations by physicists and engineers suggest that RFR would not have an appreciable effect on biological systems at nonthermal levels of exposure, primarily due to the damping effects of water molecules (Adair 2002; 2003; Sheppard et al. 2008; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2013). However, our results and the results of other experiments suggest that nonthermal exposure of cells or whole organisms to RFR may result in measurable genotoxic effects, despite varied and weak responses across studies overall (Brusick et al. 1998; Ruediger 2009; Verschaeve et al. 2010). Induction of

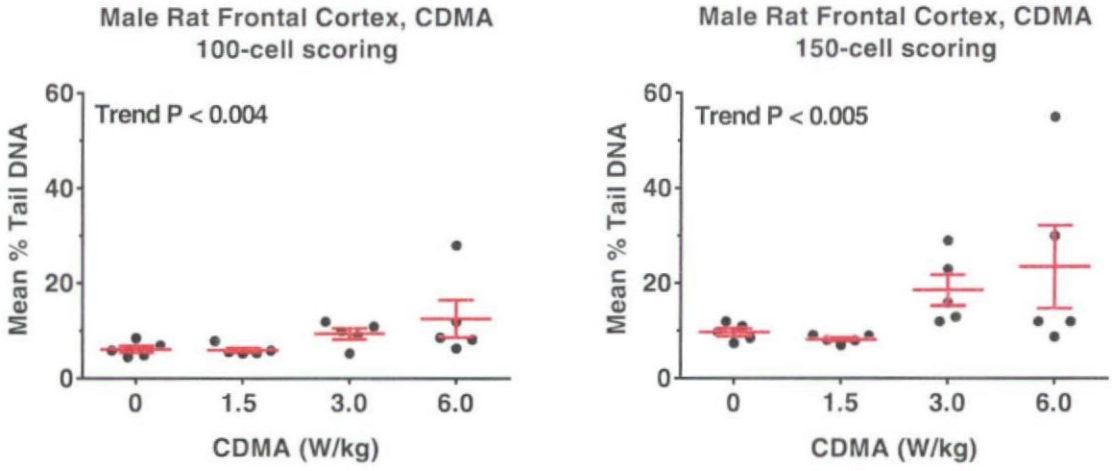

Fig. 2. (A,B) Male rat frontal cortex, CDMA, was judged to be equivocal in the comet assay using the 100-cell scoring approach (A); a similar result was obtained using the 150-cell scoring approach (B). Central horizontal bar indicates mean % tail DNA; upper and lower error bars indicate SE.

