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Effects of intermediate frequency electromagnetic fields: a review of animal studies

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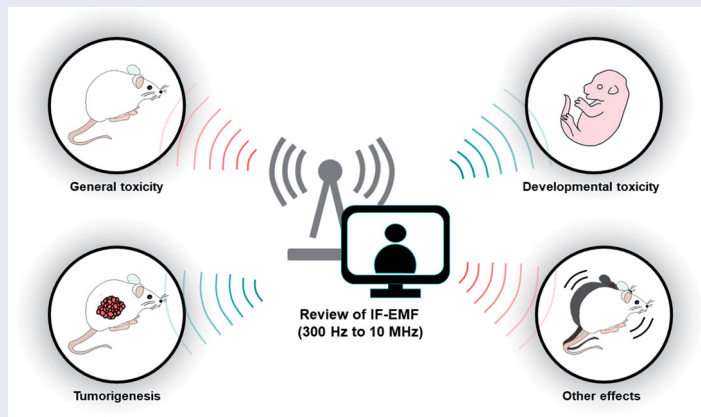
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ABSTRACT

Purpose: Many novel devices such as induction cookers or wireless power transfer produce electromagnetic fields (EMFs) in the intermediate frequency (IF) range (300 Hz to 10 MHz) and it is very meaningful for summarizing the bioeffects of IF-EMF research, particularly animal studies. This review takes into account experimental studies that used murine models to study the health effects of exposure to IF-EMF. The analyses included here use data available in the literature published from January 1988 to August 2021 including the animal studies about general adverse effects, tumorigenic effects, and effects on developmental stages. The studies that linked IF-EMF exposure during pregnancy or neonatal stage to behavioral and cognition changes were included. Additionally, this review also covers the effects of IF-EMF on gene expression patterns in the brain, behavior patterns associated with learning and memory, and immune function.

Conclusions: Although most studies have suggested that IF-EMF is harmless, some adverse effects have been reported after exposure at developmental stages and prolonged exposure. Compared to extremely low frequency (ELF) or radiofrequency (RF) EMF bands, studies on health effects with more diverse perspectives of IF-EMF have not been conducted. Therefore, performing more research should be necessary using the latest biomedical tools. From this point of view, a comprehensive review of IF-EMF studies, particularly animal studies, will provide a valuable basis for further risk analysis in humans.

GRAPHICAL ABSTRACT







Abbreviations: EMFs: electromagnetic fields; IF-EMF: intermediated frequency electromagnetic field; ELF: extremely low frequency; RF: radiofrequency; WHO: World Health Organization; EAS: Electronic article surveillance; WPT: wireless power transfer; VDT: video display terminal; KIRAMS: Korea Institute of Radiological and Medical Science; EU: European Union; GERoNiMO: Generalized EMF Research using Novel Methods; ICNIRP: International Commission on Non-Ionizing Radiation Protection; SCENIHR: The Scientific Committee on Emerging and Newly Identified Health Risks; KIEES: Korea Institute of Electromagnetic Engineering and Science; BFS: Bundesamt für

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Strahlenschutz; HF: high frequency; PubMed: Public/Publisher MEDLINE (NLM journal articles database); EMF-PORTAL: Electromagnetic Field-Portal; DMBA: dimethylbenz(a)anthracene; BP: benzo(a)pyrene; TPA: tetradecanoyl phorbol ester; IR: ionizing radiation; NMDA: N-methyl-d-aspartate; TNF α : tumor necrosis factor alpha; SD: Sprague-Dawley; AGS: audiogenic seizure; IO: iron overload

Introduction

The use of electromagnetic fields (EMFs) has become increasingly widespread since the twentieth century, which has resulted in a growing incidence of human exposure to EMFs. In turn, this has led to growing concerns regarding the potentially negative effects of EMFs on human health. In 1996, the World Health Organization (WHO) launched an international EMF project (The International EMF Project 2022) to address these concerns and protect public health. This project evaluated the scientific evidence for the potential human effects of EMFs in the frequency range up to 300 GHz. Further, this project promoted research on important issues that remain understudied and encouraged the development of international standards to limit EMF exposure.

Public concern is generally focused on exposure to extremely low frequency (ELF) EMFs which are oscillating fields defined as having frequencies below 300 Hz (e.g. electrical supply equipment including power lines) and exposure to 10 MHz to 300 GHz radiofrequency (RF) EMFs (e.g. microwave ovens, broadcasting equipment, and portable equipment with mobile phone and WiFi). The health effects of EMFs are very diverse and many studies have focused on the two aforementioned frequency bands. In contrast, very few studies have focused on the health effects of the intermediate frequency (IF) range (300 Hz to 10 MHz), which lies between the ELF and RF ranges. This is partly because there are fewer types of devices that generate EMF in this frequency range. However, there have been increasing concerns about the potentially harmful health effects of IF band technology used in homes and workplaces. These concerns include a potential link between IF-EMF exposure and employee disabilities (e.g. swelling, tingling sensation in the fingertips, and headaches).

One of the world's leading groups in research on exposure to IF-EMF is located in Korea. In 2000, the Ministry of Information and Communication in Korea established the Basic Plan for the Evaluation of EMF Effects in Humans. In accordance with this plan, Korean scientists jointly conducted animal experiments from 2002 to 2006 to analyze the effects of 20 kHz IF-EMFs. This study first sought to evaluate the relationship between chronic exposure to video display terminal (VDT) systems such as computer monitors and the occurrence of disease. Further, with the increasing adoption of wireless power transmission technology, more IF band devices are expected to be used in household and work environments, thus highlighting the need to evaluate the health risks associated with IF-EMF exposure. Nevertheless, when compared to ELF-EMF or RF-EMF, very few studies have evaluated the health effects of IF-EMF exposure in humans, and the majority of IF-EMF studies

have been conducted by the Korea Institute of Radiological and Medical Science (KIRAMS), the Electric Power Research Institute in Japan, and Kuopio University in Finland using animal models. In addition to the scarcity of animal and cellular experiments studies on the effects of IF-EMF exposure, extremely few epidemiological studies have been conducted so far. However, in 2014, there was an initiative by an international joint project of the EU (GERoNiMO – Generalized EMF Research Using Novel Methods; GERoNiMO 2022) to conduct animal and cellular experiments on IF-EMF exposure, as well as research on IF-EMF exposure environments. Further, Japan has been conducting personal/environmental IF-EMF exposure assessments and epidemiological studies since 2018.

Animal experiments remain essential to understanding the fundamental mechanisms underlying health effects or pathophysiological changes by environmental health risk factors including EMF. In this review, we limited review articles to only rodent studies although many studies were conducted in other animal species, including chick, fish, rabbit, etc., because there are difficulties in interpreting IF-EMF health effects from the various species. Mice and rats have long served as the preferred species for biomedical research animal models due to their anatomical, physiological, and genetic similarity to humans. Especially over 95% of animal studies were conducted in mice (Vandamme 2014). Well-established animal care standards for rodents help researchers repeat consistent results with similar experimental conditions. Therefore, review limited in rodent studies may help interpret and confirm bio/health effects of IF-EMF from different exposing conditions. At this point, it is very meaningful to comprehensively summarize the current state of IF-EMF research, particularly animal studies, which would provide an important basis for future risk analyses in humans.

