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## Review

# Biological and pathological effects of 2.45 GHz radiation on cells, fertility, brain, and behavior

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#### Abstract

**Purpose:** This article is a systematic review of studies on the effects of non-ionizing radiation at the microwave (MW) frequency of 2.45 GHz (2450 MHz), which is predominantly used in WLAN/Wi-Fi applications (wireless local area network) and microwave ovens. Newer WLAN standards also use the frequency ranges of 5 GHz, 6 GHz, and 60 GHz. WLAN, referred to generically in this review also as Wi-Fi, has become the technology of choice for many wireless applications because providers do not require a license, making the service free to users. To meet users' desire to be online all the time, more and more WLAN antennas (access points, femtocells, routers) emitting pulsed 2.45 GHz radiation are being installed at libraries, hospitals, hotels, airports, railway stations, shopping malls, public places, and in buses, subways, and passenger trains. Wi-Fi consoles are used to play games. Office and household appliances are also fitted with Wi-Fi antennas. Residential routers often contain two Wi-Fi transmitters. As part of its digital learning initiative, the German Conference of Ministers of Education has decided to provide all schools with Wi-Fi networks. The extensive body of research on the health risks of Wi-Fi radiation is generally not considered by policy-makers or in the public debate.

**Method:** For this review, primarily the databases LIVIVO (ZBMED) and PubMed were searched for studies, without limiting the publication date range. The selected studies have all been published in peer-reviewed journals.

**Result:** More than 100 studies on 2.45 GHz radiation were analyzed, most of which found changes compared to the control groups at levels below the safety guidelines of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) (issued as exposure limits of the 26th Federal Pollution Control Ordinance (BImSchV) in Germany). The available studies document damage to the reproductive system, impacts on the EEG and brain functions, as well as effects on the heart, liver, thyroid, gene expression, cell cycle, cell membranes, bacteria, and plants. As a mechanism of action, many studies identify oxidative stress. Adverse effects on learning, memory, attention, and behavior are the result of cytotoxic effects.

**Conclusions:** Based on the extensive body of research and the adverse health effects demonstrated in the majority of the studies, it is recommended that steps should be taken to minimize RF radiation exposure in accordance with official recommendations. Wired solutions should be given preference. Current exposure limits and SAR values do not protect from health risks associated with Wi-Fi radiation. The adverse effects on learning, attention, and behavior serve as a basis for educational institutions of all age groups to forgo the use of Wi-Fi applications. Due to cytotoxic effects, Wi-Fi technologies are not suitable for hospitals and telemedicine. Wi-Fi technologies should not be used in bedrooms, work spaces, common lounges, hospital rooms, lecture halls, classrooms, and public transport. The possible risks associated with Wi-Fi radiation could be avoided by testing alternative technologies at other frequency bands like optical VLC/Li-Fi technologies (visible light communication). When Wi-Fi cannot be avoided as a transition solution, the ALARA principle must be applied: no continuous transmission, instead Wi-Fi networks that can be turned off and feature dynamic power management.

Keywords: RF radiation, electromagnetic fields (EMF), pulsed microwaves, 10 Hz pulse, WLAN, Wi-Fi, 2.45 GHz, cell damage

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#### Introduction

Since the 1980s, when microwave ovens became a common household appliance, scientists have been interested in the effects of 2.45 GHz electromagnetic fields (microwaves) on living organisms. Since then, numerous experiments with bacteria, cell cultures, animals, plants, and also humans have found many effects caused by this type of radiation. While the short-term leakage of microwave ovens is an undesirable side effect, Wi-Fi and Bluetooth devices - a commonplace addition to households in recent years - continuously transmit at the same frequencies. Wi-Fi is now established as one of the most used frequencies, both for handheld devices such as smartphones/tablets and inhouse applications (routers, smart home, Internet of Things). Therefore, active research efforts have also started in this area. Experiments on Wi-Fi radiation usually use a sham-exposed group and exposed groups, often applying different exposure levels and durations. At the heart of the research remains the question of whether radio-frequency (RF) radiation in this frequency range has harmful effects on humans, animals, and plants.

In 2014 Springer Reference published Biology of Free Radicals and Anti-Oxidants, which includes a review on cell phone- and Wi-Fi-induced radiation by Nazıroğlu and Akman showing that even low-level Wi-Fi radiation can be harmful to health. The authors presented the current state of research at that time and already described a mechanism of damage. The Wi-Fi studies that have been published since 2014 confirm their analysis. In this new review, the study findings of 2.45 GHz and Wi-Fi radiation are presented, documenting adverse effects on cells and organs, differences between non-exposed controls and exposed samples, as well as effects on the behavior of animals and humans. Changes caused by this type of radiation were often observed at levels well below the ICNIRP exposure limits, which have been adopted in most countries (e.g. in accordance with the 26th Federal Pollution Control Ordinance (BImSchV) in Germany). It is important to distinguish between acute and chronic exposure studies; in everyday life we are dealing with chronic exposures. In studies designed to investigate short-term exposure, one can say with clear conscience: "Under these conditions, no (statistically significant) effects could be observed." However, this does not say anything about daily exposures in real life. There are various studies in which researchers tried to determine the differences in terms of biological effects between GSM (900 and 1800 MHz) and Wi-Fi (2.45 GHz) frequencies. In most cases, Wi-Fi radiation was found to result in a stronger effect compared to GSM radiation. In some animal experiments, it was observed that young mice or rats responded more sensitively to radiation than young adults or old animals. Research studies often investigate several endpoints. For example, there are studies that establish an association between oxidative stress, DNA strand breaks, and sperm damage. As a result, there is some overlap among the chapters of this review. Generally, however, each study was only assigned to one endpoint. The frequent assertion that no mechanisms of action exists has been wrong for a long time. This holds true for 2.45 GHz radiation as well as frequencies of cellular networks. This review presents many studies that explain mechanisms of action, including astonishingly frequently documented oxidative damage, DNA strand breaks, changes in ion channels, effects on transmitters in the hippocampus, and others.

#### 1 Effects on reproduction and fertility

#### 1.1 Effects on testes and sperm

Mobile devices are used close to the body. Smartphones are often carried in close proximity to the user's reproductive organs with headsets at the ear, or laptops placed directly on the user's lap. Cytotoxic effects on sperm, ovaries, and embryos at the frequencies of GSM, UMTS, and Wi-Fi have been documented by more than 130 studies. The Austrian Medical Association therefore recommends in its ten medical cell phone rules: "Do not keep the phone directly on your body when using a headset or the built-in speakerphone. Pregnant women should be especially cautious. In men, mobile phones are a risk to their fertility when carried in the pant pocket." And the cell phone company Orange warns on its home page: "Keep your mobile phone or other mobile device away from the stomach of a pregnant woman or lower abdomen of adolescents." (http://radio-waves.orange.com/en/ your-mobile/best-practice).

Akdag et al. (2016) exposed male rats for one year (longterm exposure) to Wi-Fi radiation, produced by a frequency generator, and analyzed several organs for DNA damage (brain, skin, liver, kidneys, testes, whole-body SAR 141.4 µW/kg, maximum 7127 µW/kg). They found that all organs showed increased levels of DNA damage due to the radiation exposure, but only in the testicular tissue was the increase significant. The testicular tissue of the rats apparently responded more sensitively to the 2.45 GHz radiation than other organs. The authors' recommendation: Men should be cautious about using laptops in their laps because their fertility may be impaired. Besides damage to DNA, the research team of Avendaño et al. (2012) found additional abnormalities: Sperm motility was lower in users (26-45 years old) who placed the Wi-Fi-activated laptop in their lap. The radiation exposure from the Wi-Fi-activated laptops was 3 times higher than from the Wi-Fi-deactivated laptops, and 7 to 15 times higher than in the control group (no laptop). Due to the laptop radiation, the number of immotile sperm was significantly higher and progressive sperm motility was significantly lower. Sperm quality can be lowered by Wi-Fi radiation emitted by a laptop, and as a result, fertility may become compromised. Dasdag et al. (2015) studied the long-term effects of 2.4 GHz radiation on the testicular functions of rats; a signal generator was placed at a distance of 50 cm. One group of male rats was exposed to 2.4 GHz radiation for 24 hours per day for 12 months (SAR values per point, 1 g, and 10 g of testicular and prostate tissues were 4880, 2420, and 1020 µW/kg); the other group was sham-exposed. At the end of the experiment, the weight of testes, sperm duct, prostate, and seminal vesicle as well as the motility, concentration in the epididymis, and morphological defects (tail and head defects, seminiferous tubules diameter, and tunica albuginea thickness) of sperm cells were determined by microscopic examination, which was performed blinded. Sperm shapes differed significantly between the two groups. In the exposure group, the percentage of defective sperm heads significantly increased, whereas the weight of the epididymis and seminal vesicle, the diameter of the seminiferous tubules, and the thickness of the tunica albuginea significantly decreased. All other parameters were not statistically significantly different from the controls. The authors' recommendation: Since long-term exposure to 2.4 GHz radiation can impair fertility, children and adolescents should be protected from Wi-Fi radiation. In male rats, Kumar et al. (2011) found that 2.45 GHz radiation (2 hours/day for 60 days, 0.21 mW/cm<sup>2</sup>, SAR 0.014 W/kg) caused oxidative stress and thus reduced fertility. Melatonin and testosterone levels were significantly lower in serum, whereas apoptosis rate and creatine kinase (for energy transport) were significantly higher in sperm. A pulsed electromagnetic field of 100 Hz can reduce the adverse effects because the magnetic flux induces circulating electric currents in the tissue that quench free radicals. Based on their findings, Kumar and colleagues conclude that 2.45 GHz radiation causes apoptosis during sperm development or maturation; caspase-3 seems to affect reproductive physiology via an oxidative pathway, whereby the impact of the latter can be reduced by a field pulsed at 100 Hz.

Meena et al. (2014) and several papers by the research group of Prof. M. Nazıroğlu (see chapter on oxidative stress) found adverse effects on testes caused by oxidative stress, which was reduced by melatonin. Shahin et al. (2014) found harmful effects in male mice after exposure to 2.45 GHz radiation (continuous wave, 2 hours/day for 30 days, 0.029812 mW/cm<sup>2</sup>, SAR 0.018 W/kg). In testes, they determined sperm count and motility, reactive oxygen species (ROS), nitrate and nitrite production, testosterone, nitric oxide synthase (iNOS), and the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD, an important enzyme for steroid biosynthesis). The testicular tissue had significantly changed due to radiation exposure. The seminiferous tubules had degenerated and showed a significantly smaller diameter, and the Leydig cells were damaged. The exposed animals had significantly fewer sperm cells (more dead cells) and a lower motility in comparison to the sham-exposed controls. ROS, nitrate and nitrite concentrations, lipid peroxidation, and antioxidant enzymes had changed significantly in the liver, kidneys, hypothalamus, and testes; in some cases, the change was even highly significant, for instance for ROS, especially in the liver and testes. In the testes, 3β-HSD activity and testosterone concentration levels were significantly reduced and iNOS levels had increased. The authors assume that 2.45 GHz radiation may cause infertility due to oxidative and nitrosative stress (free radicals) as well as due to the degeneration of testicular tissue. Shokri et al. (2015) used rat testes to examine how 2.45 GHz radiation (2 Wi-Fi antennas at opposite walls, one group 1 hour/day and another group 7 hours/day for 2 months) affects fertility and to determine changes in apoptosis, sperm, and tissue. The analysis was conducted under double-blind conditions. Exposed animals had a significantly lower weight of seminal vesicles, a lower number of sperm cells, and less motility; these effects were more pronounced in the higher exposure group. An intact germinal epithelium with about 5 cell layers was visible in the testes after one hour of exposure; after seven hours of exposure, statistically significant damage to the germinal epithelium of the seminiferous tubules could be observed, with statistically significantly fewer cell layers. In the 7-hour exposure group, apoptosis rate and caspase-3 activity (the enzyme that executes apoptosis once it has been initiated by other caspases) were significantly increased in the seminiferous tubules. The authors' recommendation: In view of these findings and the everincreasing number of Wi-Fi networks, exposure time to Wi-Fi radiation should be limited.

#### 1.2 Female reproduction – studies of prenatal and postnatal effects

The world-renowned Swedish Karolinska Institute in Stockholm published a press release on 3 February 2011 in which it issued a warning regarding wireless applications (cell phones, cordless phones, Wi-Fi) because children and pregnant women are at risk. The exposure limits must be lowered: "Current US and ICNIRP standards for radiofrequency and microwave radiation from wireless technologies are entirely inadequate. They never were intended to address the kind of exposures from wireless devices that now affect over 4 billion people." (Olle Johansson, Professor, Department of Neuroscience, Karolinska Institute, Stockholm, sagereports. com/smart-meter-rf/?page\_id=382).

Nakamura et al. (2000) studied the effects of continuous exposure to 2.45 GHz radiation (2 mW/cm<sup>2</sup> for 90 minutes) on pregnancy, the uterus or blood flow between uterus and placenta, hormones, and biochemical mediators (corticosterone, estradiol, prostaglandin  $E_2$ , prostaglandin  $F_2\alpha$ ). The blood flow was reduced due to the radiation exposure; an increase in progesterone and prostaglandin  $F_2\alpha$  was observed only in pregnant animals. The increase in corticosterone and the decrease in estradiol were similar in pregnant and non-pregnant animals. The disturbances in the uterus/ placenta cycle due to the 2.45 GHz radiation are probably caused by prostaglandin  $F_2\alpha$  and may be a risk factor for pregnancies. Margaritis et al. (2014) studied oogenesis in the ovaries of two Drosophila strains (fruit fly) after exposure to electromagnetic fields of various frequencies and examined if Drosophila is suitable as a test system (biomarker). To this end, reproductive capacity (fecundity) and apoptosis (with two test methods) were examined during oogenesis, which normally must pass through certain developmental stages. Another object of investigation was whether pulse modulation and continuous wave radiation cause different biological effects. The results are given for exposures to a microwave oven, Bluetooth (lowest field strength 0.3 V/m within the first 7 days), and Wi-Fi (2.44 GHz, 2.1 V/m, 10 Hz pulse, pulse duration 1 ms). Untreated and sham-exposed animals served as controls. All types of radiation, including low-level Bluetooth radiation up to 22 V/m from cell phones, showed a statistically significant increase in apoptosis rate, and at almost all frequencies, fecundity was reduced by 10 % (Wi-Fi and Bluetooth) to 30 % (cell phones and DECT cordless phones). The radiation seems to affect still unknown mechanisms at switching points during developmental stages of the oocytes. The number of pupae in the offspring was significantly lower. The authors assume that the effects are not mediated by the ELF components, because some of the devices do not have them, but by the pulses. Despite the rather low exposure level, Bluetooth, for example, caused the same damage with regard to apoptosis and reproduction as the signals without pulses put out by an FM generator, which at 13 V/m were 43 times higher than the Bluetooth exposure levels.

Özorak/Nazıroğlu (2013) studied the male offspring of female rats that were exposed to 2450, 900, and 1800 MHz (1 hour/day, 5 days/week during pregnancy and 6 weeks after birth with 12 µW/cm<sup>2</sup>, 10 V/m, whole-body SAR 0.01-1.2 W/kg, mean  $0.18 \pm 0.07$  W/kg). The double-blind study analyzed kidneys and testes for trace elements (the metals chromium, copper, iron, manganese, selenium, and zinc), because those act as cofactors in antioxidant enzymes, and the oxidative parameters of lipid peroxidation, glutathione and glutathione peroxidase, the vitamins A, E, and beta-carotene in the kidneys and testicular tissue of 4-, 5-, and 6-week-old offspring. The 4-week-old animals responded more sensitively to the radiation than the older 5- and 6-week-old animals; in the 6-week-old animals, the oxidative stress caused only significantly different levels in total antioxidant status (TAS), lipid peroxidation, copper, and iron in the kidneys compared to the controls. The authors conclude that exposure to 900, 1800, and 2450 GHz radiation can lead to oxidative stress in the kidneys and testes of young rats, which is reflected in increased levels of lipid peroxidation, oxidizable iron, and lower concentration levels of trace elements, TAS, and glutathione (GSH) in the kidneys and testes of animals that are still developing, which is comparable to puberty in humans. Shahin et al. (2013) studied female mice after exposure to 2.45 GHz radiation (non-thermal power density of 0.033549 mW/cm<sup>2</sup>, SAR 0.023023 W/kg, 2 hours/day for 45 days). After 45 days, blood and tissue homogenates were extracted from the kidneys and ovaries to determine the parameters of blood cells (erythrocytes, leukocytes, ratio of neutrophils to lymphocytes, hemoglobin), DNA breaks, NO, NO2<sup>-</sup>/NO3<sup>-</sup> (nitrite/nitrate), progesterone ( $P_4$ ), estradiol ( $E_2$ ), ROS, and antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). In exposed animals, a significant increase in ROS, hemoglobin, total count of erythrocytes and leukocytes, DNA strand breaks, and hormones (P<sub>4</sub> and E<sub>2</sub> were elevated in plasma compared to the controls, but significantly only E<sub>2</sub>) was observed as well as a significant decrease in NO and antioxidant enzyme activity in all three organs. Low-level microwave radiation produces physiological stress responses in pregnant mice, resulting in the death of embryos. In the ovaries, follicles were enlarged, the number of embryos was significantly lower, and embryonic development fell behind. The authors trace the adverse effects to oxidative stress (ROS impair the antioxidant defense system and can lead to apoptosis), a change in progesterone and estradiol levels, and DNA strand breaks. The change in the leukocyte count points to an inflammatory process. Radiation of 2.45 GHz is a potent trigger of oxidative stress.