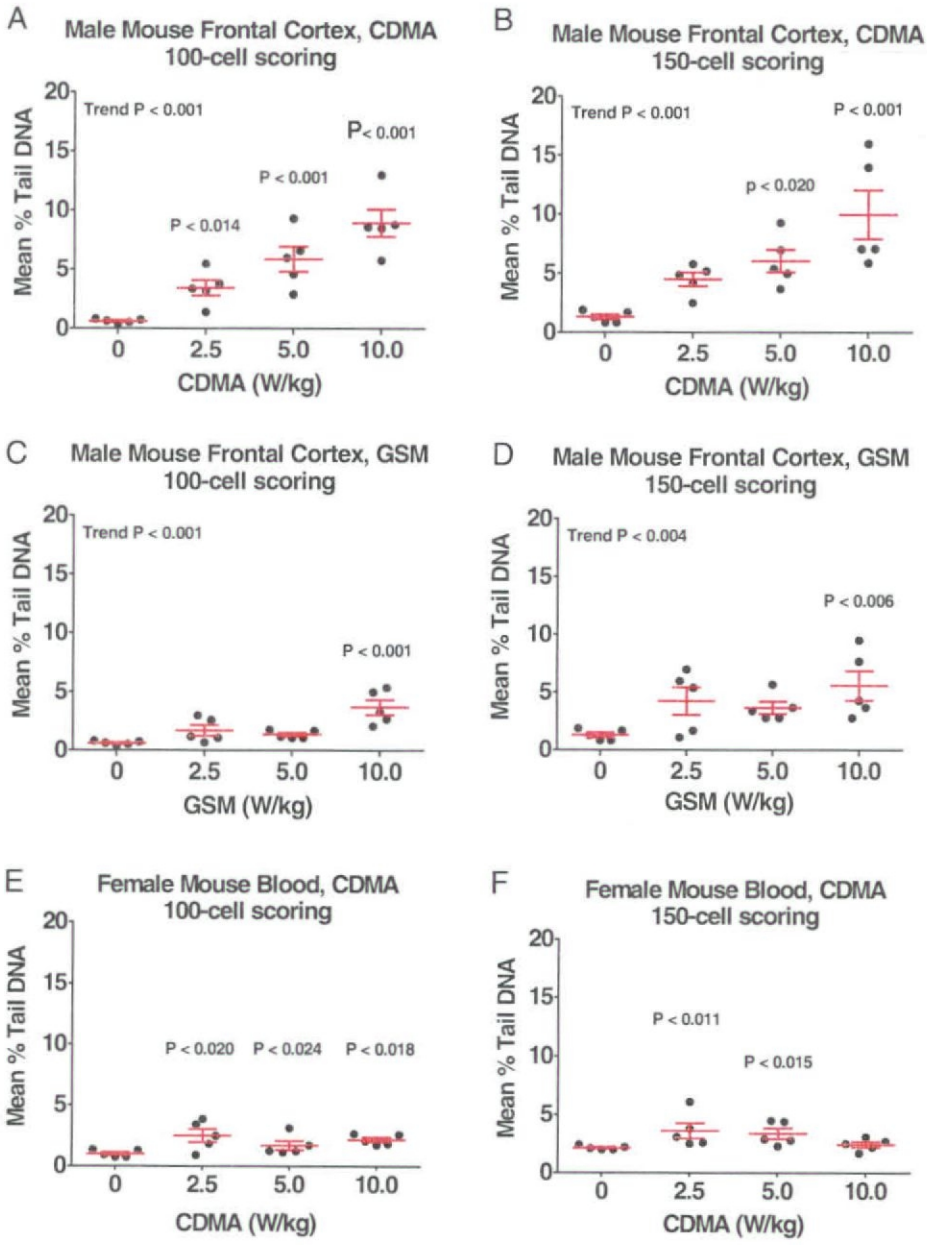

Fig. 3. (A–F) Mouse tissues judged to be positive in the comet assay using the 100-cell scoring approach. Central horizontal bar indicates mean % tail DNA; upper and lower error bars indicate SE.

oxygen radicals or interference with DNA repair processes has been proposed as possible mechanisms by which RFR could cause DNA damage (Ruediger 2009; Yakymenko et al. 2015).

NTP Technical Reports on the results of the 2-year cancer bioassay for exposure to RFR for rats (TR 595) and mice (TR 596) were finalized, peer reviewed, and made publicly available in 2018. The NTP concluded that results demonstrated clear evidence of carcinogenic activity of cell phone RFR (both modulations) based on incidences of malignant schwannomas of the heart in male rats. Malignant gliomas in the brain were also observed in male rats exposed to cell phone RFR and were considered to be related to exposure. Female rats exhibited malignant schwannomas of the heart and malignant gliomas, but incidences of these tumors were considered equivocal. The observation that cell phone RFR affects heart and brain tissue in Sprague Dawley rats after long-term exposure was replicated in a similar study (that used only the GSM modulation) by the Ramazzini Institute (Falcioni

et al. 2018). The gliomas and schwannomas observed in rats are similar to the tumor types reported in some epidemiology studies to be associated with cell phone use. The NTP bioassay findings in mice, in which different organs were affected compared to rats, were considered equivocal. Notably, spontaneous and chemically induced brain tumors are rare in rats (Sills et al. 1999), and as of 2019, only 12 out of approximately 600 test articles have shown evidence of an increase in brain tumor incidence in rats in NTP bioassays.

The U.S. Federal Communications Commission has set a guideline limit for RFR requiring that mobile devices emit an SAR of less than of 1.6 W/kg as measured in a volume containing 1 g of tissue absorbing the signal. In contrast, animals in the NTP studies received whole-body exposure to higher levels of RFR to identify potential target organs and to characterize toxicity. The highest exposure of 6 W/kg in rats and 10 W/kg in mice, for a total of 9 h 10 min a day (achieved by cycling for 10 min on, 10 min off over 18 h 20 min), produced higher exposures than experienced by humans under normal cellular phone use conditions. Thus, whether the findings in the NTP animal studies (eg, malignant gliomas in the brain and malignant schwannomas in the hearts of male rats; increased levels of DNA damage in hippocampal cells of male rats and the frontal cortex of male mice) indicate a potential for adverse health outcomes in humans remains a question. Because one of the most important questions prompted by our results concerns the mechanism(s) by which RFR might induce biological effects, follow-up studies by the NTP to investigate mechanisms of genetic damage associated with RFR exposure are underway.