IF-EMF sources

The definition of the IF range varies from country to country and from institution to institution. The WHO (WHO 2005) defines a range between 300 Hz and 10 MHz, whereas the International Commission on Non-Ionizing Radiation Protection (ICNIRP) does not provide an explicit definition (ICNIRP 1998). The European Commission (SCENIHR) defines it between 300 Hz and 100 kHz (SCENIHR 2015). In Korea, the Korea Institute of Electromagnetic Engineering and Science (KIEES), an organization that standardizes terms related to EMFs, defines the range between 300 Hz and 10 MHz. Meanwhile, the Federal Office for Radiation Protection (BfS) in Germany only considers ELF and RF fields and sets the boundary at 100 kHz (Bodewein et al. 2019). In order to evaluate the bioeffects of examined

studies from different countries, we reviewed the range of IF-EMF between 300 Hz and 10 MHz recommended by WHO.

The main sources of IF-EMF can be divided into the following areas.

- *Industry:* Dielectric heater sealers, induction and plasma heaters, broadcast and telecommunication transmitters.
- *General public:* Induction cookers, proximity readers, electronic article surveillance systems and other anti-theft devices, computer monitors, and TV sets.
- *Hospitals:* MRI systems, electromagnetic nerve stimulators, electrosurgical instruments, and other medical devices.
- *Defense:* Submarine communication transmitters and high frequency (HF) transmitters.

Human EMF exposure from IF devices is generally lower than the recommended limit established by the ICNIRP. However, some occupations such as oil and induction heater workers, as well as soldiers and technicians working in the vicinity of high-power broadcast equipment may be exposed to fairly high levels of IF-EMF (SCENIHR 2015).

Potential health effects of IF-EMF

Several thermal and non-thermal endpoints have been established to evaluate how IF-EMF (mainly electric fields) affect biological systems. Basic restriction for the general population and occupational exposure is determined based on the minimal exposure required to elicit any observable adverse effects (thermal or non-thermal). Exposure to high IF-EMF levels at relatively high frequencies can cause thermal damage (a slow process when tissues are kept hot for long periods); however, the most obvious risk in this frequency is that of cell membrane stimulation. This non-thermal effect is linked to changes in the membrane potential induced by external EMF exposure and may affect/stimulate peripheral nerves and muscle cells, to name a few examples. Another mechanism is electroporation, which is defined as a reversible or irreversible disruption of the cell membrane that occurs when IF-EMFs induce a large potential difference across the cell membrane. Although this phenomenon can cause tissue damage by electric shock, its therapeutic applications are also being studied, as short electric pulses could allow for better drug penetration in human tissues. External IF-EMF exposure can only have these effects on the human body at intensities much higher than normal environmental exposure.

Methods

This review takes into account experimental studies that used murine models to study the health effects of exposure to IF-EMF. For the interpretation of health effects of IF-EMF, subjects of experiments were limited to mice and rats which are the most common experimental animals in biomedical research. The analyses included here use data

available in the public domain, i.e. literature published in English from January 1988 to August 2021. The databases Electromagnetic Field-Portal (EMF-PORTAL 2022) and Public/Publisher MEDLINE (PubMed 2022) were searched using the terms ‘intermediate frequency,’ ‘intermediate frequency field,’ and the results were then filtered by the following terms: ‘experimental studies,’ ‘mouse,’ or ‘rat’. Total of 239 research articles were checked for the title and abstract as an initial search results. This review was limited to experimental studies investigating bio/health effects of IF-EMF on rodents (mice and rat); 80 studies were excluded for in vitro studies ($n=34$), ex vivo studies ($n=4$), and other species ($n=42$). Studies investigating therapeutic outcomes were also excluded unless they reported specific bio-effects; tumor treating fields ($n=25$), pulsed RF ($n=18$), and EMF from other medical device ($n=7$). A study investigating hypersensitivity was excluded. Next, we checked the method and result sections of the remaining 108 studies and excluded 55 studies with absent/unclear exposure levels ($n=51$) or magnetic resonance imaging ($n=4$). Furthermore, seven studies for which full-text articles could not be retrieved, six studies that did not have IF alone exposed group, and two studies investigating the dosimetry were excluded. Finally, 38 research articles were reviewed. Full-text copies of potentially relevant articles were obtained, and the results and reported outcomes were extracted (Figure 1).

Results

The review encompasses 38 experimental studies that used rats or mice to investigate the various bioeffects of IF-EMF, including effects on prolonged exposure, developmental stages, and tumorigenicity. The uniformity of IF-EMF magnetic flux density and numbers of cages or animals during the exposure are summarized in Table 1, if the description of IF-EMF exposing system was available. The exposure characteristics and biological systems investigated in experimental studies for the various bioeffects are shown in Tables 2–5.

Effects of long-term exposure

Spontaneous cancer incidence, hematologic, blood biochemical, and histological effects were observed after exposing rats to 20 kHz IF-EMFs for eight hours a day at a magnetic flux density of 6.25 μ T (five days a week for 90 days). No cancer was observed in neither the non-exposed nor the IF-EMF-exposed group, and no changes in body weight and the weight of various organs (lung, liver, brain, heart, testis, and ovary) were observed. Additionally, no alterations in hematological parameters (white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, mean red blood cell mass, mean hemoglobin concentration, and platelet count), blood biochemical parameters (total protein, hematologic nitrogen, creatine, glucose, total bilirubin, total cholesterol, and various enzymes), urine parameters (acidity, specific gravity, protein, ketone bodies, erythrocytes,

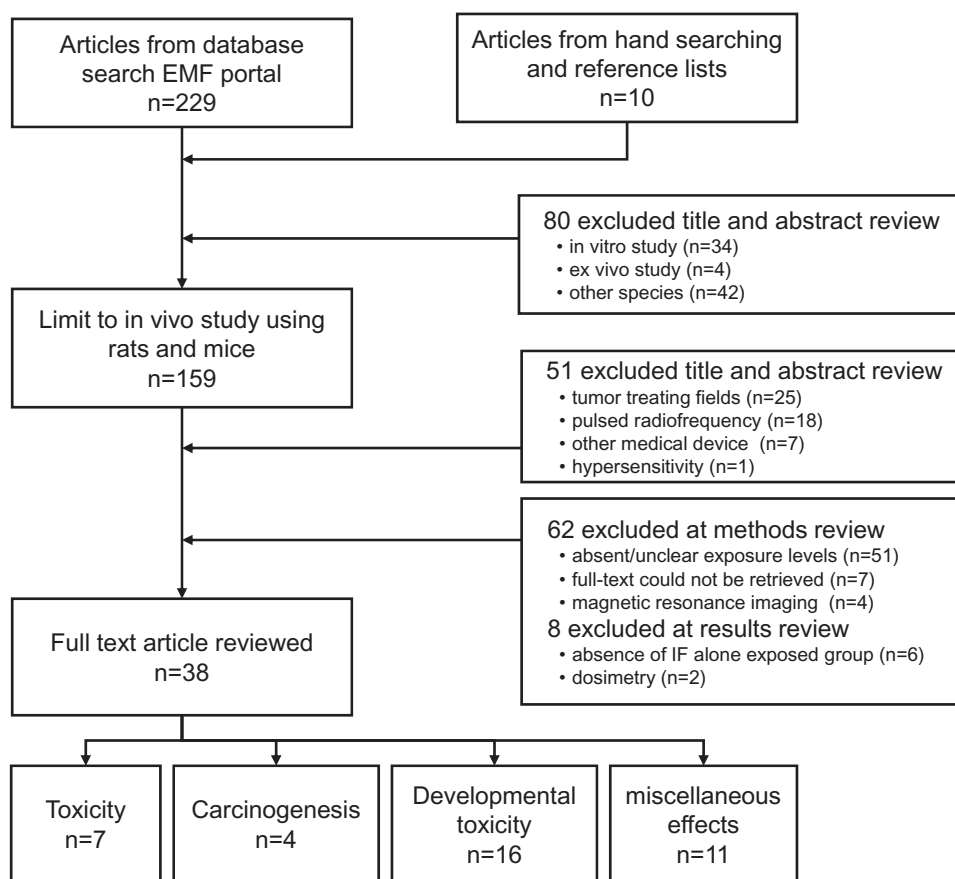


Figure 1. Flow diagram of research results review. Initially, 239 studies were considered and this review was limited to experimental studies investigating bio/health effect of IF-EMF on rodents (mice and rat).