In the first longitudinal study on this type of radiation, Sangün et al. (2015) investigated the effects of long-term exposure to Wi-Fi radiation (2.45 GHz) on the growth and development of young female rats. During embryogenesis, the most vulnerable developmental stage, severe damage can arise from external exposure to chemicals or radiation. Young females were sham-exposed and exposed prenatally and postnatally (2.45 GHz radiation for 1 hour/day until puberty with 45.5 V/m within near field, whole-body SAR 0.143 W/kg). At puberty, serum, ovarian and brain tissues were collected and the oxidative/antioxidative status determined. Chronic oxidative stress was observed in both organs. In serum, concentration levels of the following hormones were determined: follicle-stimulating hormone (FSH), luteinizing hormone (LH),  $17\beta$ -estradiol (E<sub>2</sub>), and insulin-like growth factor 1 (IGF-1). Only LH was significantly increased in serum after the pre- and postnatal exposure compared to the sham-exposed controls. In addition, the hypothalamus and ovaries were histologically analyzed for changes in the cells and tissues; no significant differences were observed. The prenatal exposure to 2.45 GHz caused impairments in growth and delayed puberty in female rats. The exposure levels in the study were within the range of international exposure limits. Chronic exposure to Wi-Fi radiation, especially during the intrauterine phase and early childhood, may have adverse effects on growth and puberty. The authors' recommendation: Precautionary measures should be implemented in the vicinity of these radiation sources and in the case of long-term exposure. Yüksel/Nazıroğlu et al. (2016) exposed female rats to 900, 1800, and 2450 MHz for one year. Dams and offspring were subsequently analyzed for their hormone status and oxidative stress (described in Chapter 6.1 on oxidative stress). In the 4-, 5-, and 6-week-old offspring, the radiation effects were more pronounced overall compared to the dams; for the 2.45 GHz exposure, the differences were particularly noticeable. The review of Desai, Kesari, and Agarwal (2009) summarizes the study findings on RF radiation (cell phone and 2.45 GHz) and its effects on the reproductive system. The authors conclude that, apart from other damage (DNA, cell membranes, calcium homeostasis, and others), male fertility may be impaired and currently valid exposure limits should be lowered. Seven other reviews come to the same conclusion, covering all frequency ranges: Adams et al. (2014), Agarwal et al. (2011), Behari/Rajamani (2012), Bellieni/Pinto (2012), British Columbia Centre for Disease Control (BCCDC) (2013), Dama/Bhat (2011), Gye/ Park (2011), La Vignera et al. (2012).

#### 2 Effects on EEG, brain, and brain development

There are already convincing studies showing that Wi-Fi radiation affects EEG results. Several research teams have carried out studies on this topic, using animals (rats, mice) and humans. Already in 1995, Lebrecht von Klitzing concluded in his study that the alpha activity (alpha waves 8-13 Hz, 5-100 µV) in humans whose EEG was recorded under the influence of low-frequency pulsed electromagnetic fields was altered, both during exposure and several hours afterwards. These effects were caused at exposure levels that were below international exposure limits. Aggarwal et al. (2013) recorded the EEG of 12 young male rats after they had been exposed to 2.45 GHz radiation (2 groups: 4 animals shamexposed and 8 animals exposed, 1 hour/day for 21 days,  $7.37 \times 10^{-4}$  mW/cm<sup>2</sup>, SAR 1.16 mW/kg). In both groups, the temperature, which was measured on days 0, 7, 14, and 21, hardly increased. All observed effects are therefore nonthermal effects. At the end of the experiment, two animals of the exposed group had died. From day 22, the EEG was recorded in the anesthetized animals for three hours. The response to the exposure over time was as follows: Only during the second hour were significant differences between the two groups observed across all frequency bands; during the third hour, in the theta and beta bands. Overall, the responses within the theta and beta bands were more pronounced than in the alpha and delta bands. Low-level chronic exposures to 2.45 GHz may therefore cause psycho-pathophysiological impairments because the electrophysiology of the neurons is altered. The synchronization/desynchronization of the firing neurons changed, which can have effects on the blood-brain barrier and concentration levels of neurotransmitters in synapses, the researchers state. Sinha, Aggarwal, and collaborators (2008) analyzed male rats for their behavior, thyroid hormone levels of T<sub>3</sub>, T<sub>4</sub>, TSH, and EEG after exposure to 2.45 GHz radiation (2 hours/day for 21 days, 16.5 µW/cm<sup>2</sup>, SAR parallel to E plane 3.6 µW/g, H plane 9.8 µW/g). Concentration levels for T<sub>3</sub> were significantly lower (100 to 78.8 ng/dl), for T<sub>4</sub> significantly higher (1.24 to 3.01  $\mu$ g/dl) in comparison to the control animals. TSH and body temperature hardly differed between the two groups. This also affected behavior (see Chapter 3 "Effects on Behavior"). Maganioti et al. (2010) carried out memory tests in 15 men and 15 women (mean age 23.7 years, high education level). The study subjects were instructed to memorize numbers after a signal tone, one time without RF exposure and another time with RF exposure (0.49 V/m at head); the Wi-Fi access point was located 1.5 m from the head of the participants. The signal tone of 3000 or 500 Hz was delivered via headphones to the test subjects who were instructed to memorize the number named thereafter. The entire test, which took 45 minutes, included 52 repetitions. These tests were carried out twice within a 2-week interval. The results showed that there were no differences in the delta and theta bands, compared to the controls or between the sexes. However, there were significant differences in the alpha and beta bands. The EEG record was similar for both sexes during sham exposure. After activating the 2.45 GHz radiation exposure, the increase of energy in men was insignificant, while in women the decrease

was significant. The alpha band is associated with long-term memory and beta activity with tension, alertness, and concentration. The findings point to a physiological change due to Wi-Fi radiation exposure, which varies in its effect on the excitability of the cerebral cortex in women and men.

In the 1980s, the team of Lai and Singh carried out many experiments in rats to elucidate the mechanisms caused by brain exposure to 2450 MHz radiation, especially the hippocampus; which neurological functions are changed and how behavior is affected. In the 1990s, experiments investigating DNA damage were added. In 1983, they examined the effect of three drugs (apomorphine, amphetamine, and morphine) during short-term exposure and determined that the animals differed in their responses. The researchers found the nature of microwave radiation on brain functions to be complex. In the following years, they studied cholinergic activity while administering various antagonists (1987a, 1987b, 1988, 1989a, 1989b, 1991, 1994, 1996b). They found that both cholinergic and endogenous opioid-neurotransmitter systems in the brain are involved in deficits of spatial learning and memory after exposure to low-level 2.45 GHz radiation. The uptake of choline (as a measure of cholinergic activity) in the hippocampus was significantly reduced, which can be inhibited by beta-funaltrexamine. Spatial learning and memory were impaired during exposure, but not when pretreated with the cholinergic agonist physostigmine or the opiate antagonist naltrexone. Pretreatment with the peripheral opiate antagonist naloxone methiodide had no effect. An increase in the concentration level of muscarinic cholinergic receptors was found in the hippocampus of rats that had been exposed to the radiation for 45 minutes; the effect could be inhibited by naltrexone. Nazıroğlu and Gümral (2009) exposed rats to 2.45 GHz radiation (1 hour/day for 28 days, 11 V/m, wholebody SAR 0.1 W/kg, whole-brain SAR 1.73 W/kg), one group each had either selenium or L-carnitine administered prior to exposure. Immediately after exposure, the EEG was recorded followed by a brain analysis, which followed a double-blind protocol. In the brains, the concentration levels of vitamins A, C, and E, lipid peroxidation (LP) and glutathione (GSH) were determined, as well as the activity of glutathione peroxidase (GSH-Px) and beta carotene. The activity of GSH peroxidase was significantly higher in the selenium-treated group; GSH and beta carotene levels did not differ significantly from the controls. Lipid peroxidation was significantly reduced with the administration of selenium, even more so with the administration of L-carnitine. The significant reduction in vitamin C and E levels due to the 2.45 GHz radiation exposure was significantly prevented by selenium and L-carnitine. These vitamins as well as L-carnitine and selenium provide protection against oxidative damage (ROS) in brain tissue. In the EEG, only a slight hyperexcitability could be observed after exposure, which was compensated by selenium and L-carnitine.

The research team of Judita Orendáčová carried out a series of studies on the generation and development of newly formed neurons; two of those studies examined rats of both sexes after exposure to 2.45 GHz radiation (2009, 2011). In the study of Orendáčová et al. (2009), newborns (7 days) and adult rats

(24 months) were exposed to pulsed 2.45 GHz radiation with a power density of 2.8 mW/cm<sup>2</sup>. It was the objective of the study to examine whether differences in the development of the cells could be observed. The adult brain contains at least two regions for neuronal growth and migration: the subventricular zone (SVZ) and dentate gyrus in the hippocampus. The newly formed cells migrate from their place of generation to other regions and mature to differentiated cells. The animals of both age groups were divided into two groups: exposure 4 hours/day for 2 days (acute) or 8 hours/day for 3 days (chronic). The researchers could demonstrate that in newborn rats the differences in cell growth between controls and exposed animals were significant, but not in 24-monthold animals. The change in cell division rates is dose- and age-dependent. On the days 7 through 10, the activity is very high, then drops to the levels of the controls, and increases again from day 14 through 21, only to drop once more to the levels of the controls on day 35. In the first week after birth, cell division rates or the development and maturation of neurons are particularly high. During this period, there is a risk of harmful effects on neurogenesis as could be shown in newborn animals. In the second study, Orendáčová et al. (2011) examined again two age groups. The rats of both sexes were exposed once on day 7 (newborns) or on day 28 (young adults) after birth (2 hours with a power density of 2.8 mW/cm<sup>2</sup>, 10 animals each). After this short exposure, the immediate early gene product fos protein, which increases in neurons at early stress, was determined as well as NO-producing cells, which regulate neurogenesis after day 7. In comparison to the controls, exposed animals showed significant differences in the expression of immediate early genes. Numerous NO-producing cells were found on day 7 in the rats exposed on day 7; in the controls, only on day 10. In the young adult rats (P28), the number of NO-producing cells was also different compared to the controls. Papageorgiou et al. (2011) tested the responses of 15 men and 15 women (mean age ca. 24 years), using EEG to record the P300 waves. The P300 component is active during thought and memory processes. The subjects heard incomplete sentences via headphones and were instructed to complete the sentences with a sensible response. There were significant differences in the responses between exposed and non-exposed persons, but also between women and men. While there were no differences between the genders in nonexposed subjects, the P300 amplitude significantly increased in men and significantly decreased in women when the Wi-Fi signals were activated (0.49 V/m).

Paulraj and Behari (2006a) studied calcium-dependent protein kinase C (PKC) in developing brain cells of young male rats, exposing the animals to 2.45 GHz radiation (2 hours/day, 35 days, 0.344 mW/cm<sup>2</sup>, SAR 0.11 W/kg). PKC is involved in very many pathological processes, including carcinogenesis in many cell types. In nerve tissue, this enzyme regulates the release of neurotransmitters and the formation of long-term memory. Brain tissue (entire brain, hippocampus, and brain without hippocampus) was examined for PKC activity; a significant drop in activity was observed in the entire brain and hippocampus compared to the controls. In the brain tissue without hippocampus, no significant differences compared to the sham-exposed controls were found. Electron microscopy revealed an increased number of glial cells. Chronic exposure to 2.45 GHz radiation may impair the growth and development of the brain at non-thermal levels. This may be one explanation for the frequently observed changes in learning and memory owing to radio-frequency radiation. Testylier et al. (2002) tested in male rats which effects 800 MHz and 2.45 GHz radiation exposures have on the behavior and concentration levels of acetylcholine (ACh) in the hippocampus. In the case of 2.45 GHz, the exposure lasted for 1 hour at 2 mW/cm<sup>2</sup> or 4 mW/cm<sup>2</sup> (whole-body SAR 3.26) or 6.52 W/kg), respectively. Seven hours after exposure, the decrease in the mean concentration level of ACh was not significant at 2 mW/cm<sup>2</sup> and significant (40 %) at 4 mW/cm<sup>2</sup>. The release of ACh began to drop as soon as exposure began, reaching its lowest level compared to the sham-exposed controls 5 hours after exposure. In the latter group, the levels continued to increase slightly. This is not a thermal effect because the decrease was the steepest several hours after exposure had ended. A video camera recorded the behavior of the animals from hour 4 to the end of the experiment. No differences were observed. Yang et al. (2010) tried to answer the question of how pulsed 2.45 GHz radiation (20 minutes, SAR 6 W/kg) affects microglia in the brains of mice (N9 microglia cell line). Microglia are immune cells in the nervous system, which are activated by injuries and infections of the nervous tissue. A certain signal pathway, the JAK-STAT signal pathway, controls the immune mechanisms, which are also activated when exposed to radiation. When the regulation is impaired, immunodeficiency and cancer may develop. The radiation exposure caused changes in the gene expression of various genes; among others, the gene expression for the tumor necrosis factor (TNF- $\alpha$ ) and inducible nitric oxide synthase (iNOS), which are involved in the inflammatory response of nervous tissue. The excessive activation of microglia may lead to degenerative diseases (ALS, Alzheimer's, Parkinson's). The findings showed that microglia were activated by exposure to 2.45 GHz radiation involving TNF- $\alpha$ , NO, und ROS; this was followed by a pro-inflammatory response, and the JAK-STAT signal pathway was activated at the same time. The radiation exposure represents an external physical factor that can lead to inflammatory processes and damage in nerve tissue through microglia activation. Yang et al. (2012) exposed adult male rats to 2.45 GHz radiation (pulsed for 20 minutes, 65 mW/cm<sup>2</sup>, SAR 6 W/kg) and chose stress-related genes for further analysis. Three hours after exposure, 41 relevant genes out of 2.048 in total were significantly changed in the hippocampus: 23 upregulated and 18 downregulated (heat shock proteins (HSP), metabolism, signal transduction, cytoskeleton, apoptosis, cell attachment, DNA repair, and others). Seven genes concern stress-related heat shock proteins or chaperone proteins, including in particular the gene for HSP27 and HSP70, whose expression was significantly higher in the hippocampus, specifically in the pyramidal cells of the Ammon's horn (CA3 region) and in the granule cells of the dentate gyrus. Both heat shock proteins were at their highest level at different times; they serve different functions. The data provide direct evidence that 2.45 GHz radiation triggers stress responses in the hippocampus of rats.

#### 2.1 Significance of the 10 Hz pulse

Wi-Fi radiation contains a 10 Hz pulse which is why the results of the Andechs bunker experiments of Dr. Rütger Wever (Max Planck Institute for Behavioral Physiology, Seewiesen and Erling-Andechs) from 1968 are so important. Wever studied the effect of the frequency of 10 Hz (within the alpha wave range of 8-13 Hz,  $5-100 \mu$ V) on the circadian rhythm in humans. In 1968, the newspaper DIE ZEIT wrote about this experiment: "After geophysicists had discovered that this electromagnetic field - an electromagnetic wave radiation of 10 Hertz – goes through a maximum and minimum over the course of an earth day, following a 'diurnal cycle,' chronobiology researchers became interested in studying if it may have an effect on the 'inner clock' of humans. Two underground bunkers mirroring each other were built, one of which was shielded against the 10 Hz field by several layers of iron. Study subjects moved in and lived in their wakesleep room under constant conditions, but above all without a clock, completely surrendering to their own inner rhythm." Being isolated from the ambient 10 Hz field affected the study subjects: "Not only is there a shift...in the alternation between activity and rest, but the synchronized autonomic functions also shift, such as the functioning of the kidneys and the maximum and minimum of the body temperature. ... Ten such experiments provide a certain result: The human body responds to an alternating electric field of 10 Hz" (http:// www.zeit.de/1968/08/im-bunker-sind-die-tage-laenger).

On his experiment, Wever writes: "The evidence of 10 Hz field effects on the human circadian rhythm also answers the question of whether such fields affect humans in general. For this question, the frequency of ca. 10 Hz is also of interest: The especially stable alpha wave component of the electroencephalogram has a frequency of 10 Hz; furthermore, the entire body surface of warm-blooded animals mechanically vibrates with a frequency of about 10 Hz (Hecht 2017)."

The medical physicist Lebrecht von Klitzing (1995, 2006) has already presented verifiable experimental results regarding the effects of the 10 Hz pulse of Wi-Fi radiation, demonstrating effects on the EEG and heart rate variability. These results still need to be replicated by other research groups. The literature search revealed that available research on the effects of the 10 Hz pulse of Wi-Fi radiation is rather unsatisfactory and that research efforts should be urgently increased. The Andechs experiment confirms that humans are electromagnetic beings and that the 10 Hz pulse could play a larger role in the brain than previously assumed.

#### 3 Effects on behavior

That the nervous system is affected by non-ionizing radiation was confirmed by the Swiss Federal Council in 2015: "Research observations of varying quality document other biological effects that cannot be attributed to thermal effects. An effect on brain waves has been sufficiently demonstrated based on scientific criteria" (Swiss Federal Council 2015). The effects on the EEG and brain are reflected in learning, memory, and behavior. These findings should be highly relevant to the debate and decision-making process regarding the implementation of Wi-Fi networks at preschools, schools, and universities as part of Germany's so-called digital learning initiative.

Cammaerts und Johansson (2014) observed the behavior of ants along their trails when exposed to various frequencies. Besides cell phone radiation, Wi-Fi routers (30 minutes at 0.06 and 0.08  $\mu$ W/cm<sup>2</sup> mean) and notebooks (5 minutes, 0.03-0.05 µW/cm<sup>2</sup>) with and without the Wi-Fi function activated were used (distance of 20-30 cm to colonies). Blinded repetition of the experiments with a different observer confirmed the results. Within a few seconds of the Wi-Fi routers being activated, the ants showed disturbed behavior; up until 30 minutes, the changed behavior pattern intensified significantly compared to non-exposed controls. It took 6 to 8 hours until the normal foraging pattern was reestablished. When the Wi-Fi function of the notebook was activated, the animals started to behave erratically, appeared to be sick within seconds; when the Wi-Fi function was deactivated, they behaved normally. Chaturvedi et al. (2011) observed the behavior of male mice after being exposed (2 hours/day for 30 days, power density 0.026 mW/cm<sup>2</sup>, SAR 0.036 W/kg; additional parameters: blood count, sperm count and motility, DNA breaks in brain cells). After 30 minutes of exposure, the behavior of the mice in the running wheel and water labyrinth was significantly different compared to the nonexposed animals. Spatial memory was impaired. Deshmukh et al. (2015) used the frequencies 900, 1800, and 2450 MHz to investigate the effects of chronic, low-level microwave radiation exposure on learning capacity, memory, heat shock proteins (HSP), and DNA damage in rat brains. For 180 days, male animals were exposed to very low levels (SAR 5.953 ×  $10^{-4}$ , 5.835 ×  $10^{-4}$ , or 6.672 ×  $10^{-4}$  W/kg, 2 hours/day, 5 days/ week). Spatial orientation as well as learning and memory performance were impaired at all three frequencies. In the brains, the level of HSP70 and the number of DNA strand breaks was significantly increased. DNA damage was also significantly increased at 1800 and 2450 MHz compared to 900 MHz. Deshmukh, Banerjee et al. (2016) exposed rats for 90 days with 900, 1800, and 2450 MHz (SAR for 2450 MHz  $6.672 \times 10^{-4}$  W/kg) and examined their behavior, HSP70, and DNA in brain tissue. The authors observed reduced brain performance as above as well as significantly higher levels of HSP70 and DNA strand breaks, especially at 2450 MHz. Hassanshahi et al. (2017) divided 80 male rats into two groups. One group was sham-exposed, the other was exposed to 2.4-2.4835 GHz for 30 days, 12 hours/day with 23.6 dBm. The activity of the exposed animals did not differ from the sham-exposed control animals, but the exposed animals were significantly worse at differentiating between known and unknown objects. The expression of the muscarinic receptor 1 (for acetylcholine) in the hippocampus doubled after Wi-Fi radiation exposure; the GABA transporter 1 (GAT1) did not differ between exposed animals and controls. Together with previous findings, we can conclude that Wi-Fi radiation has a detrimental effect on the functions of the nervous system,

both on a molecular and behavioral level. The calcium flow in neurons could be changed due to the Wi-Fi radiation. One of the most important functions of the brain is the consolidation of sensory information, which travels along different sensory channels. This process is important for experiences and the interaction with the external world. Different brain regions such as parts of the temporal lobe, which receive/ accept many sensory inputs, are involved in consolidation processes. It is assumed that acetylcholine is involved in the integration processes of the many perceptions via the muscarinic receptor. The involvement of the neurotransmitter GABA (gamma-aminobutyric acid) is also discussed in this context.