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AUTHOR CONTRIBUTIONS

K.L.W., M.E.W., G.E.K., R.R.T., and J.R.B. designed the study. S.L.S., G.E.K., K.R.S., and K.L.W. analyzed the data; S.L.S., G.E.K., K.R.S., and K.L.W. prepared the manuscript with input from C.A.H., K.G.S., R.R.T., and J.R.B. K.G.S and A.S.G. collected the tissue samples at IITRI and, together with J.W.W., conducted the genetic toxicity assays under the supervision of C.A.H. G.E.K. and K.R.S. performed statistical analyses of the data. M.E.W., M.D.S., R.R.T., C.A.H., and J.R.B. contributed important intellectual input to the study and to the manuscript, and all authors read and approved the manuscript.

CONFLICT OF INTERESTS

The authors have no competing financial interests.

REFERENCES

Adair RK. 2002. Vibrational resonances in biological systems at microwave frequencies. Biophys J 82(3):1147–1152.

Adair RK. 2003. Biophysical limits on athermal effects of RF and microwave radiation. Bioelectromagnetics 24(1):39–48.

Asanami S, Shimono K. 1997. High body temperature induces micronuclei in mouse bone marrow. Mutat Res 390(1-2):79–83.

Brusick D, Albertini R, McRee D, Peterson D, Williams G, Hanawalt P, Preston J. 1998. Genotoxicity of radiofrequency radiation. DNA/Genetox Expert Panel. Environ Mol Mutagen 32(1):1–16.

Capstick M, Kuster N, Kuehn S, Berdinas-Torres V, Gong Y, Wilson P, Ladbury J, Koepke G, McCormick DL, Gauger J, et al. 2017. A radio frequency radiation exposure system for rodents based on reverberation chambers. IEEE Trans Electromagn Compat 59(4): 1041–1052.

Cardis E, Armstrong BK, Bowman JD, Giles GG, Hours M, Krewski D, McBride M, Parent ME, Sadetzki S, Woodward A, et al. 2011. Risk of brain tumours in relation to estimated RF dose from mobile phones: Results from five Interphone countries. Occup Environ Med 68(9):631–640.

Falcioni L, Bua L, Tibaldi E, Lauriola M, De Angelis L, Gnudi F, Mandrioli D, Manservigi M, Manservisi F, Manzoli I, Menghetti I, Montella R, Panzacchi S, Sgargi D, Strollo V, Vornoli A, Belpoggi F. 2018. Report of final results regarding brain and heart tumors in Sprague-Dawley rats exposed from prenatal life until natural death to mobile phone radiofrequency field representative of a 1.8 GHz GSM base station environmental emission. Environ Res 165: 496–503.

Gong Y, Capstick M, Kuehn S, Wilson P, Ladbury J, Koepke G, McCormick DL, Melnick RL, Kuster N. 2017. Life-time dosimetric assessment for mice and rats exposed in reverberation chambers of the 2-year NTP cancer bioassay study on cell phone radiation. IEEE Trans Electromagn Compat 59(6):1798–1808.

GSMA Intelligence. 2019. Definitive Data Analysis for the Mobile Industry, Global Data: Unique Mobile Subscribers, https://www.gsmaintelligence.com/, accessed June 6, 2019.

Hardell L, Carlberg M. 2015. Mobile phone and cordless phone use and the risk for glioma—analysis of pooled case-control studies in Sweden, 1997–2003 and 2007–2009. Pathophysiology 22(1):1–13.

Hardell L, Carlberg M, Hansson Mild K. 2011. Pooled analysis of case-control studies on malignant brain tumours and the use of mobile and cordless phones including living and deceased subjects. Int J Oncol 38(5):1465–1474.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, et al. 2003. Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. Mutagenesis 18(1): 45–51.

Hobbs CA, Chhabra RS, Recio L, Streicker M, Witt KL. 2012. Genotoxicity of styrene-acrylonitrile trimer in brain, liver, and blood cells of weanling F344 rats. Environ Mol Mutagen 53(3):227–238.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2013. Non-ionizing radiation, Part 2: Radiofrequency electromagnetic fields. IARC Monogr Eval Carcinog Risks Hum 102(Pt 2):1–460.