Table 1. In vivo IF-EMF exposure systems.

References	Frequency	Uniformity for magnetic flux density	Numbers of cages or animals during exposure
Frölen et al. (1993) ^a , Svedenstål and Holmberg (1993), Svedenstål and Johanson (1995), and Dimberg (1995)	20 kHz	±1% for 15 μ T	5 cages/rack Two racks for exposure
Huuskonen et al. (1993) ^a , Huuskonen et al. (1998a, 1998b), and Juutilainen et al. (1997)	20 kHz	-6% to +17% for 15 μ T	4 cages
Kim et al. (2004) ^a , Kim et al. (2006), Lee et al. (2006, 2007, 2009, 2010)	20 kHz	4-7% for 6.25 and 30 μ T	6 cages \times 2 rows
Kumari, Capstick, et al. (2017) ^a , Kumari, Koivisto, et al. (2017), Kumari et al. (2018), and Herrala et al. (2018)	7.5 kHz	3.8% for 12 and 120 μ T	Exact number is not provided
Nishimura et al. (2011) ^a and Nishimura et al. (2012, 2016, 2019)	20 kHz 60 kHz	±3% for 100 and 200 μ T	12 cages (3-row \times 4 column)/rack Three racks for exposure
Win-Shwe et al. (2013) ^a , Win-Shwe et al. (2015), and Ushiyama et al. (2014) ^a	21 kHz	±5% for 3.8 mT	4 acrylic holders (1 rat/holder) or one rounded acryl cage with 6 divisions (for 6 mice)
Ohtani et al. (2019) ^a and Ohtani et al. (2021)	82 kHz	19.7 and 25.3 mT	One acrylic holder (1 mouse in the holder)

^aThe original reference for IF apparatus descriptions.

leukocytes, glucose, bilirubin, and urobilinogen), and organ pathology were observed (Kim et al. 2006). Similarly, almost no effects were observed when the rats were evaluated after 12-18 months of long-term exposure under the same exposure conditions; however, the number of neutrophils increased in females in the exposed group after 12 months, and the number of lymphocytes decreased in males after exposure for 18 months (Lee et al. 2006). To confirm these findings, the exposure environment and time were kept the

same and only the exposure magnetic flux density was increased from 6.25 μ T to 30 μ T. However, despite long-term exposure to 30 μ T for 18 months, abnormalities in neutrophil count and lymphocyte count were not confirmed (Lee et al. 2010).

Another research group exposed mice to 10 kHz at high magnetic flux densities of 80, 280, and 1000 μ T. The exposures were conducted for 22.6 hours a day for 14 or 90 consecutive days. However, no changes in animal behavior,

Table 2. In vivo animal studies investigating IF-EMF on toxicity.

Reference	Study subject	Frequency	Magnetic flux density	Exposure duration	Endpoint	Results
Herrala et al. (2018)	C57BL/6J mice 2-month-old male mice Sham ($n = 9$), EMF ($n = 10$)	7.5 kHz	12 μT , peak 120 μT , peak	24 h/d, 5 weeks	Genotoxicity assays in blood: comet assay and micronuclei assay	No effect
Kim et al. (2006)	SD rats 3-week-old female and male rats Sham ($n = 10/\text{sex}$), EMF ($n = 10/\text{sex}$)	20 kHz	6.25 μT	8 h/d, 5 days/week, 90 days	Body weight, organ weight Mortality Hematology parameters Serum biochemistry Urine analysis Pathological examination	No effects
Lee et al. (2006)	SD rats 3-week-old, female, and male rats 12 month ex: Sham ($n = 20/\text{sex}$), EMF ($n = 20/\text{sex}$) 18 month ex: Sham ($n = 20/\text{sex}$), EMF ($n = 20/\text{sex}$)	20 kHz	6.25 μT	8 h/d, 5 days/week, 12 and 18 months	Body weight, organ weight Mortality Hematology parameters	Increased neutrophils in 12 month-exposed female rats Decreased neutrophils in 12 month-exposed female rats No differences in the other parameters
Lee et al. (2010)	SD rats 3-week-old female and male rats Sham ($n = 20/\text{sex}$), EMF ($n = 20/\text{sex}$)	20 kHz Triangular	30 μT	8 h/d, 5 days/week, 18 months	Serum biochemistry Urine analysis Pathological examination Morbidity and mortality Body weight, organ weight and clinical observation Urine analysis Hematology parameters Serum biochemistry Histopathological examination	No effect
Nishimura et al. (2016)	CrI:CD (SD) rats 5-week-old male and female rats Sham ($n = 12/\text{sex}$), EMF ($n = 12/\text{sex}$) (*2 replications)	20 kHz Sinusoidal	200 μT	22 h/d, 14 days or 13 weeks	Clinical observation Hematological examination Clinical chemistry examination Gross pathological examination and organ weight measurement Histopathological examination	No effect
Robertson et al. (1996)	B6C3F1 mice Male and female mice Acute: control ($n = 13/\text{sex}$), EMF ($n = 6-7/\text{sex}/\text{group}$) Subchronic: control ($n = 19-20/\text{sex}$), EMF ($n = 15/\text{sex}/\text{group}$)	60 kHz Sinusoidal 10 kHz, sinusoidal	100 μT 80 μT 280 μT 1000 μT	22.6 h/d, 14 days (acute) or 90 days (subchronic)	Gross and histological examination Morbidity Body weight Clinical chemistry	No effect
Zhao et al. (2020)	KM mice 1-month-old male and female mice Sham ($n = 5/\text{sex}$), EMF ($n = 5/\text{sex}$)	47 kHz, wireless power transfer	270 μT , peak	5 h/d, 6 days/week, 12 weeks	Hematology parameters The levels of immune factors, testosterone, and progesterone in blood Histopathological examination in brain, heart, lung, kidney, liver, stomach, spleen, ovary and testis	Increased the level of TNF α ^a , IL-6 ^b and IL-1 β ^c and decreased the level of testosterone and progesterone Morphological changes observed in liver, spleen, ovary, and testes

The magnetic flux density was represented by rms value, but if it was a peak value, the value followed by peak.

^aTNF α : tumor necrosis factor- α .

^bIL-6: interleukin 6.

^cIL-1 β : interleukin 1 beta.

Table 3. In vivo animal studies investigating IF-EMF on carcinogenicity.