In the 1980s and 1990s as well as in 2000, 2004, and 2005, Henry Lai and his collaborators conducted many experiments on electromagnetic fields, including some studies on 2.45 GHz. Among other things, they studied the behavior of animals (mice and rats), physiological changes in the brain (especially the hippocampus, the area in which learning and memory are processed), and DNA damage after 45 minutes of exposure (1 mW/cm<sup>2</sup>, SAR 0.6 W/kg or 2 mW/cm<sup>2</sup>, 2 hours, SAR 1.2 W/kg). In 1996, they found single- and doublestrand DNA breaks in rat brain cells due to 2.45 GHz radiation (2 mW/cm<sup>2</sup>, 2 hours, SAR 1.2 W/kg). Some experiments revealed that the cholinergic system is involved, not after 20 minutes, but after 45 minutes of exposure to 2.45 GHz radiation, 1 mW/cm<sup>2</sup>, SAR 0.6 W/kg. Overall, the findings of the experiments showed that physiological changes in the neurotransmitter system (catecholamine, serotonin, and acetylcholine) occurred and thus affected behavior. Lai and Singh refer to experiments by Thomas et al. (1979, 1979, 1980) and Wangemann/Cleary (1976), who had already observed changes in rats and rabbits in the 1970s and 1980s. Wang/ Lai (2000) studied the behavior of rats in the water labyrinth after acute exposure to pulsed 2.45 GHz radiation. The acute exposure to 2.45 GHz microwaves had a significant impact on the behavior of the rats. The scientists attributed the changed behavior to the decrease in cholinergic activity in the brain of the animals (in the frontal cortex and hippocampus), which was caused by the 2.45 GHz radiation. Lai (2004) observed that spatial learning of rats (2.45 GHz continuous waves, 2 mW/cm<sup>2</sup>, whole-body SAR 1.2 W/kg) only changed when exposure to 2.45 GHz radiation was combined with an additional magnetic background field of 60 mG (6 µT). Li et al. (2008) studied the effect of pulsed 2.45 GHz radiation at 1 mW/cm<sup>2</sup> for 3 hours per day for 30 days (chronic exposure) and found in all rats significantly increasing deficits in spatial learning and memory. The function of glucocorticoid receptors (GR) in the hippocampus was impaired because the intracellular distribution (the relative rate of GRs in cytoplasm and nucleoplasm) for the active transmission of signals in the nucleus had shifted (5 independent experiments). In the control groups, the GR level was highest in the cytoplasm, whereas after exposure, the GR level was higher in the nucleoplasm. The scientists determined glucocorticoid levels and showed that 24 hours after exposure ceased the increase in corticosterones in the blood was highly significant compared to the controls. After exposure, the apoptosis rates in the hippocampus were highly significantly increased in all studied regions (CA1, CA3, and dentate gyrus), with a particularly clear effect in the CA1 region. These three changes can explain the learning deficits. They could be a result of the microwave exposure, which causes stress in cells. The data show that corticosterone is an important regulator regarding learning deficits due to exposure to microwaves since it has been known for a long time that increased concentration levels of glucocorticoids impair learning, both in animals and humans. The changes in corticosterone concentration levels alone cannot explain the learning and memory deficits, the researchers state, so there must be additional biological molecules that are affected by the 2.45 GHz radiation. The researchers point out that the findings contradict the findings of Cassel et al. (2004) and Cobb et al. (2004) (employees at the laboratory of the US Air Force), who had used the same exposure conditions as Li and also Lai and collaborators, but had not observed any behavioral changes in the water labyrinth. Cosquer/Cassel (2004, 2005) refer to Lai et al. and did not find any behavioral changes in rats after exposure to low-level radiation (45 minutes, 1 mW/cm<sup>2</sup>, SAR 0.6 W/kg). In contrast, experiments by Banaceur et al. (2013) demonstrated that after the long-term exposure to the radiation of an activated Wi-Fi device (2.4 GHz, 2 hours/day, 30 days, whole-body SAR 1.6 W/kg), the memory performance changed in mice which had had three Alzheimer-related genes inserted (amyloid beta, presenilin, and tau protein). For comparison, normal mice were treated in the same way (exposed and sham-exposed). After the end of the exposure, behavior with regard to spatial learning and memory, anxiety, and motor activity was observed. After exposure, hardly any differences were found in weight, body temperature, and motor activity of the four groups, but the anxiety level of the exposed Alzheimer mice was lower. The authors interpreted this finding as an improvement in brain performance. Shahin et al. (2015) exposed mice to continuous wave radiation at 2.45 GHz (15, 30, and 60 days' exposure and 60 days' sham exposure with 0.0248 mW/cm<sup>2</sup>, whole-body SAR 0.0146 W/kg 2 hours/ day). Spatial learning and memory were observed in the water labyrinth. The hippocampus tissue was analyzed for changes in oxidative/nitrosative stress (oxidation of DNA, lipids, proteins, as well as nitrite and nitrate concentration), antioxidant enzymes (SOD, CAT, GSH-Px), morphology of neurons in the microscope, apoptosis and DNA repair proteins (PARP-1) in the hippocampus regions. The level of creatine kinase was determined as a measure of the cells' energy status. The experiments were repeated once. With increasing exposure the significant impairments intensified compared to the sham-exposed controls. Degenerate neurons, antioxidant enzymes (SOD, CAT, GSH-Px) and creatine kinase significantly decreased, and the concentration levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), lipid peroxidation, oxidative damage of DNA and proteins, as well as apoptosis significantly increased. Learning and memory declined as a result of the cell damage. Besides the changed behavior and EEG in the rats due to the 2.45 GHz radiation, Sinha (2008) also found impairments in the levels of thyroid hormones (see Chapter 9 "Effects on Thyroid"). The behavior of the exposed animals significantly differed from that of the non-exposed counterparts: The exposed animals turned out to be hyperactive.

#### 4 Effects on DNA – tumor-initiating and tumor-promoting potentials

DNA damage is often the result of oxidative damage to various structures and molecules in the cells, rather than the direct consequence of radiation or chemicals. Oxidative damage can trigger many other impairments, e.g. changes in enzyme activities and cell membrane properties, singleand double-strand DNA breaks. Via this indirect pathway, frequencies of cell phone and Wi-Fi radiation can lead to metabolic changes and thus also to cancer. Damage to DNA has been verified many times; as early as the 1990s, there were several studies demonstrating as much.

#### 4.1 Effects on DNA

The studies by Lai and Singh (1995), for example, attracted worldwide attention. The researchers found single- and double-strand breaks in the DNA of rat brain cells when exposed to pulsed radiation at 2 mW/cm<sup>2</sup> (1.2 W/kg). Immediately after exposure, the strand breaks were not significant, but 4 hours later, they had increased significantly compared to the controls. After exposure to continuous fields, the increase was not significant. In 1996, DNA strand breaks were confirmed, but no significant difference between pulsed and continuous radiation exposures was observed in this study. In the 1997 study, the rats were kept under the same conditions, but prior to and after exposure, they were in addition treated with either melatonin or N-tert-butyl-alpha-phenylnitrone (PBN), both of which are very effective in scavenging free radicals. As a result, the level of DNA strand breaks was reduced (assessed by blinded analysis). These results are evidence that 2.45 GHz radiation generates oxidative stress through the formation of free radicals in the brain cells of rats. Already back then, the authors Lai and Singh said that more frequently occurring DNA breaks in brain cells can lead to neurodegenerative diseases and cancer, and free radicals to many diseases. These findings are therefore important for health issues related to microwave radiation. In 2005, Lai und Singh conducted another experiment of 1 mW/cm<sup>2</sup> (0.6 W/kg, 2 hours) and an additional magnetic field of 45 mG (4.5 µT). The exposure to pulsed and continuous radiation caused again significantly higher levels of single- and double-strand breaks; the 4.5 µT field alone caused hardly any differences compared to the controls, while the combination of the two fields reduced the level of DNA damage. Akdag et al. (2016) observed DNA damage in rat brain tissue after male animals had been exposed to Wi-Fi radiation for one year (whole-body SAR 41.4 µW/kg and 7127 µW/kg maximum). The analysis of the organs (brain, liver, kidneys, skin, and testes) revealed in all cases DNA damage in the exposed animals compared to the non-exposed control animals; however, the differences were only significant in the testicular tissue. Avendaño et al. (2012) could demonstrate that Wi-Fi radiation from laptops reduces the motility of sperm and increases the DNA fragmentation in sperm (see Chapter 1). Chaturvedi et al. (2011) found in 2011 that the DNA of neurons was significantly damaged in mice when they were exposed (0.026 mW/cm<sup>2</sup>, SAR 0.036 W/kg) for 2 hours per day for 30 days. Besides behavior, blood values (number of cells, hemoglobin, enzymes), sperm count, and motility, the authors also analyzed DNA strand breaks in the brain cells of the male mice. The levels of strand breaks were significantly higher in the exposed brains compared to the controls.

In three studies of rat brains, the research team of Deshmukh (2013, 2015) and Megha (2015) studied DNA damage from radiation well below the ICNIRP exposure limit of 2 W/kg, among others. Deshmukh et al. (2013) studied the effects of low-level microwave radiation on the brains of Fischer rats at 900, 1800, and 2450 MHz for 30 days (2 hours/day, 5 days/ week, SAR 5.953  $\times$  10<sup>-4</sup>, 5.835  $\times$  10<sup>-4</sup>, or 6.672  $\times$  10<sup>-4</sup> W/kg). DNA damage was verified by comet assay. After exposure, all parameters of the comet assay showed significant differences compared to the controls. The level of DNA strand breaks was the highest at 2450 MHz. Microwave radiation can therefore be referred to as a genotoxic agent. When DNA becomes damaged, this can lead to cell death, cancer, or neurodegenerative disease if the repair system is overwhelmed. Deshmukh et al. (2015) studied the learning ability of rats when exposed to 900, 1800, and 2450 MHz radiation for 180 days; in addition, heat shock proteins (HSP70) and DNA damage were determined at SAR levels as in the study described above (5.953 × 10<sup>-4</sup>, 5.835 × 10<sup>-4</sup>, and 6.672  $\times$  10<sup>-4</sup> W/kg). Again, the comet assay showed significantly increased levels of DNA breaks compared to the controls, but also significantly increased levels at 1800 and 2450 MHz compared to 900 MHz. In both studies, Deshmukh et al. found increased DNA damage (strand breaks) in rat brain cells after exposure to very low levels of radiation (6.672  $\times$  10<sup>-4</sup> W/kg) for 30 or 180 days, respectively. As a possible cause, the researchers discuss indirect mechanisms via oxidative processes through reactive oxygen species. In the 2015 study, the authors also observed increased levels of heat shock proteins and changes in the behavior of the animals (the exposed animals needed more time than the control animals). Besides 2450 MHz, exposures to 900 and 1800 MHz were also investigated in this experiment, and it was found that 1800 and 2450 MHz had more noticeable effects. Megha et al. (2015) studied the brains of male rats at the frequencies of 900, 1800, and 2450 MHz and at the already mentioned low levels (SAR 0.59, 0.58, and 0.66 mW/kg) after 60 days of exposure (2 hours/day, 5 days/week). In addition to the oxidative stress markers GSH, SOD, CAT, PCO (protein carbonyl), MDA (malondialdehyde), and cytokines, the DNA was also analyzed. After exposure, all parameters of the comet assay showed a significant increase; the greatest differences were measured for exposures at 2450 MHz. Besides oxidative stress and inflammatory responses, the experiments revealed DNA damage in the brains of the rats. The three studies show that three frequencies cause DNA damage and that 2450 MHz has the greatest effect. Gürler et al. (2014) studied the effect of garlic extract on rats. They tried to clarify whether garlic has a protective effect when the animals are exposed to microwave radiation at non-thermal levels (SAR 0.02 W/kg or 3.68 V/m, 1 hour/

day for 30 days). The exposure limit for the public is set at 0.08 W/kg. One group received an oral garlic extract daily one hour prior to exposure. After 30 days, lipid peroxidation (MDA), protein oxidation, and DNA oxidation (formation of 8-hydroxy-deoxyguanosine, 8-OHdG) were determined in the whole blood. In the exposed group, the concentration levels of 8-OHdG were significantly increased in brain tissue and blood plasma compared to the control group. The garlic extract prevented the increase in DNA oxidation (of 8-OHdG). The protein oxidation (level of advanced oxidation protein products, AOPP) in the blood was significantly higher compared to the controls; due to the garlic extract, the levels of the exposed group were similar to those in the control group. The brain tissue and lipid peroxidation levels were indistinguishable among the three groups. Conclusion: Lowlevel exposure to 2.45 GHz radiation can cause significant oxidative damage in the DNA and proteins of the brain tissue and blood of rats; certain substances in the garlic extract can significantly reduce the oxidative effect. The low SAR value of 0.02 W/kg may be the reason that lipid peroxidation was not found to increase.

Kesari/Behari and collaborators (2010a, 2010b, 2012) also studied rat brains after the male animals had been exposed to 2.45 GHz radiation (2 hours/day for 35 days, 0.34 mW/cm<sup>2</sup>, whole-body SAR 0.11 W/kg). In the exposed rat brains, the comet assay revealed significantly increased double-strand breaks (double-blind analysis), i.e. significant DNA damage. In addition, the activity levels of the enzymes SOD, glutathione peroxidase, and histone kinase were reduced and the catalase level had increased. The researchers point out that the significant changes, DNA damage, and oxidative stress can lead to tumor promotion. The balance between damage and the ability to repair is disturbed, which can lead to mutations or apoptosis. Already in 1993, Maes and collaborators observed a threefold increase in chromosome aberrations and micronuclei in lymphocytes of volunteers after their blood samples had been exposed to 2.45 GHz radiation (50 Hz pulse, 80 mW/ml, 75 W/kg) for 120 minutes. The increase was less after 30 minutes. Sister chromatid exchange did not occur more often in exposed samples. The authors noted that this finding was surprising because the exposure level was too low (according to proponents of the thermal dogma still prevalent today) to be able to break chemical bonds directly. Paulraj and Behari (2006b) examined the brains of 35-day-old male rats for DNA damage. The rats were exposed with 0.344 mW/cm<sup>2</sup> (1 W/kg) for 2 hours per day, 5 days a week for 35 days. In the exposed group, significantly increased single-strand breaks were found (controls 24.11  $\pm$  4.47 µm or 41.011  $\pm$  4.66 µm DNA migration). In the simultaneous experiments at 16.5 GHz, similar effects were observed. In this study, DNA damage was also seen as a possible risk for disorders, impairments of neurological functions, and the development of degenerative diseases. In 1994 Sarkar et al. found significantly increased levels of DNA breaks in the brains and testes of mice when exposed to 2.45 GHz radiation (2 hours/day, 1 mW/cm<sup>2</sup>, continuous wave, SAR 1.18 W/kg) compared to the controls, whereby the exposure levels were below the ICNIRP limits. The researchers emphasize that this is not a thermal effect and that this mutagenic effect significantly increases the risk for cancer promotion in the brain and genetic material. Already back then, they suggested that the exposure limits should be reviewed. Zotti-Martelli et al. (2000) exposed human lymphocytes of two healthy 27-year-olds to three different devices per frequency (2.45 – because this is the resonance frequency of water - and 7.7 GHz, for 15, 30, and 60 minutes, at 10, 20, and 30 mW/cm<sup>2</sup>, blinded analysis of duplicate trials), and based on micronucleus tests, found that the frequency of micronuclei in the exposed lymphocytes rose with increasing exposure duration and field strength; the effect was significant at higher field strengths and for longer exposure durations. The levels at 2.45 GHz were as follows for subjects in both studies: controls 2.5 ‰ at 30 mW/cm<sup>2</sup> and 7.5‰, 8.5‰, and 11.5‰ for the exposure durations of 15, 30, or 60 minutes. The cell cycle did not change and binucleated cells did not show any significant differences; the temperature did not increase, either. The researchers point out that neurasthenia, vascular damage in the nervous system, and cancer mortality occur more frequently in radar technicians. They recommend implementing protective measures against non-ionizing radiation.