International Telecommunication Union. 2017. ICT Development Report and Database. https://data.worldbank.org/indicator/IT.CEL.SETS?end=2017&start=1960&view=chart, accessed June 6, 2019. Geneva, Switzerland: The World Bank.

INTERPHONE Study Group. 2010. Brain tumor risk in relation to mobile telephone use: Results of the INTERPHONE international case-control study. Int J Epidemiol 39:675–694.

Kissling GE, Dertinger SD, Hayashi M, MacGregor JT. 2007. Sensitivity of the erythrocyte micronucleus assay: Dependence on number of cells scored and inter-animal variability. Mutat Res 634(1–2): 235–240.

Larjavaara S, Schuz J, Swerdlow A, Feychting M, Johansen C, Lagorio S, Tynes T, Klaeboe L, Tonjer SR, Blettner M, et al. 2011. Location of gliomas in relation to mobile telephone use: A case-case and case-specular analysis. Am J Epidemiol 174(1):2–11.

Lorenzo Y, Costa S, Collins AR, Azqueta A. 2013. The comet assay, DNA damage, DNA repair and cytotoxicity: Hedgehogs are not always dead. Mutagenesis 28(4):427–432.

National Research Council (US) Institute for Laboratory Animal Research. 1996. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academies Press (US).

National Toxicology Program (NTP). 2018a. Toxicology and Carcinogenesis Studies of GSM- and CDMA-Modulated Cell Phone Radiofrequency Radiation at 900 MHz in Sprague Dawley Rats (Whole Body Exposure). Technical Report Series No. 595. USA: The National Toxicology Program.

National Toxicology Program (NTP). 2018b. Toxicology and Carcinogenesis Studies of GSM- and CDMA-Modulated Cell Phone Radiofrequency Radiation at 1,900 MHz in B6C3F1/N Mice (Whole Body Exposure). Technical Report Series No. 596. USA: The National Toxicology Program.

OECD. 2014. Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing.

OECD. 2016. Test No. 489: in vivo Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing.

Recio L, Kissling GE, Hobbs CA, Witt KL. 2012. Comparison of Comet assay dose-response for ethyl methanesulfonate using freshly prepared versus cryopreserved tissues. Environ Mol Mutagen 53(2): 101–113.

Ruediger HW. 2009. Genotoxic effects of radiofrequency electromagnetic fields. Pathophysiology 16(2–3):89–102.

Rundell MS, Wagner ED, Plewa MJ. 2003. The comet assay: Genotoxic damage or nuclear fragmentation? Environ Mol Mutagen 42(2): 61–67.

Sato Y, Akiba S, Kubo O, Yamaguchi N. 2011. A case-case study of mobile phone use and acoustic neuroma risk in Japan. Bioelectromagnetics 32(2):85–93.

Sheppard AR, Swicord ML, Balzano Q. 2008. Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes. Health Phys 95(4):365–396.

Sills RC, Hailey JR, Neal J, Boorman GA, Haseman JK, Melnick RL. 1999. Examination of low-incidence brain tumor responses in F344 rats following chemical exposures in National Toxicology Program carcinogenicity studies. Toxicol Pathol 27(5):589–599.

Speit G, Gminski R, Tauber R. 2013. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in HL-60 cells are not reproducible. Mutat Res 755(2):163–166.

Verschaeve L, Juutilainen J, Lagroye I, Miyakoshi J, Saunders R, de Seze R, Tenforde T, van Rongen E, Veyret B, Xu Z. 2010. In vitro and in vivo genotoxicity of radiofrequency fields. Mutat Res 705 (3):252–268.

Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, Recio L. 2008. Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. Mutat Res 649(1–2):101–113.

Wyde ME, Horn TL, Capstick MH, Ladbury JM, Koepke G, Wilson PF, Kissling GE, Stout MD, Kuster N, Melnick RL, et al. 2018. Effect of cell phone radiofrequency radiation on body temperature in rodents: Pilot studies of the National Toxicology Program's reverberation chamber exposure system. Bioelectromagnetics 39(3): 190–199.

Yakymenko I, Tsybulin O, Sidorik E, Henshel D, Kyrylenko O, Kyrylenko S. 2015. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagn Biol Med 35: 1–16.

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