Reference	Study subject	Frequency	Magnetic flux density	Exposure duration	Endpoint	Results
Lee et al. (2007)	Female SD rats for the mammary tumors Sham ($n = 20$), EMF ($n = 20$), DMBA ($n = 24$), DMBA + EMF ($n = 23$)	20 kHz	6.25 μ T	8 h/d, 5 days/week, 16 weeks	Incidence of mammary tumor Histopathological examination	No adverse effect
	Newborn ICR mouse for the lung tumors Sham ($n = 40$), EMF ($n = 40$), BP ^c (carcinogen, $n = 40$), BP + EMF ($n = 40$)			8 h/d, 5 days/week, 6 weeks	Incidence of lung tumor Histopathological examination	
	Female ICR mice for the skin tumors Sham ($n = 20$), EMF ($n = 20$), DMBA (carcinogen, $n = 20$), DMBA + TPA ($n = 20$), DMBA + TPA + EMF ($n = 20$)			8 h/d, 5 days/week, 19 weeks	Incidence of skin tumor Histopathological examination	
Lerchl et al. (2021)	CD-1 IGS mouse 3-month-old female mice Sham ($n = 80$), EMF ($n = 80$)	20 kHz	360 μ T	24 h/d, 7 days/week, max 13 months (behavioral test at 10 months)	Body mass Tumor incidence Behavioral changes in 8 arm radial maze, rotarod and open field tests	Higher levels of alertness in open field test No differences in the other parameters
	CByB6F1-Tg (HRAS)2Jic mice 6-week-old male and female mice Sham ($n = 25$ /sex) EMF ($n = 25$ /sex) MNUe (carcinogen, $n = 10$ /sex) (*2 replications)			Sinusoidal	22 h/d, 26 weeks	Morbidity Clinical observation Body weight Tumor incidence Gross pathology and organ weight Histopathology
Nishimura et al. (2019)						
Svedenstål and Holmberg (1993)	CBA/S mice 40-day-old female mice Control ($n = 47$) PMF ^a ($n = 53$) 5.24 Gy of X-ray ($n = 64$) 5.24 Gy of X-ray + PMF ($n = 63$)	20 kHz, sawtooth PMF, pulsed vertical	15 μ T, peak	Lifetime exposure (max 131 weeks in control group)	Hematological parameters Incidence of lymphoma	No adverse effect

The magnetic flux density was represented by rms value, but if it was a peak value, the value followed by peak.

^aPMF: pulsed magnetic field.

^bDMBA: 7,12-dimethylbenz[a]anthracene.

^cBP: benzo[a]pyrene.

^dTPA: tetradecanoylphorbol ester.

^eMNU: N-methyl-N-nitrosourea.

body weight, morbidity, or biochemical and hematological parameters were observed (Robertson et al. 1996). Similarly, a study by a Japanese research team on rats did not identify changes in body weight, organ weight, or hematological parameters after exposure at 20 kHz for 22 hours a day at a magnetic flux density of 200 μ T for 14 days or 13 weeks (Nishimura et al. 2016). Additionally, Svedenstål et al. exposed newborn, young, and adult mice to 20 kHz IF-EMFs for 40 days, after which an increase in body weight was observed (Svedenstål and Johanson 1998). Kumari et al. observed sperm cells from cauda epididymis after exposing

mice to 7.5 kHz IF-EMF at 12 and 120 μ T for 5 weeks. The 120 μ T exposure group exhibited significant increase in sperm motility (Kumari, Capstick, et al. 2017). Herrala et al. assessed the genotoxic effects of IF-EMF in vitro and in vivo. Rat primary astrocytes were exposed to a magnetic flux density of 30 or 300 μ T for 24 hours. Male C57BL/6 J mice were continuously exposed for five days at 12 or 120 μ T. The results from comet assays and micronuclei counts showed no genotoxicity or co-genotoxicity of 7.5 kHz IF-EMFs at magnetic flux densities of up to 300 μ T in vitro or in vivo (Herrala et al. 2018). Recently, Zhao et al.

Table 4. In vivo animal studies investigating IF-EMF on fetus.

Reference	Study subject	Frequency	Magnetic flux density	Exposure duration	Endpoint	Results
Chiang et al. (1995)	Swiss Webster mouse Con ($n = 24$, pregnant) EMF ($n = 21$, pregnant) Ara-C ^a ($n = 22$, pregnant) EMF + Ara-C ($n = 22$, pregnant) Wistar rats	15.6 kHz Sawtooth	40 μ T, peak	4 h/d, G6b-G17 Ara-C injection at G9	Number of malformations in fetuses Body mass and length of living fetuses	No adverse effect on fetuses in EMF alone Increased cleft palate and/or cleft lip in the combination of EMF and Ara-C group than the Ara-C group No adverse effect
Dawson et al. (1998)	Set I: pregnant rats ($n = 14-16$ /group, 4 groups) Set II: male rats ($n = 5$ /group, 4 groups) Set III: female rats ($n = 16-17$ /group, 4 groups)	10.8 kHz Sinusoidal	95 μ T 240 μ T 950 μ T	20-23.5 h/d Set I: G0-G22 Set II: 30-72 days before mating Set III: 45-58 days before mating	Dam: Weight gain, litter size, implantation sites, resorptions, blood chemistry and hematology Fetus: deaths, weight, sex, external and internal morphology, skeletal abnormalities, histology, hematology Adult male: body weight, clinical signs, mortality, hematology, clinical chemistry, gross pathology at necropsy, reproductive organ weights and histology Adult female: estrous cycle, ovulation, weight gain Brain weights	
Dimberg (1995)	C57/B1 mice Sham ($n = 15$, pregnant) EMF ($n = 15$, pregnant)	20 kHz Asymmetric sawtooth	15 μ T, peak	24 h/d, G1-G19	Concentration of protein, DNA, AChE, NGF, CNPe at PD1f, PD21, PD308	Decrease in the whole brain weight on PD308 Decrease in DNA level and increase in the activities of CNP, AChE, and NGF protein in the cortex region on PD21 Reduction of the amount of DNA in cerebellum but enhanced CNP activity in cortex on PD308
Frölen et al. (1993)	CBA/S mice G1-G19: sham (mated $n = 173$, pregnant $n = 154$), EMF (mated $n = 245$, pregnant $n = 211$) G1-G19: sham (mated $n = 127$, pregnant $n = 111$), EMF (mated $n = 172$, pregnant $n = 142$) G2-G19: sham (mated $n = 114$, pregnant $n = 99$), EMF (mated $n = 136$, pregnant $n = 117$) G5-G19: sham (mated $n = 109$, pregnant $n = 98$),	20 kHz Pulsed	15 μ T, peak	24 h/d, G1-G19	Number of implants, placental resorptions, living fetuses, dead fetuses Number of malformations in living and dead fetuses Body mass and length of living fetuses	Increased placental resorption rate in all IF EMF exposed groups, except G7-G19 exposed group. Reduced body mass and length in G7-G19 exposed group. Increased dead fetuses in G1-G19 exposed group.

(continued)

Table 4. Continued.