#### 4.2 Effects on cancer (cells)

Cig B, Nazıroğlu M (2015) studied the effect of distance to the radiation source on apoptosis, oxidative stress, and calcium accumulation in the cytosol via TRPV1 channels as caused by cell phones (900 and 1800 MHz) and Wi-Fi (2450 MHz) in breast cancer cells. The TRPV1 channel - a channel permeable to cations, in this case Ca<sup>2+</sup> ions – opens in the presence of harmful heat, oxidative stress, and capsaicin (CAP, trigger of heat in pepper/chili). The analysis of calcium signals, ROS production, cell survival, and apoptosis revealed that Wi-Fi and cell phone radiation at 10 cm distance can produce significant oxidative responses and apoptosis in cancer cells. A distance greater than 10 cm may provide a certain protection against oxidative stress, apoptosis, and too high levels of intracellular Ca<sup>2+</sup> ions. There were no significant differences at the distances of 20 and 25 cm. Czerska et al. (1992) exposed human lymphocytes from fresh blood to pulsed and continuous radiation of 2.45 GHz (12.3 W/kg) and examined the cells for degeneration (microscopy by three independent examiners under blinded conditions). Controls were sham-exposed at 37 °C, and heat-treated cells (0.5, 1.0, 1.5, and 2 °C above) served as positive controls. At 37 °C, exposure to continuous radiation caused no increase in degenerate cells, but cell damage was observed; with an increase in temperature, the number increased. The pulsed radiation without any increase in temperature generated a significant increase in degenerate cells. With an increase in temperature, the pulsed radiation caused the highest number of degenerate cells. Continuous and pulsed radiation generate degenerate lymphocytes in different ways (lymphoblastic transformation). In a review paper, Nazıroğlu, Tokat, and Demirci (2012c) discussed the role of melatonin in the context of oxidative stress caused by EMF (ELF and RF) in connection with the calcium (Ca<sup>2+</sup>) signal pathway in breast cancer. Calcium (Ca2+) homeostasis is one of the most important factors for the physiological functioning of cells because it is involved in cell growth, signal transmission, and apoptosis. It is regulated by ion channels in cell membranes and many other cell components, including melatonin. Melatonin also acts as a radical scavenger for oxidative stress. The researchers reviewed 89 studies and concluded that melatonin plays an important role as an antioxidant, for the calcium influx into cells, and as a hormone. In the case of a disturbed calcium (Ca2+) homeostasis and melatonin concentration level, women who work at night could be at higher risk of developing estrogen-dependent breast cancer because exposure to electromagnetic fields at night lowers the production of melatonin. Szmigielski et al. (1982) exposed mice to 2.45 GHz radiation (2 hours/day, 6 days/week for 1 to 6 months, 5 or 15 mW/cm<sup>2</sup>), and for comparison, a sham-exposed group was used with animals that were chronically stressed and treated with the carcinogenic substance benzopyrene. Over the course of a year, it became obvious that the radiation exposure lead to a significantly faster growth of skin tumors and that the number of tumors was twice as high after 10 months compared to the non-exposed controls.

#### 5 Effects on cardiac activity

Wi-Fi radiation also affects cardiac activity and blood pressure. Saili et al. (2015) studied the impact of radiation on the heart of rabbits. The effects on cardiac activity were recorded while the radiation exposure (1 hour) was provided by an access point at a distance of 25 cm; there was a parallel group of non-exposed animals. In the exposed rabbits, a significant increase in heart rate and blood pressure were observed; Wi-Fi radiation affected the heart rate variability. This shows that 2.45 GHz radiation changes the regulation of the cardiovascular system. The ECG was unchanged. The 2.45 GHz radiation of Wi-Fi devices most likely acts on receptors, which leads to a change in the bond between receptor and ligand. The impact of the radiation on heart rate variability causes the higher heart rate and higher blood pressure. Zhu et al. (2016) found a plausible mechanism for the apoptosis in heart muscle cells of rats due to microwave radiation. Exposure to 2.45 GHz radiation was carried out at 50, 100, 150, and 200 mW/cm<sup>2</sup> for 6 minutes. The following parameters were investigated: ATP activity, respiratory chain in the mitochondria, the enzymes creatine kinase (CK), (lactate dehydrogenase) LDH, and aspartate transaminase (AST), troponin I, the oxidative parameters SOD, GSH, and lipid peroxidation, apoptosis proteins (MAP kinase pathway), and morphology. All biochemical parameters showed significant dose-dependent changes, some of which were highly significant – the higher the dose, the greater the change. Tissue analysis under the optical and electron microscopes showed dose-dependent significant morphological changes such as irregular striations of heart muscle cells, discoloration of cell nuclei, fewer mitochondria with destroyed membranes, and other degenerative manifestations. Furthermore,

significantly increased levels of apoptosis proteins and rates were found which means that the radiation facilitated the apoptosis of the heart muscle cells via the mitochondrial pathway. Overall, radiation exposure leads to an oxidative imbalance (oxidative stress), malfunctioning mitochondria, and apoptosis in heart muscle cells. For the effects on cardiac activity, further evidence is provided by the studies of Kim MJ, Rhee SJ (2004) (see Chapter 8.2) and by two studies of Lebrecht von Klitzing (2014, 2016).

#### 6 Mechanism of action – oxidative cell stress

The formation of free radicals or reactive oxygen species (ROS) is a process that occurs in all cells during normal metabolism. The cells can counteract this process using antioxidant molecules. Both processes are usually in balance. Should this balance be disturbed too greatly by external factors, cells can initiate apoptosis, the programmed cell death. These processes involve, among others, reactive oxygen species, including oxygen (O2<sup>-</sup>) radicals, hydroxyl (OH) radicals, and hydrogen peroxide  $(H_2O_2)$ . They also pave the way for the first steps of carcinogenesis such as initiation and promotion as well as damage to cell membranes and DNA. Oxidative stress is one of the most frequently studied and documented effects of non-ionizing and non-thermal radiation. Oxidative mechanisms, which occur in biological systems, are well understood and reputable scientists do not deny them (Becker 2007, Hecht 2015, Hensinger/ Wilke 2016, Warnke/Hensinger 2013). Oxidative stress occurs when oxidative processes due to free radicals exceed the capacity of the antioxidant processes to neutralize, shifting the balance toward oxidation. In cells, various inflammatory injuries can be caused by, for example, oxidation of unsaturated fatty acids, proteins, and DNA: "Free radicals are chemical entities characterized by a high reactivity. The formation of free radicals during the metabolism of xenobiotics is therefore an important mechanism of action through which some toxic agents may cause cellular damage. ... The interaction of free radicals with cellular components may lead to the formation of secondary radicals derived from proteins, lipids, or nucleic acids. These may, in turn, react with other cellular macromolecules, and initiate and thus maintain a chain reaction. Consequently, cellular damage may be exacerbated to a large extent. ... Radicals may have immediate effects, such as cellular necrosis and, eventually, fibrosis. They may, however, also result in delayed long-term effects, for example, tumorigenesis" (Marquardt 1994: Lehrbuch der Toxikologie).

#### 6.1 WLAN/Wi-Fi leads to oxidative cell damage

One of the most important review papers on ROS was published by Yakymenko and colleagues (2016). The paper discusses the mechanism of damage due to microwave radiation exposure: The formation of ROS in cells and the oxidative damage of DNA as a result of the excessive formation of free radicals due to radiation exposure interfere with cell signaling paths and thus may lead to carcinogenesis. The findings are remarkable: The review of 100 scientific papers available at the time found that 93 showed oxidative damage or oxidative stress in biological systems - in vitro, in plants, animals, and humans. Among these papers, 12 studied the effects of 2.45 GHz. All of these studies were carried out at exposure levels below the ICNIRP exposure limits. The number of micronuclei, for example, increased already at 0.5 W/kg (exposure limit 2 W/kg), and ROS formed already at 0.1 µW/cm<sup>2</sup> or 0.3 W/kg. A series of enzymes are involved in metabolic and oxidative processes (NADH oxidases, ornithine carboxylase (ODC), catalase, superoxide dismutase (SOD), cytochrome oxidase, and Na/K-ATPase). Cell structures are damaged (DNA, membranes, mitochondria, ion channels, electron transport chain, proteins, and others) as are Ca<sup>2+</sup>dependent signal pathways and conformation changes of proteins. Once the damage reaches a certain level, apoptosis may be initiated. Extreme overproduction of ROS and oxidative DNA damage can cause cells to degenerate into malignant tumors. Oxidative stress is associated with carcinogenesis. Atasoy et al. (2013) observed adverse effects on the growth of rat testes due to common Wi-Fi devices (2.437 GHz). They exposed young animals for 24 hours per day for 20 weeks at a maximum SAR level of 0.091 W/kg and examined various parameters in the blood serum and testicular tissue. In most cases, there were significant differences between the exposed and non-exposed groups in terms of the measured parameters and levels of oxidative/ antioxidant molecules and enzymes (malondialdehyde MDA, activities of the enzymes xanthine oxidase, superoxide dismutase, catalase, glutathione peroxidase). The results show that chronic exposure of the whole body to a Wi-Fi router causes oxidative damage to developing testes. For this study, still developing animals were chosen because their organs, in particular their testes, respond more sensitively to radiation and because children in school and at home spend many hours in front of their computers and laptops. Chauhan et al. (2017) also observed oxidative stress in male rats after the animals had been exposed to a microwave oven at 0.2 mW/cm<sup>2</sup> (whole-body SAR 0.14 W/kg) for 2 hours per day for 35 days. The brain, liver, kidneys, testes, and spleen (triplicate trials) were examined for lipid peroxidation and tissue changes. In the brain, liver, and spleen, a significant increase in lipid peroxidation was found, and under the optical microscope, tissue changes were also found in the brain, liver, testes, kidneys, and spleen compared to the non-exposed control animals. The latter had normal tissue, while the radiation caused degenerate testicular tissue and damaged sperm cells - changes which could result in infertility. The authors suspect that these changes may also occur in humans. Degenerative changes were observed in the brain, including in the hippocampus, which is responsible for learning and memory. Likewise, in the spleen, liver, and kidneys of all exposed animals, structural changes were observed. In 2013 Eser et al. examined rat brains which had been exposed to 900, 1800, and 2450 MHz for oxidative stress in the frontal cortex, cerebellum, and brain stem, as well as inflammatory processes (interleukin-1ß) and apoptosis (caspase-3). Exposure at 1.04 mW/cm<sup>2</sup> (SAR 1.04 W/kg) lasted for 2 months with 1 hour per day. After exposure, almost all parameters were significantly changed compared to the non-exposed controls: Tissue changes in the frontal cortex, brain stem, and cerebellum, oxidative status, apoptosis (caspase-3), and inflammation (interleukin 1 beta (IL-1β)). After exposure to 2.45 GHz radiation, the degeneration of neurons and apoptosis rate were highest in the frontal cortex and brain stem. The authors state that these changes may lead to functional impairments and the development of cancer. Kumari et al. (2012) examined antioxidant enzymes in adult male rats (2.45 GHz radiation, 2 hours/day for 35 days at a power density of 0.22 mW/cm<sup>2</sup>, whole-body SAR 0.15 W/kg). After exposure, the liver tissue was examined. The 2.45 GHz radiation induced an overproduction of free radicals, which led to the suppression of antioxidant enzymes and ultimately to oxidative stress in the liver, whereby the liver function was impaired (see Chapter 8 "Effects on Liver"). Othmann et al. (2017) observed in rats that the offspring suffered from oxidative stress when their mothers were exposed to Wi-Fi radiation from standard Wi-Fi routers (2.45 GHz). Exposed animals only had half the number of offspring compared to the control animals. In the first two weeks after birth, the exposed young animals showed delayed reactions, and their sense of balance and maturation of the motor system seemed to be impaired. The antioxidant enzymes in the brain were significantly changed. Changes in cholinesterase activity could affect the corresponding neurotransmitters and thus behavior. The Wi-Fi radiation temporarily disturbed the redox balance through oxidative stress; the oxidative impairments due to being exposed in utero are apparently compensated in adulthood. In adult animals, no abnormal motor or emotional behavior was observed. The study shows that exposure of pregnant mothers to 2.45 GHz Wi-Fi radiation has various adverse effects on the offspring. Brain development is delayed, and enzyme activities due to oxidative stress are changed without visibly lasting changes in the behavior of the adult animals. Özorak et al. (2013) studied in female rats how radiation exposures at 900, 1800, and 2450 MHz (Wi-Fi) affect the oxidative status in the kidneys, testes, and offspring. Pregnant animals and their offspring were exposed (1 hour/day, 5 days/week for 6 weeks, power density 12  $\mu$ W/cm<sup>2</sup>, 10 V/m, whole-body SAR 0.18 ± 0.07 W/kg); the assessment followed a double-blind protocol. The male offspring's development was examined in week 4, 5, and 6 after birth. In the kidney and testicular tissues, the following parameters were measured: lipid peroxidation, glutathione, enzyme activity of glutathione peroxidase, and the antioxidant vitamins A, E, and beta carotene, protein level, and trace elements (Cr, Fe, Cu, Mg, Mn, Se, Zn). Many lab values were significant or even highly significant compared to the non-exposed controls; others were not significant. The researchers concluded that RF radiation exposure contributes to significant levels of oxidative stress in the kidneys and testes during development and that the offspring may experience early onset of puberty. Yüksel et al. (2016) examined the hormone status of pregnant rats and their offspring after long-term exposure to 900, 1800, and 2450 MHz radiation. The dams were exposed for 1 hour per day for 5 days a week throughout the entire pregnancy, and

the offspring were exposed for a whole year (52 weeks, 11 V/m, whole-body SAR 0.1 W/kg). The blood serum and uterine tissue were analyzed for lipid peroxidation and enzyme activities, GSH, antioxidant vitamins A, C, and E in blood plasma of the dams, as well as the hormones prolactin, estrogen, and progesterone in serum. In addition, the body temperature of the pregnant animals was measured. The assessment was conducted under double-blind conditions. In the adult animals, the findings showed hardly any differences in the oxidative ratios; only the overall oxidative status had significantly increased after exposure. In the offspring, lipid peroxidation was significantly higher in utero of the exposed group, GSH-Px activity was significantly lower, and GSH levels did not differ significantly. Concentration levels of the hormones were as follows: Almost all measured values were different between the control and exposed groups. For prolactin and estrogen, the decrease was more pronounced in adult animals exposed to 2450 MHz than to 900 and 1800 MHz. In the offspring, prolactin levels were also significantly lower, most significantly for 2450 MHz exposure. The body temperature significantly differed between the non-exposed and exposed groups. After long-term exposure (1 hour/day, 5 times per week for 1 year), all three frequencies caused changes in hormone concentration levels and oxidative stress in the pregnant animals and their offspring. The researchers do not rule out the possibility of additional changes during longer exposure, which may also manifest in humans, especially young teenagers who often use their cell phone for several hours a day.

#### 6.2 Substances that protect cells

As a response to oxidative stress, living organisms have developed antioxidant defense mechanisms; furthermore, antioxidant substances can be absorbed from food. These include catechins, selenium, the vitamins A, C, and E, beta carotene, and other plant components. Though we hardly need additional evidence to demonstrate that radio-frequency electromagnetic fields cause oxidative effects or microwaves produce free radicals and peroxides, experiments with antioxidant substances are well suited to confirm this fact. To demonstrate the oxidative effect of RF radiation exposure as well as the ability of different substances to protect against the effects of oxidative stress, various research teams have used extracts of garlic, basil, olive leaves, and the vitamins A, E, and C. The following studies confirm both the oxidative and antioxidant mechanisms in cells and tissues, the damage potential of Wi-Fi radiation and effect of protective substances. Aweda et al. (2003) examined in rats what effects an exposure level of 6 mW/cm<sup>2</sup> (2.45 MHz for 8 weeks) may have on lipid peroxidation. A control group was sham-exposed, and among the exposed groups, some also had additional ascorbic acid (vitamin C) and tocopherol (vitamin E) administered. Furthermore, the level of lipid peroxidation was determined in blood plasma. The analysis of free radicals and lipid peroxidation showed that the levels in exposed animals were significantly higher compared to the control animals; the administration of the vitamins C and E an increase in lipid peroxidation within 24 hours; after about one week of exposure, the levels dropped to the levels of the control group. When the animals were pretreated with the antioxidants vitamin C (ascorbic acid) and vitamin E (alpha tocopherol) before exposure to RF radiation, the levels dropped below the levels of the control group and increased within the study period. This means that RF radiation generates oxidative stress, which can be compensated by antioxidants. The research group of Aynali et al. (2013) observed oxidative damage in mucous membranes after exposure to 2.45 GHz radiation (1 hour/day for 28 days). Lipid peroxidation was significantly increased; the administration of melatonin significantly reduced this effect as well as glutathione peroxidase activity. Ceyhan et al. (2012) exposed the skin of two groups of male rats (2.45 GHz pulsed radiation similar to Wi-Fi, 1 hour/day for 4 weeks, SAR 64 mW/kg on the skin) and examined whether beta-glucan has a protective effect against oxidative stress in the skin. The activity of the antioxidant enzymes SOD, GSH-Px, and catalase as well as lipid peroxidation levels were measured in skin samples. The RF radiation produced significantly increased levels of lipid peroxidation and catalase activity as well as significantly decreased SOD and GSH-Px activity. The treatment with betaglucan slightly increased catalase activity and did not significantly prevent the decrease in GSH-Px activity compared to the controls. Lipid peroxidation was significantly lower due to beta-glucan, almost at the level of the control group. This study demonstrates the protective effect of an additional substance against oxidative stress due to RF radiation. After exposure to 2.45 GHz radiation (1 hour/day for 28 days, 11 V/m, SAR 0.1 W/kg), Gümral and Nazıroğlu (2009) measured oxidative stress in blood plasma of male rats, which could be prevented by L-carnitine; selenium did not have a protective effect.

reduced the oxidative effect. RF radiation exposure caused

Kim and Rhee (2004) fed rats with green tea catechins and discovered that the latter protect rats from microwave-induced oxidative damage in cardiac tissue (see also Chapter 5 "Effects on Cardiac Activity"). Cardiac tissue is particularly susceptible to oxidative stress, which can lead to diseases like high blood pressure and cardiovascular disorders. The polyphenol component catechin, which is found in green tea, is a highly effective antioxidant that scavenges free radicals. The oxidase system was activated and the levels of superoxide radicals, lipid peroxides, oxidized proteins, and lipofuscin increased. The antioxidant defense system of the cardiac tissue was weakened in the rats exposed to RF radiation, but the oxidative damage was significantly reduced by the administration of catechin. Over the last years, the research group of Prof. Mustafa Nazıroğlu has carried out several experiments on oxidative stress, some of which included the administration of substances that can compensate the harmful effects of microwave radiation. Besides exposing rats to 2.45 GHz radiation (1 hour/day for 28 days), Türker et al. (2011) administered selenium and L-carnitine and determined the concentration levels of lipid peroxidation and the antioxidant vitamins A, C, and E in cardiac tissue. The exposed groups showed significantly increased levels of lipid peroxidation, which were significantly reduced by selenium and L-carnitine. The concentration levels of the vitamins C, A, and E were decreased in the exposed animals; however, they were higher in those treated with selenium and L-carnitine. Likewise, the activity of the enzyme glutathione peroxidase was higher. Overall, exposure to 2.45 GHz radiation caused oxidative stress in the hearts of rats. The administration of selenium and L-carnitine could reduce the oxidative effect of the 2.45 GHz radiation. Meena et al. (2014) studied in male rats whether melatonin has a protective effect when the animals are exposed to 2.45 GHz radiation (2 hours/day for 45 days, 0.21 mW/cm<sup>2</sup>, SAR 0.14 W/kg). Due to the RF radiation exposure, a very high level of ROS production was observed in the testicular tissue; melatonin decreased the ROS levels. In sperm cells, DNA strand breaks and apoptosis rates were very high due to the 2.45 GHz radiation exposure; the administration of melatonin reduced these levels, even though they were still significantly above the control levels. The RF radiation exposure caused significantly lower testosterone levels in the testes, but they were significantly higher with the administration of melatonin. Under the microscope (blinded analysis), the exposed tissues showed disorganized seminiferous tubules and inflammation in the testicular tissue, effects which were prevented by melatonin. The non-exposed controls showed normal reproductive and Sertoli cells with a normal developmental cycle. Melatonin reduced the changes in the enzymes lactate dehydrogenase isoenzyme (LDH-X) and xanthine oxidase (XO) in the testes caused by lipid peroxidation and protein oxidation. The oxidative stress in the testicular tissue generates DNA damage and affects the development of sperm. The authors wonder whether microwave radiation may affect the polarization of cell membranes and that, to a certain extent, melatonin has an impact on xanthine oxidase whose enzyme activity was significantly increased by the radiation exposure. Melatonin could be used as a therapeutic agent. Oksay et al. (2014) also observed in the testicular tissue of rats that the oxidative damage due to 2.45 GHz radiation at 0.1 µW/cm<sup>2</sup> (1 hour/ day, 30 days, pulse 217 Hz, 10 V/m, SAR ca. 0.143 W/kg) can be reduced by the administration of melatonin. Melatonin is an efficient radical scavenger that protects testes from lipid peroxidation when 2.45 GHz radiation causes oxidative damage in the testicular tissue of rats. Salah et al. (2013) studied the effectiveness of olive leaf extracts (daily administration during exposure) on disturbances of the glucose metabolism and oxidative stress in the liver and kidneys of rats caused by exposure to Wi-Fi signals (2.45 GHz, 1 hour/ day for 21 days). The cages were placed underneath an access point of a Wi-Fi device. The following parameters were measured: MDA, the enzymes GSH-Px, catalase, SOD, and thiol group levels, tissue changes, total protein in plasma, cholesterol, triglyceride, alanine transaminase (ALT), aspartate transaminase (AST), uric acid, creatinine, and iron level. The RF radiation exposure caused diabetes-like conditions and almost all parameters measured in blood plasma were significantly changed. The olive leaf extract prevented disturbances of the glucose metabolism and restored the enzyme activity of GSH-Px, catalase, and SOD and the thiol group level in the liver and kidneys. The increased MDA levels after

exposure were reduced by the olive leaf extract in the liver, but not in the kidneys. This means that the olive leaf extract reduced the oxidative stress and thus the disturbances of the glucose metabolism. Histological examination revealed damaged liver cells after exposure; damage was reduced by the olive extract. The data show that subchronic exposure to 2.45 GHz radiation causes metabolic disorders and especially disturbs the glucose metabolism; the olive leaf extract improved the glucose metabolism and minimized the adverse effects of oxidative reactions caused by the radiation exposure. Tök L, Nazıroğlu M (2014) examined the effectiveness of melatonin on the eye lenses of male rats when exposed to 2.45 GHz radiation (217 Hz pulsed, 1 hour/day for 30 days, 0.1 µW/cm<sup>2</sup>, 11 V/m, corresponds to a wholebody SAR of about 0.1 W/kg). The analysis of the oxidative parameters (lipid peroxidation, GSH, GSH-Px activity) in the homogenate of the lenses revealed some significant and some non-significant results. The results show that Wi-Fi radiation led to a non-significant increase in the lipid peroxidation level in the eye lenses, and melatonin to a significant decrease. The GSH-Px activity was significantly decreased during exposure, and the administration of melatonin significantly increased the activity compared to the controls. After exposure, GSH was non-significantly decreased, while the addition of melatonin significantly increased the GSH concentration levels compared to the Wi-Fi-exposed group.

#### 7 Effects on cell cycle

Cleary et al. (1996) wanted to clarify whether continuous 2.45 GHz and 27 MHz radiation have a non-thermal effect on the cell cycle. They exposed synchronized ovarian cells of hamsters (2 hours, SAR 5 and 25 W/kg). In cell cultures, the cell cycle, division, and growth were examined from 2 hours to 4 days after exposure. It was shown that both frequencies have different effects: 2.45 GHz radiation causes changes during all stages of the cell cycle and its effect was twice as high compared to exposure at 27 MHz under the same temperature conditions (37 °C). In the hamster cell line V79, Ballardin et al. (2011) studied the non-thermal effects of 2.45 GHz (5 and 10 mW/cm<sup>2</sup>) on cell division (in particular, the spindle apparatus) and survival rate. Apart from the RF radiation exposure, the cells were also subjected to heating (15 minutes 38-41 °C). Under exposure conditions, the number of cells with defective spindle apparatus was much higher, more so at 10 than at 5 mW/cm<sup>2</sup> compared to the controls. The number of cells with apoptosis was significantly increased at both field strengths. Up to 41 °C, the temperature had no impact on the correct functioning (assembly) of the spindle apparatus; at 40 and 41 °C, the apoptosis rate increased. From these results, we can conclude that, despite the very short exposure duration, the changes are due to the RF radiation exposure and not thermal effects. The researchers assume that short-term radiation exposure with the resonance frequency of water in V79 cells causes reversible changes in the spindle apparatus and the cells return to a normal cell cycle, while longer exposure periods could cause irreversible effects.

#### 8 Effects on the liver

Holovska et al. (2015) examined the livers of rats for structural and ultrastructural changes under the optical and electron microscope following exposure to 2.45 GHz radiation (3 hours/day for 3 weeks, power density 2.8 mW/cm<sup>2</sup>). In the exposed livers, they observed more blood in the vessels, sporadic necrotic liver cells, dilation of liver sinusoids; the cell nuclei were irregular and contained condensed chromatin. Some hepatic lobules showed small foci of inflammation. The electron microscope revealed changes in the membrane structures, some had binucleated cells, significantly more vesicles in the cells, irregularly shaped cell nuclei, and significantly more lipid droplets of varying sizes. RF radiation exposure can therefore have adverse effects on the liver cells of rats. Kumari et al. (2012) studied antioxidant enzymes and liver function tests in adult male rats exposed to 900 MHz and also 2.45 GHz from a microwave oven (2 hours/day for 35 days at 0.22 mW/cm<sup>2</sup>, whole-body SAR 0.15 W/kg). After exposure, many parameters of liver tissue were examined: activity of the antioxidant enzymes GSH-Px, SOD, catalase, and lipid peroxidation (MDA) to determine damage to the cell membranes, and for the liver function test total bilirubin (TBIL), alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), as well as total protein, albumin, and globulin. It was observed that a slight increase in ROS leads to an increased activity of antioxidant enzymes, while high concentration levels of ROS inhibit these enzymes so that they cannot carry out their protective function against ROS. Antioxidant activity and ROS suppression are important to protect the liver from radiationinduced damage. The 2.45 GHz radiation exposure produced significantly reduced levels of GSH-Px (13.33 ± 069 to 22.12 ± 0.82 nmol/min/ml) and liver SOD activity (32.50  $\pm$  1.52 to 52.31  $\pm$  1.52 U/ml), whereas the catalase level in the liver was significantly increased (24.74 ± 2.31 to 17.73 ± 2.42 nmol/min/ml). As an indication of cell membrane damage, the MDA concentration levels were significantly increased (3.92 ± 0.44 to 1.87 ± 0.55 nmol/min/ml). The concentration levels of TBIL, ALP, ALT, and AST were significantly higher than in the control group. These findings show that 2.45 GHz radiation causes excessive production of free radicals, which inhibit the antioxidant enzymes and thus lead to oxidative stress in the liver and damage cells, tissues, and organs. Similar results were also found for 900 MHz.

#### 9 Effects on the thyroid

**Misa-Agustiño et al. (2012)** found that heat shock protein levels of HSP70 and HSP90 were changed due to 2.45 GHz radiation exposure below the thermal threshold after 90 minutes and 24 hours (average SAR at 0.046, 0.104, and 0.482 W/kg in the thyroid or whole-body SAR at 0.0169, 0.0364, and 0.161 W/kg). They examined the thyroids of rats and noticed that the cells did not show any damage and that the HSP concentration levels were significantly reduced after 90 minutes of exposure, but no apoptosis mechanisms (chromatin condensation, cell nucleus fragmentation) were

creased again, but remained below the control levels. The 2.45 GHz radiation generated stress in the thyroid; however, without initiating apoptosis in the thyroid tissue. Misa-Agustiño et al. (2015) studied the effects of 2.45 GHz radiation on thymus cells in rats under similar conditions as above (mean SAR in thymus 0.046, 0.104, and 0.482 W/kg, whole-body SAR 0.0169, 0.0364, and 0.161 W/kg) at 1.5, 3.0, and 12 W for 30 minutes. Ninety minutes after the RF radiation exposure, protein concentration levels, HSP90, HSP70, and GR levels (glucocorticoid receptors) were determined and morphological changes examined in thymus tissue and cell extracts. Under the microscope, many changes were found: increased permeability of the endothelium (red blood cells outside the vessels), new formation of vessels, significantly more reticular epithelial cells and red blood cells. The number of glucocorticoid receptors (GR) in the thymus cortex of the exposed animals had also increased. Furthermore, the HSP90 level was lower and in one sample, the HSP70 level was higher. The changes are an indication of cell stress and an impaired immune response due to the radiation exposure, as well as impairment of maturity and apoptosis in thyroid tissue and development of thymocytes. The changes in HSP levels mean that the functions of apoptosis and immune response are not properly regulated. Besides the changes in behavior and EEG in the rats due to 2.45 GHz radiation exposure, Sinha (2008) also found impaired thyroid hormone levels (TSH, T<sub>3</sub>, T<sub>4</sub>). The non-thermal exposure (2 hours/day for 21 days, 16.5 µW/cm<sup>2</sup>) caused a 2.1 % increase in the water content of the brain, leading to swelling of 6.97 %. Parallel to the significant changes in behavior (higher motor activity and others), T<sub>3</sub> was significantly decreased in the blood on days 16 and 21, T<sub>4</sub> was significantly increased on day 21, and the TSH level differed non-significantly compared to the controls. The low field strengths may be harmful; they are sufficient to change thyroid hormone levels and the responses of the animals. There could be a connection between changes in hormone levels and behavior because thyroid hormones work together with other neurohormonal systems and neurotransmitters. The behavior of the exposed animals significantly differed from that of the non-exposed ones; the exposed animals turned out to be hyperactive.

initiated. After 24 hours, the HSP concentration levels in-

#### 10 Effects on gene expression

Lee et al. (2005) found that pulsed 2.45 GHz radiation of a magnetron device (SAR 10 W/kg, pulse 133 W/kg for 155 µs, 320 V/m) changed the gene expression in human cell cultures of the HL60 cell line (promyelocytic leukemia cell line) after 2 and 6 hours of exposure. The gene expression of 221 genes was changed after 2 hours of exposure (early genes); after 6 hours, 896 genes were affected, of which 742 were known and 154 unknown. Among them, genes for apoptosis (6), metabolism, polysaccharide biosynthesis, RNA functions, and translation were upregulated; genes for transport, metabolism, RNA functions and cell cycle (23) were downregulated. Overall, the gene expression of heat shock proteins had not significantly increased even though some genes were significantly different. The results demonstrate that 2.45 GHz radiation changes the gene expression via non-thermal mechanisms in cell cultures.

#### 11 Effects on cell membranes

The study by Panagopoulos et al. (2015), published in the Scientific Reports of the Nature Publishing Group, proposed the hypothesis that polarization, which is the fixed spin direction of the electric field vector of a wave, is a crucial factor in understanding biological effects of electromagnetic fields at low exposure levels. Since polarization, like the pulse modulation of microwave radiation, is a property independent of a given power density level, it can also cause damage associated with this type of radiation at extremely low exposure levels. Panagopoulos et al. found that, after only 4.8 minutes of exposure, the Wi-Fi frequency at a "low" power density level of 0.8 µW/cm<sup>2</sup> can also lead to irregular responses in the ion channels of cell membranes, which is caused by polarization. Calcium (Ca<sup>2+</sup>) homeostasis is one of the most important factors for the physiological functioning of cells because it is involved in cell growth, signal transmission, and apoptosis. It is regulated by ion channels in cell membranes. Nazıroğlu et al. (2012a) found in the brain tissue of male rats (double-blind analysis) that melatonin acts and regulates via calcium (Ca<sup>2+</sup>) ion channels when exposure to pulsed 2.45 GHz radiation (0.1  $\mu$ W/cm<sup>2</sup>, SAR 0.1434) produces oxidative stress in neurons and increases the Ca<sup>2+</sup> influx. Voltage-gated calcium channels (VGCC) and voltage-independent, calcium-permeable cation channels (TRPM2) are involved. This means that the radiation of Wi-Fi devices causes oxidative damage via calcium channels and an increase in intracellular Ca<sup>2+</sup> ions. Melatonin protects brain tissue from damage by counteracting oxidative stress. Nazıroğlu M, Cig B et al. (2012b) also studied the impact of a Wi-Fi device (2.45 GHz pulsed, 0.1-2.5 W/kg for 1, 2, 12, or 24 hours) on oxidative stress and calcium (Ca<sup>2+</sup>) influx into human leukemia cells. The RF emissions of the device caused oxidative stress and increased cell growth through a significantly increased Ca2+ influx into the cytoplasm of human leukemia cancer cells, the researchers conclude (see also Cig B (2015), Nazıroğlu M (2012c)).

#### 12 Effects on bacteria

At the moment, there are only a few studies on the effects of 2.45 GHz radiation on bacteria. In 2015 and 2017, Taheri and collaborators studied antibiotic resistance of the bacterial strains Listeria monocytogenes, Escherichia coli, and Klebsiella pneumonia after exposure to radiation. They observed that exposure increases resistance. In 2015, the researchers studied the response of Klebsiella to five different antibiotics after exposure to 2.45 GHz radiation from a Wi-Fi router (1 W) at a distance of 5 cm up to 8 hours after the radiation exposure. Up to 4.5 hours, the sensitivity of the bacteria to the radiation increased; thereafter, the sensitivity decreased, that is, resistance started to develop. The researchers attribute this to the fact that bacteria must reach a certain damage threshold to have their defense mechanisms activated, in this case, resistance to all five antibiotics. This follows a nonlinear response curve, which means that there is a so-called window effect. In such a case, certain conditions (frequency, dose, etc.) must be met to elicit a significant effect, and lower or higher values have no effect. Since all antibiotics in this study attack the cell walls of the bacteria, the cell walls could have been made more permeable by the RF radiation and thus would allow molecules to pass through the channels, which, for example, could be used by antibiotic therapies. After exposure to 900 and 2450 MHz (standard Wi-Fi router 2.45 GHz, SAR 0.13 W/kg at 14 cm distance to bacterial suspension), the growth rates of Listeria monocytogenes and Escherichia coli significantly increased as did the antibiotic resistance to six antibiotics in this study from 2017 study. The exposed cells grew faster and in the case of the Listeria, the cell density was higher at the end of the experiment compared to the controls. Escherichia coli developed resistance to all six antibiotics; in the case of the Listeria, a significant resistance was observed for only one antibiotic compared to the controls. It follows that different frequencies trigger different responses. Exposure to 900 MHz and 2.45 GHz microwave radiation affects ion channels, membranes, DNA repair systems, and probably also ion pumps in cell membranes. These findings are a reason for concern since this development may pose a possible health threat.

#### **13 Effects on plants**

Chen et al. (2009) exposed wheat seeds (Triticum aestivum) to salt stress and 2.45 GHz radiation to improve the yield. Wheat does not grow well under salt stress since it increases the formation of reactive oxygen species (ROS), which is followed by damage to the cells. Plants defend themselves against this type of cell damage by, for example, increasing the release of antioxidant enzymes such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD), NO synthase, as well as nonenzymatic substances such as glutathione, ascorbic acid (vitamin C), proline, nitric oxide (NO), and other responses. The seeds were divided into four groups: non-exposed control group, exposed group, in addition salt stress with and without radiation exposure; the exposure at the center of the seeds reached 126 mW/cm<sup>2</sup> and lasted for 0, 5, 10, 15, 20, or 25 seconds. The following measurements were taken: length of the roots and shoots, MDA, in leaves the enzymes SOD, POD, CAT, GR, protein concentration level, oxidized and reduced glutathione, NOS, and NO. The microwave exposure caused a significant increase in root and shoot length three days after treatment, except for when exposure lasted 25 seconds. The effect was most pronounced at 10 seconds, which is why this time was subsequently used. The enzyme activities were the lowest in the salt-stress group and the highest in the microwave-exposed group (significantly compared to the control group).

The researchers conclude that the ROS-induced decrease in shoot and root lengths under salt stress conditions can be improved by treating the seeds. The microwave radiation exposure increases the tolerance of the wheat seeds against salt stress by stimulating the antioxidant defense system that produces NO as well as enzymatic and nonenzymatic antioxidants. In agriculture, this could be an advantage to reduce crop losses. Soran et al. (2014) found that herb or vegetable plants, some of which are also medicinal plants, show stress responses to microwave radiation exposure. Three weeks after sowing, many changes could be documented in parsley (Petroselinum crispum), celery (Apium graveolens), and dill (Anethum graveolens) after exposure for three weeks to 900 MHz of a cell phonelike signal generator (10  $\mu$ W/cm<sup>2</sup>) or to 2.45 GHz of a Wi-Fi router (7 µW/cm<sup>2</sup>). Plants generally produce essential oils to fend off pathogenic agents and pests, whereby the levels are greatly dependent on environmental conditions. Under the electron microscope (cell wall, chloroplasts, and mitochondria), photosynthesis and assimilation were analyzed. In all cases, the water transport was significantly decreased, most noticeably during the Wi-Fi exposure. The release of essential oils and volatile compounds from the leaves (odoriferous substances) was increased, especially the release of monoterpenes. The thickness of cell walls, length of chloroplasts, and size of mitochondria were dramatically smaller when exposed to Wi-Fi radiation (e.g. Anethum controls 1.57 μm, 900 MHz 0.55 μm, Wi-Fi 0.25 μm). Wi-Fi radiation exposure resulted in decreased concentration levels of essential oils. There was a direct link between structural and chemical changes in the three plant species. Conclusions: Microwave radiation produces stress in plants which is why plants increasingly release fragrances and terpenes, and the oil content and leaf anatomy also change. Wi-Fi radiation produces more stress than 900 MHz radiation.