Reference	Study subject	Frequency	Magnetic flux density	Exposure duration	Endpoint	Results
Huuskonen et al. (1993)	EMF (mated $n = 147$, pregnant $n = 137$) G7-G19: sham (mated $n = 85$, pregnant $n = 81$), EMF (mated $n = 103$, pregnant $n = 100$) Wistar rat Sham ($n = 72$, pregnant) EMF ($n = 72$, pregnant)	20 kHz Sawtooth	15 μT , peak	24 h/d, G0-G20	Number of malformations in fetuses Body mass and length of living fetuses	No increased malformations or resorption Increased minor skeletal abnormalities No adverse effect
Huuskonen et al. (1998a)	CBA/S mouse Phase 1: sham (mated $n = 60$, pregnant $n = 46$), MF (mated $n = 59$, pregnant $n = 46$) Phase 2: sham (mated $n = 42$, pregnant $n = 34$), MF (mated $n = 43$, pregnant $n = 40$) CBA/Ca mice	20 kHz Sawtooth	15 μT , peak	24 h/d, G0-G18	Hematological parameters Micronuclei analysis Measurement of corpora lutea	No increased malformations or resorption Increased minor skeletal abnormalities No adverse effect
Huuskonen et al. (1998b)	Pregnant mice control ($n = 45$), EMF ($n = 56$) Pregnant CBA/Ca mice Control ($n = 41$), EMF ($n = 53$) Pregnant CBA/S mice Control ($n = 75$), EMF ($n = 84$) ICR mice	20 kHz, sawtooth	15 μT , peak	24 h/d, G0-G18	Body weight, skeletal variations, number of implantation sites and living fetuses Hematology and bone marrow analysis of maternal mice	No increased malformations or resorption Increased fetuses with at least 3 skeletal variations in all IF EMF
Juutilainen et al. (1997)	Pregnant CBA/Ca mice Control ($n = 41$), EMF ($n = 53$) Pregnant CBA/S mice Control ($n = 75$), EMF ($n = 84$) ICR mice	20 kHz, pulsed	15 μT , peak	24 h/d, G0-G18	Body weight, skeletal variations, number of implantation sites and living fetuses	No effect on the resorption rate in CBA/Ca mice. Increased resorption rate in CBA/S mice compared to control group. No adverse effect
Kim et al. (2004)	Pregnant mice Sham ($n = 18$), EMF ($n = 16$) C57BL/6J mice 8-week-old male mice	20 kHz Sawtooth	6.25 μT	8 h/d, G2.5-G15.5	Number of implantation, resorptions, sex, death and living fetuses Body mass and size of fetuses Incidence of external, visceral and skeletal malformations	No adverse effect on fertility indicators Increased sperm motility in 120 μT group.
Kumari, Capstick, et al. (2017)	Sham ($n = 30$), EMF ($n = 20$) (*combined 3 cohorts) ICR mice	7.5 kHz	12 μT , peak 120 μT , peak	24 h/d, 5 weeks	Body and organ weight. Sperm motility and sperm counts, morphological analysis	No adverse effect on fertility indicators Increased sperm motility in 120 μT group.
Lee et al. (2009)	Pregnant mice: sham ($n = 11$), EMF ($n = 10$) EMF ($n = 10$) Crj:CD (SD) rats Pregnant rats Sham ($n = 25$), EMF ($n = 25$)	20 kHz Triangular waves	30 μT	8 h/d, G2.5-G15.5	Number of implantation, resorptions, death and living fetuses Body mass and size of fetuses Incidence of external, visceral and skeletal malformations	No adverse effect
Nishimura et al. (2011)	Crj:CD (SD) rats Pregnant rats Sham ($n = 25$), EMF ($n = 25$)	20 kHz 60 kHz	200 μT 100 μT	22 h/d, G7-G17	Number of implantation, resorptions, sex, death and living fetuses Body mass and size of fetuses Incidence of external, visceral and skeletal malformations	No adverse effect

Nishimura et al. (2012)	(*2 replications) Cr:CD (SD) rats Sham ($n = 24$), EMF ($n = 24$)	20 kHz Sinusoidal	200 μ T	22 h/d, 14 d prior to mating, during mating, until G7	Clinical signs and gross pathology Gross observations in mated rats Number of live fetuses, dead fetuses, implantation sites, resorptions Weight of testes and epididymis Sperm motility Histopathology of epididymis Hematological parameters and differential counts of bone marrow of dams Weight of dams, fetuses and placentas Number of resorptions and pups Fetal abnormalities Number of implants, placental resorptions, living fetuses, dead fetuses	No adverse effect
Stuchly et al. (1988)	(*2 replications) SD rat	60 kHz Sinusoidal 18 kHz	100 μ T	7 h/d, 2 weeks prior to and throughout pregnancy	A significant decrease in maternal lymphocyte count for 66 μ T but within the normal range	
Svedenstål and Johanson (1995)	Sham ($n = 26$) EMF ($n = 26$) CBA/S mice	Sawtooth 20 kHz	5.7 μ T, peak 23 μ T, peak 66 μ T, peak 15 μ T, peak	24 h/d G1-G5.5 or G1-G7	Decreased in the length and body weight of living embryos exposed for 5.5 days	
Wiley et al. (1992)	G1-G5.5: sham ($n = 114$ mated, $n = 100$ pregnant), EMF ($n = 125$ mated, $n = 113$ pregnant) G1-G7: sham ($n = 126$ mated, $n = 103$ pregnant), EMF ($n = 132$ mated, $n = 120$ pregnant) CD-1 mouse Sham (mated $n = 185$, pregnant $n = 141$) EMF (mated $n = 186$ /group, pregnant each $n = 151$, 163, 144) (*4 replications)	Sawtooth 20 kHz sawtooth	3.6 μ T, peak 17 μ T, peak 200 μ T, peak	20-21 h/d, G1-G18	Elevation in the level of progesterone on day 3 and calcium on day 13 in exposed dams, respectively	No adverse effect

The magnetic flux density was represented by rms value, but if it was a peak value, the value followed by peak.

^aAra-C: cytosine arabinoside (a teratogen).

^bG: gestation.

^cACHe: acetylcholine esterase.

^dNGF: nerve growth factor.

^eCNP: 2',3'-cyclic nucleotide 3'-phosphodiesterase.

^fPD: postnatal day,

Table 5. Other in vivo animal studies investigating bioeffects of IF-EMF.

Reference	Study subject	Frequency	Magnetic flux density	Exposure duration	Endpoint	Results
de Kleijn et al. (2016)	Balb/c mouse	ELF/IF-EMF signal contained multiple frequencies (20–5000 Hz)	10 μ T	1, 4, and 24 h/day, 1 week or 15 weeks	Immune phenotyping of blood cells	Increased numbers of neutrophils and CD4+ lymphocytes in the 24-h exposed group Significantly lower in POMC expression and plasma adrenocorticotrophic hormone.
Juutilainen et al. (1988)	6 week-old male mice 4 groups; sham ($n = 12$) Short-term EMF ($n = 12$) Long-term EMF ($n = 20$) KM rat (susceptibility to audiogenic seizures)	10 kHz, 28 kHz, sinusoidal 100 Hz	1.254 μ T	1 h	Strength and duration of seizures, seizure latency	No effects
Kumari, Koivisto, et al. (2017)	1-year-old female rats Sham ($n = 16$), EMF ($n = 16$) C57BL/6J mice	7.5 kHz	12 μ T, peak 120 μ T, peak	24 h/d, 5 weeks	Behavioral tests: spontaneous exploratory activity, rotarod, marble burying, isolation-induced aggression, Morris swim task, passive avoidance	Mild impairment of learning and memory performance was observed in Morris swim task and the passive avoidance task in the group exposing over 48 h of 120 μ T IF EMF Increased expression of the proinflammatory cytokine TNF α mRNA in the 120 μ T group
Maaroufi et al. (2011)	Male mice sham ($n = 20$), EMF ($n = 20$ /magnetic flux density) Wistar rats 1-month male rat	150 kHz	6.25 μ T	1 h/d, 21 consecutive days	q-PCR for BDNF, TNF α Immunohistochemistry for GFAP Lipid peroxidation level of MDA Enzyme activity of SOD and catalase	Significantly increased of the lipid peroxidation in the cerebellum in the IF EMF exposure group.
Ohtani et al. (2019)	5 groups; saline ($n = 8$), iron overload (IO) ($n = 8$), EMF exposed ($n = 6$), EMF-IO ($n = 6$), sham-without iron ($n = 6$) C57BL/6NCR-Slc mice	82 kHz	1.97×10^4 μ T 2.35×10^4 μ T	1 h/d, 5 days/week, 2 weeks	Microarray analysis in brain and liver Real time RT-PCR	No effects
Ohtani et al. (2021)	5-week-old male mice EMF: 19.7 mT ($n = 5$), 25.3 mT ($n = 10$) C57BL/6NCR-Slc mice 5-week-old male mice Sham ($n = 5$), EMF ($n = 15$) X-ray irradiation ($n = 4$)	Wireless power transfer 82 kHz Wireless power transfer	1.97×10^4 μ T 2.35×10^4 μ T	1 h/d, 5 days/week, 2 weeks	Biochemical and hematological parameter of blood Micronuclei assay Pig-a mutation assay in mature erythrocytes and reticulocytes Nerve regeneration	No effects
Rusovan et al. (1992)	SD rat female weighing 200 g with crush lesion of the right sciatic nerve (group sizes and age of animals and group size was not described)	500 Hz, 1000 Hz, 1500 Hz, and 2000 Hz Sinusoidal	100 μ T	3, 4 or 6 days	Nerve regeneration	Increased in the nerve regeneration rate at 1000 Hz
Ushiyama et al. (2014)	SD rats 4–5-week-old male rats	21 kHz	3.8×10^3 μ T	1 h/d, 14 days	Biochemical and hematological parameter of blood Real time chemotactic assay Phagocytic activity	No effects

<p>Win-Shwe et al. (2013)</p>	<p>Cage control ($n = 11$), sham ($n = 12$), EMF ($n = 12$) Animals were restrained in acrylic holders</p>	<p>21 kHz</p>	<p>$3.8 \times 10^3 \mu\text{T}$</p>	<p>1 h/d, 2 weeks</p>	<p>No effects</p>	<p>Immune function Cytotoxic activity T-cell population Body weight and organ weight Hippocampal mRNA expression levels in NMDA^a receptor subunits such as NR1b, NR2A, and NR2B, CaMK-IVc, CREB-1d, NGF, and BDNF Histological examination</p>
<p>Win-Shwe et al. (2015)</p>	<p>Recovery (exposed to EMF 2 weeks and then left for one week in home before sample collection) ($n = 3/\text{sex}$) C57BL/6J mice Pregnant mice</p>	<p>21 kHz Sinusoidal</p>	<p>$3.8 \times 10^3 \mu\text{T}$</p>	<p>1 h/d, G7-G17, or G7-G17 + PND27-PND48</p>	<p>Expression levels of memory function-related genes such as NMDA receptors (NR1, NR2B) and their signal transduction pathway molecules (CaMK-IV, CREB-1) were significantly upregulated in the hippocampus of 7-week-old male mice exposed to IF EMF during organogenesis and adolescent periods and these changes are transient and recover after termination of exposure without histopathological changes.</p>	<p>Quantification of mRNA expression level in hippocampus Histological examination Immunohistochemistry</p>

The magnetic flux density was represented by rms value, but if it was a peak value, the value followed by peak.

^aNMDA: N-methyl-D-aspartate.

^bNR: NMDA receptor.

^cCaMK-IV: calcium/calmodulin-dependent protein kinase-IV.

^dCREB-1: cyclic AMP responsive element binding protein-1.

developed a wireless power transmission system and exposed mice to 47 kHz (270 μ T), after which they calculated the peak value of the induced electric field strength and temperature rise of each organ in mice. The heart tissue structure did not change, but the liver, spleen, ovary, and testes were affected. Additionally, the liver showed the greatest morphological changes, which was consistent with the highest peak electric field strength of 1.43 V/m (Zhao et al. 2020) (Table 2).

Tumorigenic effects

The KIRAMS also conducted animal experiments to evaluate the effects of IF-EMF on tumor development. This research group estimated that humans would be most likely exposed to 20 kHz EMFs around the chest area, and therefore sought to link IF-EMFs to breast, lung, and skin cancer. First, 20 or more female rats per group were examined to evaluate the effect of IF-EMFs on the development of mammary tumors. Specifically, this study evaluated the effects of IF-EMF exposure alone and in combination with known environmental carcinogens such as dimethylbenz(a)anthracene (DMBA) on the incidence of mammary tumors. The organisms were exposed to IF-EMFs for 14 weeks, five days a week, for eight hours a day. IF-EMFs alone did not induce mammary tumors, and exposure to IF-EMFs in combination with DMBA did not significantly increase the incidence of mammary tumors caused by DMBA. Although the number of tumors per animal and tumor size were evaluated, the authors could not conclusively confirm the effect of IF-EMF co-exposure on tumorigenesis. Additionally, further analyses confirmed that 6.25 μ T IF-EMF exposure did not adversely affect the development of mammary malignancies caused by DMBA. Therefore, the authors concluded that IF-EMFs did not induce cancer development, nor did they play a role in accelerating or exacerbating the progression of mammary tumors induced by known carcinogens. However, this study failed to evaluate the effect of IF-EMF exposure on genetic breast cancer predisposition, which is often seen in humans.

Next, the effect of IF-EMFs on lung cancer development was evaluated in both male and female mice. Lung tumors were induced by benzo(a)pyrene (BP), a carcinogen found in cigarette and automobile smoke. BP was injected into newborn mice within 24 hours of birth and pulmonary adenomas were observed 9 weeks later. Exposure to IF-EMF alone did not induce the development of pulmonary adenomas and co-exposure with BP did not affect the development of pulmonary adenoma produced by BP alone. Moreover, IF-EMF exposure did not affect the number of lung tumors per animal. Nevertheless, this study only observed the short-term effects of IF-EMF exposure on the development of pulmonary adenomas, and therefore the development of malignant tumors could not be monitored. Moreover, similar to the mammary tumor model, the effect of IF-EMF on the occurrence of lung cancer due to genetic alterations was not observed.

Lastly, DMBA was applied to the skin to evaluate the effect of tetradecanoyl phorbol ester (TPA) and IF-EMF co-

exposure on the incidence of skin cancer. When the mice were co-exposed to DMBA and TPA, skin cancer occurred more quickly and the cancer became malignant. One week after DMBA exposure, TPA was applied to the skin of female adult mice and the mice were exposed to IF-EMFs for 19 weeks. TPA was applied to the skin once a week for 19 weeks. Exposure to IF-EMFs alone did not induce skin cancer, nor did DMBA exposure alone. In contrast, a 100% incidence of skin cancer occurred when DMBA and TPA were applied together; however, this effect was not exacerbated by IF-EMF exposure. Moreover, IF-EMF exposure did not change the number of skin tumors per animal. One of the limitations of this study was that it was difficult to identify whether IF-EMF treatment interacted with DMBA and TPA co-treatment, as the combination of these two compounds had already resulted in a 100% skin cancer incidence rate. Despite these limitations, the authors ruled out any association between IF-EMFs and the occurrence of skin cancer, as IF-EMFs alone did not induce any skin malignancies. Therefore, the authors concluded that an IF-EMF of 20 kHz at a magnetic flux density of 6.25 μ T did not affect the occurrence of mammary tumors, lung adenomas, and skin tumors (Lee et al. 2007).