#### 14 Studies that found "no effects"

Questions are raised by cell experiments with very high to extremely high SAR levels of 50 to 200 W/kg, which often find no effects, i.e. no differences between the exposed and sham-exposed controls, or which attribute changes to thermal effects (e.g. Wang et al. 2005, 2006, Takashima et al. 2006, Komatsubara et al. 2005, Fortune et al. 2010). Except for Fortune, all other researchers are members of the same single research team: Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, Miyakoshi J, Takashima Y, and Wang J. Since these high levels of exposure do not occur in real life, it is fair to ask what purpose these experiments serve. A question that needs scientific clarification is which phenomena or mechanisms underlie the observation that extremely high SAR levels do not trigger changes, but also do not seem to lead to cell death in certain cases. In various studies, the authors point out that low to very low exposure levels have effects, but higher levels do not. This not only applies to studies using 2.45 GHz, but also to other frequencies for which window effects have long since been discussed. Industry and government agenevidence that the use of Wi-Fi is safe. Commissioned by the wireless telecommunications industry, the authors compiled an overview of the current state of scientific studies on Wi-Fi radiation in 2013. After a comprehensive discussion of the technology, the authors claim that there was "overwhelming consensus" that no health risks exist below the exposure limits. They refer to the thermal effect as the allegedly only known and recognized one despite the fact that the International Agency for Research on Cancer (IARC) determined a possible carcinogenic effect in 2011. Foster and Moulder establish criteria according to which, in their opinion, studies are useful or not for evaluating the effects relevant to human health. In Table 4 of their paper, seven useful studies are listed that did not find any effects; these papers were authored by two research teams (two by an Italian team and five by a French one). The papers listed in Table 4 are authored by research teams who rarely or never find effects, almost like a group of who's who in the "no effects" research world. Some of these studies were industryfunded or involve team members who are associated with the industry (e.g. with the telecommunications company Orange). In Tables 5 and 6, studies with effects are listed, but are regarded as weak according to the authors. They argue that the weak studies would have no suitable controls, all of them had other conditions, and no positive controls (which 4 of the 7 "good" studies in Table 4 also do not have), and this is why no comparisons could be made. Apart from this, the two small tables give the impression that until 2013 there were only these few studies, even though the literature at the end lists 71 references, most of which, however, have nothing to do with Wi-Fi radiation.

cies often cite the study by Foster and Moulder (2013) as

In 2013, Foster also writes an article for the IEEE Microwave Magazine in which he discusses the technology and lists the devices (microwave oven, phones, Bluetooth, smart meter, etc.). They are supposedly safe with regard to health because all of them operate at low levels well below the exposure limits. Foster claims that there are no biological mechanisms and that it would consequently be difficult to develop a research program. This is one of the common statements made to ignore risks that have been shown to exist in ranges below exposure limits. The paper was supported by the Federal Communications Commission (FCC) and Electric Power Research Institute (EPRI). In a 2015 commentary, Foster and Moulder ask the question as to whether Wi-Fi affects brain functions and provide nine references from the literature. Three of the references refer to Wi-Fi frequencies, the other five to cell phone radiation, and the ninth is Foster's discussion of the technology and his opinion regarding health risks (2013). Studies that were funded by industry or similar stakeholders and apparently found "no effects of Wi-Fi" are problematic. The results of studies funded by third parties are frequently dependent on the funders' interests as was documented by Blank (2014), Huss et al. (2007), Prasad et al. (2017), and Slesin (2006). Furthermore, negative research results cannot disprove positive findings of correctly conducted studies. They do not prove that positive initial findings are incorrect.

#### 15 Discussion and conclusions

This overview shows that effects of 2.45 GHz radiation have been found by different research teams, repeatedly, in different study objects, and at different exposure levels. A broad spectrum of issues has been investigated such as the effects of 2.45 GHz radiation emitted by a Wi-Fi router on the release of mercury from amalgam fillings. The mercury levels were significantly increased (Paknahad et al. 2016).

To date, there are a few long-term studies, very few in humans and even fewer epidemiological studies, apart from the studies on laptops with small numbers of study subjects. It is also far too early to generate reliable figures at this time. However, there are indications that especially newborns, children, or adolescents are particularly vulnerable as has been presented in detail by the research teams of Nazıroğlu, Atasoy, Margaritis/Panagopoulos, Orendáčová, Othmann, Özorak, Sangün, Shahin and Yüksel. The experiments were carried out with rats or mice, in some cases as long-term studies (up to 1 year). In this context, it is important to note that rats and mice used in laboratories have a life expectancy of perhaps two years. This at least allows us to infer that human children and adolescents have to be protected from possible increased risks. In the study of Margaritis et al. (2014), the authors point out that the exposure levels from Bluetooth (0.3 V/m) and Wi-Fi routers (here 2.1 V/m) showed greater effects than cell phone radiation sources with much higher field strengths. This may coincide with the findings of the papers by von Klitzing, which stated that the power-dependent pulse of 10 Hz (1 ms) from Wi-Fi routers triggered reactions. Kumari et al. observed in a study from 2012 that higher levels of ROS in the liver suppress antioxidant enzymes and that lower levels cause an increase. This could be a key to further mechanisms as to how or whether tissue damage occurs or perhaps not. Likewise, the polarization of RF radiation (Meena et al. 2014, Panagopoulos et al. 2015) should also receive additional attention.

This review presents the findings of more than 100 studies that were published in reputable scientific journals. Most of these studies confirm potential health impacts as were summarized in the joint "Nicosia Declaration on Electromagnetic and Radiofrequency Radiation" by the Cyprus and Austrian medical associations in 2017: "Potential health impacts of non-ionizing radiation from EMF/RF (electromagnetic fields/radiofrequencies) of 30 KHz-300 GHz include carcinogenicity (Class B, IARC 2011), developmental neurotoxicity, effects on DNA, fertility, hypersensitivity and other serious effects are well documented in peer reviewed studies. RFR can increase oxidative stress in cells and lead to increase of pro-inflammatory cytokines and lower capacity to repair DNA single- and double-strand breaks. Cognitive impairments in learning and memory have also been shown. These effects can occur at levels well below existing limits of ICNIRP. ... Exposure to EMF/RF at an early developmental stage is of particular concern due, amongst other, to greater absorption and potential effects on the developing brain, nervous system as well as their reproductive system, may induce cancer, cognitive effects, etc." (www.diagnose-funk.org/publikationen/artikel/detail&newsid=1242 and www.cyprus-child-environment.org/easyconsole.cfm/ id/428).

The potential health impact of Wi-Fi, even at low exposure levels, can no longer be called into guestion or relativized away, not even by those studies that found no effects. The decision-makers in government, school boards, and health agencies have a responsibility to deal with the available body of research and not to be deceived by the arguments of the industry lobby or boilerplates of government institutions. Health risks are a reality. It would be particularly important to carry out further research regarding the effects on the brain and young people. The application of the precautionary principle, which is recognized in all European countries, only allows for one conclusion: Wi-Fi must not be used continuously and close to the human body. It is no coincidence that the user guide of the Telekom Wi-Fi router states: "The integrated antennas of your Speedport transmit and receive wireless signals, for example, to provide Wi-Fi connectivity. Avoid placing your Speedport in close proximity to bedrooms, children's rooms, as well as common rooms and lounges to keep the exposure to electromagnetic field as low as possible." In their joint appeal with regard to Wi-Fi, the Cyprus and Austrian medical associations call on decision-makers to "promote age-related rational application of digital technology and not allow at schools, particular at preschool, kindergarten and elementary schools wireless networks and opt for wired connections" (ibid). Lawmakers are called upon to adjust protective legislation to the current state of research and to support research into alternatives to Wi-Fi such as VLC technologies (visible light communication, Li-Fi).

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# Study findings on 2.45 GHz radiation

Authors	Experimental conditions	Findings	Notes
Aggarwal et al. 2013	Male rats, sleep EEG; 2.45 GHz, modulated at 1 kHz, 7.37 × 10 <sup>-4</sup> mW/cm <sup>2</sup> , SAR 1.16 mw/kg; 1 hour/day for 21 days; EEG recorded 9 a.m. to 12 noon	Two exposed animals died, tempera- ture increase only 0.7 °C, non-thermal effect EEG: In first hour no significant changes in EEG amplitudes, in second hour significant in beta range, also in delta and alpha. In theta range, more significant changes than in alpha and delta ranges in the second and third hour	Possible psycho-pathophysiological disturbances, possibly BBB, synaptic properties, neurotransmitter concen- tration, changes in synchronization/ desynchronization of firing neurons so that chronic exposure to 2.45 GHz can have adverse effects
Akdag et al. 2016	Male rats; 2.45 GHz, whole-body SAR 141.4 μW/kg, (maximum 7,127 μW/kg); long-term exposure for 1 year; brain, skin, liver, kidneys, testes for DNA damage	Increased DNA damage, significant in testicular tissue	
Avendaño et al. 2012	Semen samples from 29 healthy male subjects; laptop with activated Wi-Fi connection, for 4 hours; DNA, sperm	Significant DNA and sperm damage	
Aweda et al. 2003	Rats; 2.45 GHz, MW generator (Toshiba ER6660E), 6 mW/cm <sup>2</sup> , for 8 weeks; lipid peroxidation, administration of vitamins C and E	Significant increase of lipid peroxida- tion within 24 hours, decreased within one week; administration of vitamins C and E; administration of vitamins C and E has compensatory effect	Exposure causes significant oxidative stress, which can be prevented by antioxidants
Atasoy et al. 2013	Developing rats; 2.437 GHz, Wi-Fi gateway, max SAR 0.091 W/kg, 24 hours/day for 20 weeks; 8-hydroxy-2'-deoxyguanosine and 8- hydroxyguanosine in blood, histological and oxidative parameters in testicular tissue	In most cases, significant differences in growth of rat testes and oxidative parameters	Children and adolescents could be particularly at risk
Aynali et al. 2013	Male rats; 2.45 GHz Wi-Fi, 217 Hz pulse, 1 hour/day for 28 days; oxidative stress status in laryngotracheal mucosa	In mucosa, oxidative damage, lipid peroxidation significantly increased; significantly decreased by administra- tion of melatonin, as well as the activity of glutathione peroxidase	
Ballardin et al. 2011	Hamster cell line V79, 2.45 GHz, continuous wave, 5 and 10 mW/cm <sup>2</sup> ; apoptosis, cell cycle	Apoptosis rates significantly increased, non-thermal effect on spindle apparatus	
Banaceur et al. 2013	Triple transgenic mice destined to develop Alzheimer's-like cognitive impairment; 2.4 GHz Wi-Fi device, whole-body SAR 1.6 W/kg, long-term exposure at 2 hours/day for 30 days; cognitive behavior	Learning and memory performance of Alzheimer mice were changed	
Cammaerts, Johansson 2014	Ants; Wi-Fi router: 0.06 and 0.08 µW/cm <sup>2</sup> (mean) for 5 or 30 minutes, notebook: 0.03–0.05 µW/cm <sup>2</sup> with Wi-Fi function activated for 5 minutes, distance of 20–30 cm to colonies	Disturbed behavior, changed locomo- tion patterns; after 6–8 hours back to normal foraging pattern	
Ceyhan et al. 2012	Male rats; Wi-Fi-like 2.45 GHz, pulsed, SAR 64 mW/kg on skin, 1 hour/day for 4 weeks; oxidative/antioxidative parameters (SOD, KAT, GSH-Px, LPO), beta-glucan regarding protective effect in skin	Significantly increased lipid peroxida- tion and catalase activity, significantly decreased SOD and GSH-Px activity; beta-glucan slightly increased catalase activity and did not significantly prevent decrease of GSH-Px activity; due to beta-glucan lipid peroxidation significantly lower, almost at level of controls	

Chaturvedi et al. 2011	Mice; 2.45 GHz, continuous wave, 0.02564 mW/cm <sup>2</sup> , SAR 0.03561 W/kg 2 hours/day for 30 days	Differences in weight, activity, spatial memory between exposed and non- exposed group; daily rhythm affected, blood count changes, DNA strand breaks in brain cells	
Chauhan, Kesari et al. 2017	Rats; 2.45 GHz, microwave oven, 0.2 mW/cm <sup>2</sup> , whole-body SAR 0.14 W/kg, 2 hours/day for 35 days	Significantly increased lipid peroxidation; under optical microscope: tissue changes in brain, liver, testes, kidneys, spleen	Possible consequence: infertility
Cig, Nazıroğlu 2015	Breast cancer cells; 900, 1800, 2450 MHz, 217 Hz pulsed, at distance of 0, 1, 5, 10, 20, and 25 cm, $12 \mu$ W/cm <sup>2</sup> , SAR 0.36 ± 0.02 mW/kg, for 1 hour; calcium concentrations, ROS production in cell plasma, cell survival, apoptosis, caspase-3 and -9	At distance up to 10 cm, intracel- lular calcium concentrations, ROS, apoptosis, and mitochondria damage significantly increased; cell survival rates significantly decreased	As a result of exposure, TRPV1 ion channels become more permeable to Ca <sup>2+</sup> ions without heating of cell culture, which leads to increases in ROS and apoptosis
Cleary et al. 1996	Hamster, ovarian cells; continuous wave at 2.45 GHz and 27 MHz, SAR 5 and 25 W/kg, for 2 hours; cell cycle, division, and growth from 2 hours up to 4 days after exposure	2.45 GHz exposure caused changes in all cell cycle phases; twice as effective as exposure at 27 MHz	
Dasdag et al. 2015	Male rat sperm, testicular tissue; WLAN generator, SAR point, 1 g, and 10 g tissue of testes and prostate at 4880, 2420 and 1020 μW/kg, 24 hours/day for 12 months	Changes in function of testes, sperm shape significantly different between both groups; significant increase in percentage of defective sperm heads after exposure; significantly decreased weight of epididymis and seminal vesicle; changes in diameter of and thickness of tunica albuginea highly significant	Long-term exposure can affect fertility
Deshmukh, Banerjee et al. 2013	Rats; 900, 1800, 2450 MHz, at 2450 MHz SAR 6.672 $\times$ 10 <sup>-4</sup> W/kg, 2 hours/day for 30 days, 5 days/week; DNA strand breaks in brain tissue	DNA strand breaks significantly increased	Microwave exposure can cause DNA damage in brain tissue below expo- sure limit of 2 W/kg, probably due to free radicals
Deshmukh, Banerjee et al. 2015	Rats; 900, 1800, 2450 MHz, at 2450 MHz SAR $6.672 \times 10^{-4}$ W/kg, 2 hours/day 5 days/week for 180 days; behavior, HSP70, and DNA in brain tissue	Spatial orientation, learning and memory performance significantly im- paired; HSP70 levels and DNA strand breaks significantly increased; DNA damage was significantly increased at 1800 and 2450 MHz in comparison to 900 MHz	
Deshmukh, Banerjee et al. 2016	Rats; 900, 1800, 2450 MHz, at 2450 MHz SAR MHz 6.672 × 10 <sup>-4</sup> W/kg, for 90 days; behavior, HSP70, and DNA in brain tissue	Decreased brain performance like above, significantly increased levels of HSP70 and DNA strand breaks, high- est impact at 2450 MHz	
Eser et al. 2013	Rats; 900, 1800, and 2450 MHz, 1.04 mW/cm², SAR 1.04 W/kg, 1 hour/day 2 months; brain regarding histology, oxidative status, IL-1β, and apoptosis	At 2450 MHz, the strongest effects in most cases: significant degeneration of neurons, significant decrease in antioxidant capacity, significant increase in IL-1 $\beta$ concentration, significant increase in apoptosis	Due to these changes, functional disorders and cancer may develop
Gümral, Nazıroğlu 2009	Rats, blood plasma after exposure; 2.45 GHz, 217 Hz pulse, 11 V/m (32 μW/cm <sup>2</sup> ), SAR 0.1 W/kg, 1 hour/day for 28 days	Oxidative stress, prevented by L-carnitine; selenium did not exert any protective effect	
Gürler et al. 2014	Rats; 2.45 GHz, WLAN system, 217 Hz pulse, 1.04 mW/cm <sup>2</sup> , 3.17–4.88 V/m, SAR 0.02 W/kg, 1 hour/day for 30 days	Damage of DNA and proteins due to ROS; increased concentration levels of 8-OHdG in blood plasma and brain tissue; garlic prevented increased concentration levels of 8-OHdG in brain tissue	