Svedenstål and Holmberg monitored lymphoma development in mice exposed to 5.24 Gy ionizing radiation (IR) and a 20 kHz IF-EMF (15 μ T) but no effects were identified. Specifically, the authors reported that there was no statistically significant difference between the number of animals with lymphoma in the control and IF-EMF-exposed groups, as well as between the IR-exposed and IR + IF-EMF-exposed groups (Svedenstål and Holmberg 1993). Recently, Nishimura et al. investigated the carcinogenicity of 20 kHz IF-EMF in duplicate experiments in transgenic *rasH2* mice and demonstrated that chronic exposure (200 μ T, 22 hours/day for 26 weeks) did not have carcinogenic effects (Nishimura et al. 2019). Lerchl et al. assessed the long-term effects of IF-EMF in CD-1SG mice. Female CD-1 IGS mice were exposed to 20 kHz (360 μ T, 24 hours/day) for 10 months, after which body mass analysis, behavioral tests, and histopathology revealed that long-term to this IF-EMF did not have any adverse effects on body mass, learning/memory behaviors, and tumor incidence. However, IF-EMF-exposed mice exhibited significantly higher alertness in behavioral tests compared to sham-exposed mice. The results of animal studies using rodent models confirm that IF-EMFs are not significantly carcinogenic (Lerchl et al. 2021) (Table 3).

Effects on developmental stages

In addition to the potentially tumorigenic effects of IF-EMFs, the impact of these frequencies on early development and gestation are among the most important public concerns. Early developmental stages are known to be especially vulnerable to environmental insult. The KIRAMS thus exposed pregnant mice to IF-EMF during the organogenesis stage to investigate its effects on fetal formation. The females were exposed to a 20 kHz IF-EMF at a magnetic flux density of

6.25 μT for eight hours a day from the 2.5th to the 15.5th day of gestation. Cesarean sections were performed on the 19th day of pregnancy to observe the fetuses. The number of implantations, the number of live and dead fetuses, the sex, weight, and length of the fetuses, the length of the head, and the width and weight of the placenta were then measured. Additionally, embryo resorption, embryonic death, and fetal death were also investigated. No statistically significant differences in the number of implantations, the number of live and dead fetuses, fetus sex, and placental weight were linked to IF-EMF exposure. Additionally, the number of fetuses with growth retardation showed a weak increasing tendency in the group exposed to IF-EMF, but there was no statistical significance. The body weight and head width were not affected by IF-EMF exposure. IF-EMF exposure was also not statistically linked to external or internal malformations. Moreover, no skeletal abnormalities were observed in response to IF-EMF exposure (Lee et al. 2009).

Several studies have been conducted to evaluate the effects of IF-EMFs on fetuses, including teratogenicity, reproduction, and development in mice and rats; however, these results have been inconsistent. Wiley et al. reported that exposure to a 20 kHz IF-EMF (up to 200 μT) had no influence on the number of implantations, fetal death, resorption, malformation, and body mass of CD-1 mice fetuses (Wiley et al. 1992). In contrast, Frölen et al. reported that CBA/S mouse fetuses exposed to IF-EMFs (20 kHz, 15 μT) had lower body masses during early gestation (days 1–5.5), as well as higher overall resorption rates (Frölen et al. 1993). Juutilainen et al. attempted to replicate former results by Frölen using CBA/Ca and CBA/S mice and reported that the resorption rate is not affected in CBA/Ca but increased in CBA/S mice (Juutilainen et al. 1997). Juutilainen et al. described difficulties in interpreting inconsistent results in two mouse strains and suggested the animal strains as a crucial variable in bioelectromagnetic research. Svedenstål and Johnson found no increase in resorption but significant decreases in body weight and length in living CBA/S mouse fetuses exposed to a 20 kHz IF-EMF (15 μT) during pregnancy (gestational days 0–5.5 or 0–7) (Svedenstål and Johanson 1995). Other studies (Huuskonen et al. 1993, 1998a, 1998b) also sought to evaluate the early adverse effects of IF-EMF, but there were no significant differences in the maternal weight, number of corpora lutea, implantations, resorptions, and number of dead and live fetuses in CBA/Ca and CBA/S mice when exposed to a 20 kHz IF-EMF (15 μT) from gestational days 1 to 18 (Table 4).

Others

The effects of IF-EMFs on the brain and behavior of rodents have also been investigated. Win-Shwe et al. exposed mice to a 21 kHz IF-EMF at a magnetic flux density of 3800 μT for one hour every day for 2 weeks. The mice were allowed to recover for a week, after which the hypothalamus was removed and the expression of genes related to memory was determined. However, no abnormalities were observed

(Win-Shwe et al. 2013). Furthermore, other studies have suggested that exposing mice to IF-EMFs during postnatal development may cause genetic/biochemical alterations. IF-EMFs transiently affected the N-methyl-D-aspartate (NMDA) receptor, which is related to crucial signaling pathways and inflammatory mediators in the hippocampus of young adult mice; however, the mice recovered after the termination of exposure without visible histopathological changes (Win-Shwe et al. 2015). Ohtani et al. exposed mice to very high magnetic flux density (84 kHz, 2.35×10^4 μT) for 2 weeks (1 hour/day, 5 days/week); however, this exposure level did not affect transcriptional responses in the brain or liver (Ohtani et al. 2019). Kumari et al. exposed mice to a 7.5 kHz IF-EMF (12 or 120 μT for 5 weeks) and assessed behavioral and histopathological endpoints. The mice exposed to the IF-EMF for 48 hours in the 120 μT groups displayed impaired memory in passive avoidance tests and the Morris swimming assay, which coincided with a significant increase in the mRNA levels of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF α). However, the IF-EMF did not affect body weight, spontaneous activity, motor coordination, level of anxiety or aggression, or histopathology of the hippocampus (Kumari, Koivisto, et al. 2017). Lerchl et al. assessed memory-associated tasks in the eight-arm maze in CD-1 mice exposed to a 20 kHz IF-EMF (360 μT) for 7 months, but no significant differences were observed (Lerchl et al. 2021).

Furthermore, three studies investigated the effects of IF-EMFs on hematological parameters and immune modulation in animals. Ushiyama et al. reported that whole-body exposure of Sprague-Dawley (SD) rats to a 21 kHz IF-EMF (3800 μT) for 14 consecutive days (1 hour/day) did not affect immune function in juvenile rats, including biochemical, hematological, and immunological parameters (Ushiyama et al. 2014). Kleijn et al. exposed Balb/c mice to ELF- and IF-EMF signals spanning from 20 to 5000 Hz for 1, 4, and 24 hours/day in short-term (1 week) and long-term (15 weeks) experiments to investigate the whole-body effects of these treatments on the level of stress regulation and immune response (de Kleijn et al. 2016). Ohtani et al. examined genotoxicity in the blood from mice exposed to high magnetic flux density (84 kHz, 2.35×10^4 μT) for 2 weeks (1 hour/day, 5 days/week) and no adverse effects were observed in the hematological parameters, micronuclei assay and pig-a mutation assay (Ohtani et al. 2021).