Hassanshahi et al. 2017	Rats; 2.4–2.4835 GHz, Wi-Fi device, 23.6 dBm, 12 hours/day for 30 days; behavior, muscarinic receptor 1	Capability to distinguish objects significantly reduced, expression of muscarinic receptor 1 significantly increased	
	(for acetylcholine), GABA transporter 1 (GAT1) in hippocampal tissue		
Holovska et al. 2015	Rat liver cells; 2.45 GHz pulsed, power density 2.8 mW/cm <sup>2</sup> , 3 hours/day for 3 weeks; optical and electron microscopy	Major changes in the structure of membranes, cell nuclei and organelles, necrotic cells, blood vessels	
Kamali et al. 2017	Human sperm samples from 40 men 19–40 years of age (mean age 28.5 years); 3G and Wi-Fi modem activated by 50-min download to laptop; SAR < 1.6 W/kg; sperm, fertility	Non-significant decrease of motility class A and B, significant decrease in motility class A and B, significant decrease in class C, significantly higher in class D; sperm velocity significantly lower	Impairments below the exposure limit considered safe by the FCC; in the case of longer exposure periods, changes in pH, morphology, and motility could be more pronounced
Kesari, Behari, Kumar 2010a	Rats; 2.45 GHz, 0.34 mW/cm <sup>2</sup> , SAR 0.11 W/kg, 2 hours/day for 35 days; antioxidant enzymes, DNA breaks, histone kinase in brain tissue	Significant increase in DNA strand breaks, significant difference in enzyme levels compared to controls	Authors assume that free radicals due to ROS overproduction change enzyme activity
Kesari, Behari 2010b	Rats; 2.45 GHz, microwave oven, 0.34 mW/cm <sup>2</sup> , SAR 0.11 W/kg, 2 hours/day for 35 days; number of cells, antioxidant enzymes, apoptosis in sperm cells	Number of cells, apoptosis and antioxidant enzymes significantly different from controls	Genetic changes may occur
Kesari, Kumar, Behari 2012	Rats; 2.45 GHz, 0.21 mW/cm <sup>2</sup> , SAR 0.14 W/kg, 2 hours/day for 45 days; rat brain, pineal gland for melatonin, all brain tissue for creatine kinase, caspase-3, and calcium ion concentration	Significant increase in creatine kinase and calcium ion concentration in brain tissue, significant decrease of melatonin, caspase-3 levels hardly changed	Authors conclude that this may lead to significant brain damage
Kim, Rhee 2004	Rats, heart tissue; microwave oven, continuous wave, 40 mW/cm <sup>2</sup> , SAR 9.2 W/kg, for 15 minutes; administration of green tea catechins, 6 days later oxidative/antioxidative parameters analyzed	Significant activation of oxidase sys- tems, formation of superoxide radicals, lipid peroxidation, oxidized proteins, weakening of antioxidant defense system of heart tissue, significantly reduced oxidative damage due to administration of catechins	
Kumar et al. 2011	Male rats; 2.45 GHz, 100 Hz pulse, 0.21 mW/cm <sup>2</sup> , SAR 0.014 W/kg, 2 hours/day for 60 days; oxidative parameters, apoptosis, melatonin, testosterone	Significant decrease in melatonin and testosterone, significantly increased apoptosis rate and creatine kinase level	
Kumari, Kesari et al. 2012	Male rats; 900 and 2450 MHz (microwave oven), 0.22 mW/cm <sup>2</sup> , whole-body SAR 0.15 W/kg, 2 hours/day for 35 days; antioxidant and liver enzymes	At 2.45 GHz significant changes in GSH-Px, liver SOD activities, significantly increased liver catalase and MDA in exposed group; significant increase in TBIL, ALT, AST, and ALP compared to control group	2.45 GHz exposure-induced overpro- duction of free radicals, inhibits anti- oxidant enzymes and causes oxidative stress in liver; MDA activity shows cell membrane damage
Lai et al. 1983	Male rats; 2.45 GHz, polarized, 2-microsecond pulses, 500 pulses/second, 1 mW/cm <sup>2</sup> , whole-body SAR 0.6 W/kg, for 45 minutes; effect of psychoactive drugs apomorphine, amphetamine, and morphine	Apomorphine-induced hyperthermia and stereotypy enhanced by 2.45 GHz irradiation, amphetamine-induced hyperthermia attenuated by irradia- tion, stereotypy unaffected compared to control group; morphine-induced lethality and catalepsy enhanced by irradiation at certain dosages	Since these drugs have different modes of action on central neural mechanisms and the effects of micro- waves depend on the particular drug studied, these results show the com- plex nature of the effect of microwave irradiation on brain functions
Lai et al. 1994	Rats; 2.45 GHz, 2-microsecond pulses, 500 pulses/second, 1 mW/cm <sup>2</sup> , whole-body SAR 0.6 W/kg, for 45 minutes; behavior	Deficits in spatial learning, but not after pretreatment with cholinergic agonist physostigmine or opiate antagonist naltrexone; pretreatment with peripheral opiate antagonist naloxone methiodide	Both cholinergic and endogenous opioid neurotransmitter systems of the brain are involved in deficits of spatial learning after exposure to low-level fields at 2.45 GHz
Lai, Singh 1996a	Male rats; 2.45 GHz, continuous wave and pulsed, 2-microsecond pulses, 500 pulses/ second, 2 mW/cm <sup>2</sup> , SAR 1.2 W/kg, for 2 hours; DNA strand breaks in brain	Significant increase in single- and double-strand DNA breaks 4 hours after exposure to pulsed as well as continuous radiation in rat brain cells	

Lai et al. 1996b	Rats; 2.45 GHz, 2-microsecond pulses, 500 pulses/second, 1 mW/cm <sup>2</sup> , SAR 0.6 W/kg, for 45 minutes; cholinergic activity in hippocampus, effect of beta-funaltrexamine (opioid receptor antagonist)	2.45 GHz radiation caused significant decrease in cholinergic activity in hip- pocampus of rats, beta- funaltrexamine blocked this inhibitory effect; microwaves are stressors	Authors try to understand the mechanisms of how microwaves affect neurological functions and behaviors in CNS, confirming previous results that endogenous opioid receptors are targets of action
Lai, Singh 1997	Rats; 2.45 GHz, 2-microsecond pulses, 500 pulses/second, 2 mW/cm <sup>2</sup> , SAR 1.2 W/kg, for 2 hours; DNA strand breaks, effect of melatonin and N-tert-butyl-a-phenylnitrone (PBN)	Significant increase in double-strand DNA breaks 4 hours after exposure stopped; pretreatment of cells with free radical scavengers prevents DNA breaks; treatment with melatonin or PBN prior to or immediately after irradiation prevents microwave- induced effects	Since both melatonin and PBN are efficient free radical scavengers, it is hypothesized that free radicals are involved in DNA damage in the brain. DNA strand breaks can lead to neurodegenerative diseases and cancer; see hypothesis of Litovitz 1997
Lai 2004	Rats; 2.45 GHz, continuous wave, 2 mW/cm <sup>2</sup> , SAR 1.2 W/kg, and one ex- posure group with additional incoherent background magnetic field of 60 mG ( $6.0 \mu$ T), for 1 hour; behavior after training tests	Significant deficits of spatial learning in exposed animals without background magnetic field; with background field, similar behavior to control animals	See hypothesis of Litovitz 1997
Lai, Singh 2005	Rats; 2.45 GHz, continuous wave, 1 mW/cm <sup>2</sup> , SAR 0.6 W/kg, one exposure group with additional incoherent back- ground magnetic field of 45 mG (4.5 µT), for 2 hours; DNA strand breaks in brain tissue 4 hours after exposure stopped	Significantly increased single- and double-string DNA breaks only in case of exposure to 2.45 GHz radiation; the background magnetic field by itself and both fields together showed almost no differences compared to sham-exposed control group	Incoherent background field could have disturbed the reaction of the cells to the microwave exposure; see hypothesis of Litovitz 1997
Lee et al. 2005	Human promyelocytic leukemia cell line (HL-60); 2.45 GHz, pulsed at duration of 155 microseconds, SAR 10 W/kg (mean), SAR 133 W/kg or 320 V/m (peak); gene expression after 2 and 6 hours	Gene expression of 221 genes was changed after 2 hours (early genes); after 6 hours, 896 genes including genes for apoptosis, metabolism, polysaccharide biosynthesis, RNA functions, translation, transport, metabolism, and cell cycle	Genes for HSPs were not involved; this means that these are non-thermal responses
Li et al. 2008	Rats; 2.45 GHz pulsed, 1 mW/cm <sup>2</sup> , 3 hours/day for 30 days (chronic exposure); learning/memory, apoptosis, corticosterone, glucocorticoid receptors (GR) in hippocampus	Highly significant increase in corticos- terone levels, apoptosis rates in hip- pocampus; glucocorticoid receptors changed	Learning and memory deficits due to changes in corticosterones
Maes et al. 1993	Human blood-derived lymphocytes of volunteers; 2.45 GHz, 50 Hz pulse, 80 mW/ml, SAR 75 W/kg, for 30 and 120 minutes; chromosome aberrations, micronuclei, sister chromatid exchange, and cell kinetics	Significant 2- and 3-fold increase in chromosome aberrations and micronu- clei after 30 and 120 minutes; sister chromatid exchange und cell kinetics hardly affected	
Maganioti et al. 2010	15 men and 15 women, WLAN access point, 0.49 V/m (0.064 $\mu$ W/cm <sup>2</sup> ) at head; memory tests and EEG	Significant differences in alpha and beta ranges, but also significant differences between genders	Physiological changes due to Wi-Fi exposure
Margaritis et al. 2014	Drosophila oogenesis; many RF sources, including microwave oven, Bluetooth (lowest field strength of 0.3 V/m (0.024 $\mu$ W/cm <sup>2</sup> ) during first 7 days), and Wi-Fi (2.4 GHz, 2.1 V/m (1.2 $\mu$ W/cm <sup>2</sup> ), 10 Hz pulse, pulse duration 1 ms, for 1 hour/day); reproductive capacity, apoptosis, offspring	Statistically significant increase in apoptosis rate, fecundity decreased by about 10 % for Wi-Fi and Bluetooth exposure, offspring had significantly decreased number of pupae	Type of pulse seems to be responsible for damage since RF sources without pulse, but at higher field strengths, had less of an effect
Meena, Kesari et al. 2014	Male rats, testicular tissue; microwave oven, modulated at 50 Hz, 0.21 mW/cm <sup>2</sup> , SAR 0.14 W/kg, 2 hours/day for 45 days; melatonin, sperm, testosterone, DNA, apoptosis, oxidative parameters	Significantly increased apoptosis, DNA strand breaks, and MDA and ROS concentration levels in testes; significant decrease in testosterone; disorganized seminiferous tubules and inflammation in testicular cells; melatonin prevented oxidative stress	There are considerations whether microwave radiation may affect the polarization of cell membranes and that, to a certain extent, melatonin af- fects xanthine oxidase, whose activity had been significantly increased due to the exposure; melatonin could be administered as a therapeutic agent

Megha, Banerjee et al. 2015	Rats; 900, 1800, 2450 MHz, SAR 0.59, 0.58, and 0.66 mW/kg, 2 hours/day, 5 days/week for 60 days; oxidative parameters, DNA, interleukins in hippocampus	ROS formation, significantly decreased antioxidant status, cytokine levels significantly increased, significant DNA damage; 2450 MHz had the highest damage potential in all tests	
Misa-Agustiño et al. 2012	Rats, thyroid; in thyroid: SAR 0.046, 0.104, und 0.482 W/kg, whole-body SAR 0.0169, 0.0364, and 0.161 W/kg; HSP70, HSP90	HSP concentrations after 90 minutes significantly decreased, after 24 hours increased again, but below control levels	
Misa-Agustiño et al. 2015	Spleen cells of female rats; 2.45 GHz, in spleen: SAR 0.046, 0.104, and 0.482 W/kg, whole-body SAR 0.0169, 0.0364, and 0.161 W/kg; 30-minute exposure once or ten times within 2 weeks; 90 minutes and 24 hours after exposure: morphology, HSP70, HSP90, glucocorticoid receptors	Many morphological changes, permeability change in endothelium and glucocorticoid receptors; more blood vessels and red blood cells outside of vessels; HSP 90 decreased	Findings indicate cell stress
Nakamura et al. 2000	Rats; 2.45 GHz, continuous wave, 2 mW/cm <sup>2</sup> , for 90 minutes; pregnancy, uterus, blood flow between uterus and placenta, hormones and biochemical mediators (corticosterone, estradiol, prostaglandin $E_2$ , and prostaglandin $F_2\alpha$ )	Blood flow decreased due to exposure; progesterone and prostaglandin $F_2\alpha$ increased in pregnant animals; increase of corticosterone and decrease of estradiol was similar in pregnant and non-pregnant animals	Disturbances in uterus/placenta cycle due to 2.45 GHz, probably caused by prostaglandin $F_2\alpha$ and could be a risk for pregnancies
Nazıroğlu, Gümral 2009	Rats; 2.45 GHz, 217 Hz pulse, 1 hour/day for 28 days 11 V/m, whole-body SAR 0.1 W/kg, whole-brain SAR 1.73 W/kg; EEG and brain tissue (cortex) regarding oxidative parameters, administration of selenium or L-carnitine, vitamin A, C, and E	Slight hyperexcitability in EEG, compensated by selenium and L-carnitine; decreases vitamins A, C, and E, changes in oxidative parameters, protection against oxidative damage through L-carnitine and selenium	Both the vitamins and L-carnitine and selenium have a protective effect against ROS oxidative damage in brain tissue; L-carnitine and selenium seem to protect the antioxidant vitamins from the inhibitory effect of 2.45 GHz exposure
Nazıroğlu et al. 2012a	Brain tissue of male rats; Wi-Fi device 1 mW/m <sup>2</sup> (0.1 μW/cm <sup>2</sup> ), whole-body SAR 0.143 W/kg, 10 V/m, 1 hour/day for 30 days; EEG, survival rate, Ca <sup>2+</sup> concentration, oxidative parameters, beta carotene, vitamins A, C, and E, administration of melatonin	Wi-Fi radiation caused oxidative stress in neurons, EEG, vitamin levels changed, Ca2+ influx increased, melatonin reduced damage and de- crease in vitamin E; voltage-gated and TRPM2 calcium channels are involved in Ca <sup>2+</sup> influx	RF radiation from Wi-Fi devices causes oxidative damage via calcium channels and Ca <sup>2+</sup> increase in cells; melatonin protects brain tissue from damage by counteracting oxidative stress
Nazıroğlu et al. 2012b	Human leukemia cells; 2.45 GHz, pulsed, Wi-Fi device, 0.1–2.5 W/kg, for 1, 2, 12, or 24 hours; oxidative parameters and calcium influx into cells	Wi-Fi device caused oxidative stress and increased cell growth due to significantly increased Ca <sup>2+</sup> influx into cytoplasm of human leukemia cells	
Özorak, Nazıroğlu 2013	Rats, male offspring of mothers exposed during pregnancy, 2450, 900, and 1800 MHz (217 Hz pulse, for 1 hour/day, 5 days/week in utero, and up to 6 weeks after birth with 12 $\mu$ W/cm <sup>2</sup> , 10 V/m, whole-body SAR 0.01–1.2 W/kg, mean 0.18 ± 0.07 W/kg; analysis of kidneys and testes of offspring at age 4, 5, and 6 weeks for trace elements (chromium, copper, iron, manganese, selenium, and zinc), antioxidant parameters, vitamins A, E, and beta carotene	4-week-old animals respond more sensitively than older animals, oxidative stress levels were higher; increased lipid peroxidation, oxidizable iron and low concentration levels of trace elements, TAS, and GSH	The authors conclude that 900, 1800, und 2450 MHz can lead to oxidative stress in kidneys and testes of young rats; these are animals that are still developing, comparable to humans in puberty
Oksay et al. 2014	Rats, testicular tissue; 2.45 GHz, 0.1 µW/cm <sup>2</sup> , 217 Hz pulse, 10 V/m, ca. SAR 0.143 W/kg, 1 hour/day for 30 days; lipid peroxidation, vitamins A, C, and E, administration of melatonin	Increased lipid peroxidation in testes, administration of melatonin decreased damage	

Orendáčová et al. 2009	Newborn (7 days old) and adult rats (24 months); 2.45 GHz, pulsed, 2.8 mW/cm <sup>2</sup> , 4 hours/ day for 2 days (acute) or 8 hours/day for 3 days (chronic); hippocampus regions	Significant differences in cell growth in newborn rats, not in adult rats	Harmful effects on neurogenesis, especially in newborns
Orendáčová et al. 2011	Rats of 2 age groups: 7-day-olds (newborn) and 28-day-olds (young adults); 2.45 GHz, pulsed, 2.8 mW/cm <sup>2</sup> , for 2 hours; early gene product Fos protein and NO-producing cells	Fos protein and NO-producing cells significantly increased in exposed animals, earlier emergence than in controls	In very young rats, development changed; they seem to go through a kind of early maturation
Othman et al. 2017	Female rats; common Wi-Fi router, 2 hours/day throughout pregnancy; in offspring, behavior, brain development, oxidative parameters, cholinesterase activity	50% fewer offspring; in young animals delayed development of responses, sense of equilibrium, and maturation of motor system; significant changes in antioxidant enzymes in brain, impair- ment of cholinesterase activities; later compensated in adult offspring	Temporary disturbance of redox balance (oxidative stress) in young offspring could translate into delayed brain development; impaired cholines- terase activity could affect neurotrans- mitters
Paknahad et al. 2016	Teeth, amalgam; router-laptop system, router at 30 cm distance, laptop at 20 m, exposure for 20 minutes	After exposure, more mercury in saliva compared to controls	
Panagopoulos et al. 2015	Natural fields of solar irradiation between 8 and 24 mW/cm <sup>2</sup> , artificial fields e.g. of a cell phone in calling mode < 0.2 mW/cm <sup>2</sup> ; effect on ion channels in cell membrane	Due to their polarization, artificial fields are more biologically active than non- polarized natural fields; artificial fields exert an effect on all charged particles (ions, membranes) through additional electrostatic forces	Living organisms have evolved on the basis of weak natural fields; strong artificial fields impair membranes
Papageorgiou et al. 2011	15 men and 15 women each (mean age 24); Wi-Fi signal, 0.49 V/m (0.064 μW/cm <sup>2</sup> ); P300 component in EEG	Significant differences between exposed and non-exposed persons; women and men also responded differently when Wi-Fi signal was turned on; P300 component in men significantly lower, in women significantly higher	P300 is involved in alertness; Wi-Fi affects nerve activity, genders respond differently
Paulraj, Behari 2006a	Young rats, brain tissue; 2.45 GHz, 0.344 mW/cm <sup>2</sup> , SAR 0.11 W/kg, 2 hours/day for 35 days; calcium-dependent protein kinase C, histology	Significant decrease in protein kinase C activity in hippocampus, non-thermal response, increased number of glia cells	Findings could explain learning and memory deficits; protein kinase C is also involved in carcinogenesis
Paulraj, Behari 2006b	35-day-old male rats; 0.344 mW/cm <sup>2</sup> , 1 W/kg, 2 hours/day 5 days/week for 35 days; DNA damage in brain	Significantly increased level of single-strand breaks	Possible risk for impairment of neurological functions and development of degenerative diseases
Saili et al. 2015	Rabbits; 2.45 GHz, Wi-Fi access point, at distance of 25 cm, exposure 1 hour/day	Heart rate variability affected, higher blood pressure	
Salah et al. 2013	Rats, liver and kidneys; 2.45 GHz, Wi-Fi access point, 1 hour/day for 21 days; tissue changes, oxidative stress, liver enzymes among others	Diabetes-like disturbances of glucose metabolism and oxidative stress in liver and kidneys, olive leaf extract mitigated damage	
Sangün et al. 2015	Rats; 2.45 GHz, Wi-Fi, pulsed, 45.5 V/m (550 μW/cm²), whole-body SAR 0.143 W/kg, 1 hour/day	Exposure during prenatal phase leads to limited growth and delayed puberty in postnatal phase; ROS increased	Young rats respond sensitively to 2.45 GHz
Sarkar, Ali, Behari 1994	Mice, brain and testes; 2.45 GHz, continuous wave, 1 mW/cm <sup>2</sup> , SAR 1.18 W/kg, 2 hours/day; DNA strand breaks	Significantly increased DNA breaks	DNA breaks occur below ICNIRP exposure limits under non-thermal conditions
Shahin, Chaturvedi et al. 2013	Female mice; 2.45 GHz, continuous wave, 0.033549 mW/cm <sup>2</sup> , SAR 0.023023 W/kg, 2 hours/day for 45 days; liver, kidneys, ovaries, blood cells, ROS, hormones, DNA	Significant changes in ROS, hemo- globin, total number of erythrocytes and leucocytes, DNA strand breaks, hormones and enzyme activities, lower number of embryos	Inflammatory and physiological stress responses in pregnant mice, resulting in death of embryos

Shahin et al. 2014	Male mice; 2.45 GHz, continuous wave, 0.029812 mW/cm <sup>2</sup> , SAR 0.018 W/kg, 2 hours/day for 30 days; liver, kidneys, hypothalamus, testes, sperm, testosterone, oxidative parameters	Significant changes in all parameters investigated	Infertility could develop due to oxidative and nitrosative stress (free radicals) as well as degeneration of testicular tissue
Shahin, Banerjee, Chaturvedi et al. 2015	Mice; 2.45 GHz, continuous wave, 0.0248 mW/cm <sup>2</sup> , SAR 0.0146 W/kg, 2 hours/day for 15, 30, und 60 days; behavior, in hippocampus: oxidative parameters, creatine kinase, morphology, apoptosis	Learning and memory deficits increased with increasing duration of exposure, increased oxidative/ nitrosative stress and increased apoptosis; reduced dendrites and branching	Oxidative/nitrosative stress in hippocampus leads via p53 to apoptosis and loss of spatial memory because of reduced development of neurons
Shahin, Banerjee, Chaturvedi et al. 2017	Adult male mice; 2.45 GHz, continuous wave, 0.0248 mW/cm <sup>2</sup> , SAR 0.0146 W/kg, 2 hours/day for 15, 30, and 60 days; behavior, corticosterone, expression of GR, CRH, iNOS, iGluRs, PSD-95-nNOS system and PKA-PKCε-ERK1/2-pERK1/2 in hippocampus	Significantly reduced learning and memory performance, significantly increased concentration levels of corticosterone and expression of CRH, CRH-R1, and iNOS, while the expres- sion of iGluRs, nNOS, PSD-95,PKCε, PKA, ERK-p-ERK, CREB, and p-CREB decreased	Oxidative stress (free radicals) leads to learning and spatial memory deficits due to involvement of corticosterone via the iGluR/ERK/CREB signal path
Shokri et al. 2015	Rats, testes, sperm cells; 2.45 GHz, 2 Wi-Fi antennas at opposite walls in a given space, 1 hour/day or 7 hours/day for 2 months; apoptosis, sperm and tissue changes	Testicular tissue significantly changed, apoptosis significantly increased, more so after 7 hours of exposure	Researchers recommend to limit time exposed to Wi-Fi radiation
Sinha et al. 2008	Rats; 2.45 GHz, modulated at 1 kHz, 16.5 μW/cm <sup>2</sup> , 2 hours/day for 21 days; sleep EEG, behavior; determination of T3 and T4	Sleep EEG and sleep-wake param- eters differ significantly from controls, body temperature hardly different, thermal effect unlikely; T3 significantly lower, T4 significantly higher, exposed animals were hyperactive	
Sinha RK 2008	Male rats; 2.45 GHz, modulated at 1 kHz, 16.5 μW/cm <sup>2</sup> ; determination of hormones T3, T4, and TSH, behavior	Thermal effect unlikely; T3 significantly lower, T4 significantly higher; TSH changed little; increased water content of brain tissue (swelling by 6.97 %); behavior significantly changed compared to controls, hyperactive	Possible connection between behavior and thyroid hormone functions because they interact with neurohormones and neurotransmitters
Soran et al. 2014	Parsley, dill, celery; 2.45 GHz, Wi-Fi router, 10 μW/cm <sup>2</sup> , 7 μW/cm <sup>2</sup>	Reduced size of organelles, decline in photosynthesis, thinner cell walls, and others	
Szmigielski et al. 1982	Skin of mice; 2.45 GHz, 5 or 15 mW/cm <sup>2</sup> , 2 hours/day 6 days/week for 1 to 6 months; different controls, benzopyrene	Exposure produced twice as many skin tumors and significantly faster growth of skin tumors	
Taheri et al. 2015	Klebsiella pneumonia for antibiotic resistance; 2.45 GHz, Wi-Fi router, for 3, 4.5, and 8 hours	Wi-Fi radiation increased sensitiv- ity to 5 antibiotics after 4.5 hours of exposure; thereafter development of resistance	Window effect, cell walls could become more permeable due to exposure
Taheri et al. 2017	Listeria monocytogenes and Escherichia coli; 900 and 2450 MHz, common 2.45 GHz Wi-Fi router, SAR 0.13 W/kg, 14 cm distance to bacteria suspension; 6 antibiotics	After exposure, significantly increased growth and significantly increased development of resistance against 6 antibiotics	Within an exposure window of field strengths, antibiotic resistance develops
Testylier 2002	Rats; 2.45 GHz, 2 and 4 mW/cm <sup>2</sup> , for 1 hour (short-term); ACh release in hippocampus	Significantly lowered ACh release at 4 mW/cm <sup>2</sup>	As a neurotransmitter, acetylcholine (ACh) is involved in learning processes
Tök, Nazıroğlu 2014	Rats, eye lenses; 2.45 GHz, 217 Hz pulse, 1 hour/day at 0.1 μW/cm <sup>2</sup> for 30 days, 11 V/m, whole-body SAR ca. 0.1 W/kg; lipid peroxidation, GSH, GSH-Px, melatonin	Significant changes in oxidative parameters, melatonin mitigates dam- age	

Türker, Nazıroğlu et al. 2011	Rats, heart tissue, selenium and L-carnitine in addition to 2.45 GHz exposure (217 Hz pulse, 1 hour/day for 28 days); determination of lipid peroxidation and concentration levels of vitamins A, C, and E in heart tissue	Significantly higher lipid peroxidation and glutathione peroxidase, significantly decreased by selenium and L-carnitine; concentration levels of vitamins C, A, and E were decreased in exposed animals, but higher levels when treated with selenium and L-carnitine	2.45 GHz exposure caused oxidative stress in heart tissue of rats; the administration of selenium and L-carnitine could reduce the oxidative effect of 2.45 GHz radiation
Wang, Lai 2000	Rats; 2.45 GHz, pulsed, 2-microsecond pulses, 500 pulses/second, 2 mW/cm <sup>2</sup> , whole-body SAR 1.2 W/kg, for 1 hour; behavior	Acute exposure of 2.45 GHz micro- wave radiation significantly affects behavior of rats	The changed behavior could be traced back to the decrease in cholinergic activity in the brain (in frontal cortex and hippocampus) of the animals
Yang et al. 2010	Mice; 2.45 GHz, pulsed, SAR 6 W/kg radiation, for 20 minutes; microglia in brains	Microglia were activated by 2.45 GHz radiation with involvement of TNF- $\alpha$ , NO, and ROS, followed by a pro-inflammatory response, at the same time JAK-STAT signal pathway was activated	RF radiation represents an external physical factor that can lead to inflammatory processes and damaged nerve tissue via activated microglia.
Yang et al. 2012	Adult male rats; 2.45 GHz, pulsed, 65 mW/cm <sup>2</sup> , SAR 6 W/kg, for 20 minutes; stress-related genes	Out of 2,048 genes in the hippocampus, 41 relevant genes were significantly changed 3 hours after exposure; of those 23 upregulated, and 18 downregulated; 7 genes concern stress-related heat shock proteins or chaperones, of those especially the gene for HSP27 and HSP70, whose expression was significantly higher in the hippocampus, specifically in the pyramidal cells of the Ammon's horn (CA3 region) and in the granule cells of the dentate gyrus	The data provide direct evidence that 2.45 GHz radiation triggers stress responses in the hippocampus of rats; both HSPs were increased maximally at different points in time; they have different functions
Yüksel, Nazıroğlu, Özkaya 2016	Pregnant rats and their offspring, long-term exposure with 900, 1800, and 2450 MHz, 1 hour/day 5 days/week throughout the entire pregnancy, the offspring for a total of 1 year (52 weeks, 11 V/m (32 µW/cm <sup>2</sup> ), whole-body SAR 0.1 W/kg); blood serum and uterus tissue, lipid peroxidation, GSH, enzyme activities, antioxidant vitamins A, C, and E in blood plasma, prolactin, estrogen, and progesterone in serum	After long-term exposure (1 hour/day 5 times/week for 1 year), all 3 frequencies produced changes in hormone concentrations and oxidative stress in the dams and their offspring; effect stronger at 2.45 GHz than at 900 and 1800 MHz	The researchers do not rule out that for longer exposure durations further changes could emerge that also may manifest in humans, especially in young teenagers who often use their cell phones for several hours per day
Zhu et al. 2016	Heart muscle cells of rats; 2.45 GHz, 50, 100, 150, and 200 mW/cm <sup>2</sup> , for 6 minutes; ATP activity, mitochondrial respiratory chain, enzymes CK, LDH, and AST, troponin I, oxidative parameters SOD, GSH, and lipid peroxidation, apoptosis proteins (MAP kinase cascade), and morphology	Significant changes of all biochemical parameters, some of them highly significant; the higher the exposure, the greater the change; also morphological changes, e.g. irregular striations of heart muscle cells, discoloration of cell nuclei, fewer mitochondria with disturbed membranes, other degenerative manifestations; significantly increased apoptosis proteins and apoptosis rates	RF radiation produces apoptosis in heart muscle cells via mitochondrial pathway
Zotti-Martelli et al. 2000	Human lymphocytes of two healthy 27-year-old persons; 2.45 and 7.7 GHz, continuous wave, 3 different devices per frequency, 10, 20, and 30 mW/cm <sup>2</sup> , for 15, 30, and 60 minutes; blinded assessment of duplicate data sets, micronucleus test	Frequency of micronuclei in exposed lymphocytes increased at both frequencies with duration and field strength, significantly increased for 30- and 60-minute exposure duration and the highest field strength; cell cycle was not affected	2.45 GHz is the resonance frequency of water; the temperature did not increase in the experiment; neurasthenia, vascular damage in the nervous system, and cancer mortality occur more often in radar technicians; researchers recommend protective measures against non-ionizing radiation

### References / endnote / abbreviations

Endnote regarding the studies of Ballardin and Zotti-Martelli

In the studies of Ballardin and Zotti-Martelli, 2.45 GHz is referred to as the resonance frequency of water. Microwaves can excite water molecules at any frequency to stimulate dipole and multipole oscillations, resulting in a temperature increase. Strictly speaking, there is no resonance frequency for this absorption mechanism, but a broad range of microwave frequencies around 30 GHz, i.e. above 2.45 GHz, at which absorption is relatively high (see also www1.lsbu.ac.uk/water/microwave\_water.html). Among other things, this range depends on temperature. The lowest resonance frequency, which is the lowest frequency with the highest absorption, is at ca. 22 GHz for free water molecules. The use of 2.45 GHz in microwave ovens is a compromise between absorption rate, which is not quite as high, but higher penetration depth into the cooked food, because penetration depth increases with increasing frequency. For the whole-body absorption of microwaves in living organisms, the size of the exposed object plays an important role besides the frequency of the microwave radiation. Maximum absorption of microwave radiation is reached when the body size is about half the wavelength and the electric field strength component of the microwave is parallel to the longitudinal axis of the body (worst case). At higher and lower frequencies, absorption decreases again. In adults (ca. 2 m tall), the maximum absorption is at ca. 70 MHz, in smaller persons, children, and babies, the frequency is correspondingly higher, and in primates at ca. 300 MHz and in mice at 2.45 GHz, because their body size of 6 cm corresponds to approximately half the wavelength of the latter frequency. Since most studies use mice or rats (similar size), 2.45 GHz represents the frequency with the highest absorption rate (absorption maximum) in those animals (according to Bernhardt, a mouse absorbs 1.8 W/ kg at a power density level of 1 mW/cm<sup>2</sup>). Higher or lower frequencies result in lower absorption rates. (Source: Bernhardt J H (1995): Mobilfunk und Elektrosmog. Biologische Wirkung von elektromagnetischer Strahlung. Phys Bl 51 (10), 947-950).

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#### Index of studies based on endpoints

(Author, date of publication/chapter in review)

**Apoptosis:** Ballardin 2011/7, Cig 2015/4, Deshmuk 2013/4, Kumar 2011/1, Margaritis 2014/1, Meena 2014/6, Misa-Agustiño 2015/9, Shahin 2015/3, Shokri 2015/1, Zhu 2016/5

**Calcium ion channels (Ca2+):** Cig 2015/4, Hassanshahi 2017/3, Kesari 2012/4, Nazıroğlu 2012c/4 & 2012a/11, Panagopoulos 2015/11, Taheri 2015/12 & 2017/12

Cancer: Czerska 1992/4, Eser 2013/6, Sarkar 1994/4, Szmigielski 1982/4, Yang 2010/2

Cell growth and cycle: Ballardin 2011/7, Cleary 1996/7, Nazıroğlu 2012b/11, Orendáčová 2009/2

DNA damage: Akdag 2016/4, Avendaño 2012/1,4, Chartuvedi 2011/4, Czerska 1992/4, Deshmuk 2013,4 & 2015/3,4, Gürler 2014/4, Kesari 2010a, 2010b, 2012/4, Lai 1996&1997, Lai/ Singh 1995/4 & 1996/7 & 1997/3 & 2005/3,4, Meena 2014/6, Megha 2015/4, Paulraj/Behari 2006/4, Sakar 1994/4, Shahin 2013/1, Taheri 2015/12 & 2017/12, Zotti-Martelli 2000/4

**EEG/brain development:** Chauhan 2017/6, Maganioti 2010/2, Nazıroğlu /Gümral 2009/2, Othmann 2017/6, Papageorgiou 2011/2, Paulraj/Behari 2006/2,4, Sinha 2008/2, Testylier 2002/2, von Klitzing 1995/2 & 2016/2, Yang 2010/2, Yang 2012/2

Embryo/pregnancy/reproductive capacity: Cleary 1996/7, Margaritis 2014/1, Nakamura 2000/1, Özorak 2013/6, Othmann 2017, Sangün 2015/1, Shahin 2013/1, Yüksel 2016/6

Gene/gene expression: Kesari 2010b/4, Lee 2005/10, Orendáčová 2011/2, Yang 2010/2, Yang 2012/2

Glucose metabolism: Salah 2013/6

Heart: Kim/Rhee 2004/6, Saili 2015/5, von Klitzing 2014/5 & 2016/5, Zhu 2016/5

Heat shock proteins (HSP): Deshmuk 2015/3, Misa-Agustiño 2012/9 & 2015/9, Yang 2012/2

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Kidneys: Özorak 2013/1,6

Liver: Chauhan 2017/6, Holovska 2015/8, Kumari 2012/6,8, Salah 2013/6

Memory / learning / behavior: Cammaerts/Johannson 2014/3, Chartuvedi 2011/3, Deshmuk 2015/3, Hassanshahi 2017/3, Lai 2004/3 & 1987a & 1987b & 1988 & 1989a & 1989b & 1991 & 1994 & 1996b/2, Li 2008/3, Othmann 2017/6, Orendáčová 2011/2, Paulraj 2006a/2, Sangün 2015/1, Shahin 2015/3, Sinha 2008/3,9, Thomas 1980/3, Wang/Lai 2000/3

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**Sperm / testes:** Akdag 2016/1, Atasoy 2013/6, Avendaño 2012/1,4, Dasdag 2015/1, Meena 2014/1,6, Oksay 2014/6, Özorak 2013/6,1, Sarkar 1994/4, Shahin 2014/1, Shokri 2015/1

Spleen: Chauhan 2017/6

Testosterone: Meena 2014/6

Thyroid glands: Misa-Agustiño 2013/9 & 2015/9, Sinha 2008/3,9

## Abbreviations

Wi-Fi, a brand name for IEEE 802.11 wireless networks, and WLAN (wireless local area network), any of several different wireless network protocols, are commonly used interchangeably, as in this review.

3β-HSD	3β-hydroxysteroid dehydrogenase
8-OHdG	8-hydroxy-deoxyguanosine
ACh	acetylcholine
ALARA	as low as reasonably achievable
ALP	alkaline phosphatase
ALT	alanine transaminase
AOPP	advanced oxidation protein products
AST	aspartate transaminase
ATP	adenosine triphosphate
BImSchV	Bundes-Immissionsschutzverordnung (Federal Pollution Control Ordinance)
CAP	capsaicin
CAT	catalase
ск	creatine kinase
DECT	digital enhanced cordless telecommunications
ECG	electrocardiogram
EEG	electroencephalogram
ELF	extremely low frequency
EMF	electromagnetic field
E plane	electric field plane
FSH	follicle-stimulating hormone
GABA	gamma-aminobutyric acid
GAT1	GABA transporter 1
GSH	glutathione
GSH-Px	glutathione peroxidase
GSM	global system for mobile communications
H plane	magnetic field plane
HSP	heat shock protein
IARC	International Agency for Research on Cancer
ICNIRP	International Commission on Non-Ionizing Radiation Protection
IEEE	Institute of Electrical and Electronics Engineers
IGF-1	insulin-like growth factor 1
<b>IL-1</b> β	interleukin 1 beta
iNOS	nitric oxide synthase

JAK-STAT	Janus kinase-signal transducers and activators of transcription
LDH	lactate dehydrogenase
LH	luteinizing hormone
Li-Fi	wireless network using visible light; term coined in the style of Wi-Fi
LP	lipid peroxidation
МАРК	mitogen-activated protein kinase
MDA	malondialdehyde
MW	microwave
NADH	nicotinamide adenine dinucleotide hydrogen
NO	nitric oxide
ODC	ornithine carboxylase
PBN	N-tert-butyl-alpha-phenylnitrone
PCO	protein carbonyl
РКС	protein kinase C
POD	peroxidase
RF	radio-frequency
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAR	specific absorption range
SOD	superoxide dismutase
svz	subventricular zone
TAS	total antioxidant status
TBIL	total bilirubin
TNF-α	tumor necrosis factor alpha
TRPM2	transient receptor potential melastatin 2
TRPV1	transient receptor potential vallinoid 1
тѕн	thyroid-stimulating hormone
UMTS	universal mobile telecommunications system
VGCC	voltage-gated calcium channel
VLC	visible light communication
WLAN	wireless local area network
Wi-Fi	a brand name for IEEE 802.11 wireless networks using radio-frequency radiation