Most in vivo studies compare the effects of IF-EMF-treated animals to those of sham-exposed animals; however, some studies have implemented specific research models to identify the health effects of IF EMF exposure. For instance, Juutilainen et al. exposed audiogenic seizure (AGS)-susceptible rats to 10 kHz and 28 kHz frequencies as well as a 100 Hz sinusoidal magnetic field. The time from the beginning of the sound stimulus to the onset of the seizure (seizure latency) and the duration of the convulsion was then measured. There was no difference between the sham and IF-EMF exposed groups (Juutilainen et al. 1988). Rusovan et al. studied the effects of IF-EMFs on the regeneration of the sciatic nerve in rats. Female SD rats with crush lesions

of the right sciatic nerve were exposed to various IF-EMF frequencies (50–2000 Hz) at 100 μT , after which regeneration rate was measured with a pinch test and neurofilaments were characterized via immunohistochemistry. Interestingly, a 1000 Hz IF-EMF increased the regeneration of the sciatic nerve in rats (Rusovan et al. 1992). Maaroufi et al. investigated the possible relationship between iron levels, EMF exposure, and brain oxidative stress in young adult rats by exposing rats to high iron concentrations and/or a 150 kHz IF-EMF. Iron overload (IO) did not induce any oxidative stress in young adult rats and instead stimulated antioxidant defenses in the cerebellum and prefrontal cortex. However, IF-EMF exposure both alone or coupled with IO stimulated lipid peroxidation mainly in the cerebellum, thus demonstrating that IF-EMFs may be harmful to young adults by impairing the antioxidant defense system (Maaroufi et al. 2011) (Table 5).

Discussion

In this paper, initially 239 studies were considered and this review was limited to experimental studies investigating bio/health effects of IF-EMF on rodents. One hundred and eight articles were selected excluding *in vitro* and *ex vivo* experimental articles, articles not in mice and rat studies, articles on cancer treatment effect, pulsed RF and other medical device papers, and hypersensitivity articles. Finally, 38 research articles were targeted, excluding articles with uncertain exposure levels or magnetic resonance imaging, articles that were not full text, and articles with inaccurate dosimetry.

In vivo biological effects of IF-EMFs from 38 research articles can be divided into the following categories: analysis of (1) tumorigenesis; (2) animal studies after long-term exposure; (3) effects on developmental stages during gestation; (4) effects on sperm fertility; and (5) recent research that linked IF-EMF exposure during pregnancy or neonatal stage to behavioral and cognition changes. Studies on the effects of IF-EMFs have also evaluated gene expression in the brain after IF-EMF exposure, learning and memory behaviors, and immune function.

Most studies have reported that IF-EMFs do not induce any observable adverse effects. However, some studies have linked the adverse effects of IF-EMF exposure (15 and 66 μT) on early development in mice, including increased malformation rates, decreased fetus brain weight, increased placental resorption rate, increased fetus mortality, and reduced body mass and length of fetuses (Stuchly et al. 1988; Frölen et al. 1993; Dimberg 1995; Svedenstål and Johanson 1995; Huuskonen et al. 1998b). However, no adverse effects were reported in some studies using higher exposures (up to 200 μT) (Wiley et al. 1992; Dawson et al. 1998; Nishimura et al. 2011, 2012). In addition to developmental effects, some adverse effects were reported such as increased or decreased neutrophil count (6.5 μT), increased concentrations of some cytokines (10 μT), and decreased concentrations of testosterone and progesterone (270 μT), although no correlation was observed between the level of

exposure of IF-EMF and the induction of adverse events (Lee et al. 2006; de Kleijn et al. 2016; Zhao et al. 2020). Changes in mRNA or protein expression in the brain at various inconsistent exposure levels of IF-EMF have also been reported (Rusovan et al. 1992; Maaroufi et al. 2011; Win-Shwe et al. 2015; Kumari, Koivisto, et al. 2017) (Figure 2). Although various biological effects have been reported in some experimental studies, these results were not independently reproduced and did not depend on the degree of IF-EMF exposure. In addition, animal studies could not identify an exposure threshold of IF-EMF. Available animal studies have provided no confirmed evidence that IF-EMF is associated with bioeffects relevant to human health. Therefore, we concluded that IF-EMF exposure within ICNIRP limits (ICNIRP reference levels: 27 μT for the general public and 100 μT for occupational exposure; ICNIRP 2010) did not produce harmful effects on animals. We also suggest that future experimental studies should be continued to monitor health effects in animals related to IF-EMF to provide adequate and sufficient information for a meaningful safety assessment.

Nevertheless, our knowledge about the effects of IF-EMFs remains very limited compared to our understanding of the ELF or RF bands. In fact, the ICNIRP recommendations for IF-EMF were also based on research on the RF and ELF bands. In addition, based on the characteristics of IF-EMFs elucidated so far, these frequencies appear to mainly affect tumorigenesis, reproduction, and development. Moreover, unlike RF- and ELF-EMFs, the role of IF-EMF in degenerative brain disease, neurological diseases, and immune-related diseases is still unknown. Additionally, IF-EMF studies using cell systems are also very lacking, and studies adopting a mechanistic approach to evaluate the effects of IF-EMFs are extremely rare. Therefore, as the use of IF-EMF-based home appliances increases, evaluating the potential health impacts of these frequencies and their exact role in tissue and organ system becomes more urgent.

Conclusions

As the use of IF-EMF-based devices such as induction cookers and wireless power transfer increases, it is meaningful to comprehensively summarize the biological effects of IF-EMF studies conducted so far, especially animal studies. We reviewed a total of 38 experimental studies published from databases EMF Portal and PubMed between January 1988 and August 2021 that used rats or mice to investigate the effects of IF-EMF on prolonged exposure, developmental stages, tumorigenicity, effects on sperm fertility, and other effects that linked IF-EMF exposure during pregnancy or neonatal stage to behavioral and cognition changes. In addition, studies on gene expression in the brain, learning and memory behaviors, and immune functions by IF-EMFs have also been evaluated. Even though most studies have reported that IF-EMFs do not induce any adverse effects, some studies have linked harmful effects on early development in mice. The adverse effects on neutrophil count, cytokines, and the concentrations of testosterone and progesterone, and the alteration of mRNA or protein expression levels in

Adverse effects of IF-EMF in animal studies

General toxicity

Lee et al. (2006)

- Increased neutrophils in 12 month-exposed female rats (6.25 μ T)
- Decreased lymphocytes in 18 month-exposed female rats (6.25 μ T)

Zhao et al. (2020)

- Increased level of TNF α , IL-6 and IL-1 β and decreased level of testosterone and progesterone (270 μ T, peak)
- Morphological changes observed in liver, spleen, ovary, and testes (270 μ T, peak)

Fetus

Stuchly et al. (1988)

- A significant decrease in maternal lymphocyte count but within the normal range (66 μ T, peak)

Frölen et al. (1993)

- Increased placental resorption rate (15 μ T, peak)
- Reduced body mass and length (15 μ T, peak)
- Increased dead fetuses (15 μ T, peak)

Svedenstål et al. (1995)

- Decreased in the length and body weight of living embryos (15 μ T, peak)
- Elevation in the level of progesterone and calcium in exposed dams (15 μ T, peak)

Dimberg (1995)

- Decrease in the whole brain weight (15 μ T, peak)
- Decrease in DNA level and increase in the activities of CNP, AChE, and NGF protein in the cortex region (15 μ T, peak)
- Reduction of the amount of DNA in cerebellum but enhanced CNP activity in cortex (15 μ T, peak)

Huuskonen et al. (1998)

- Increased fetuses with at least 3 skeletal variations (15 μ T, peak)

Kumari et al. (2017a)

- Increased sperm motility (120 μ T, peak)

Others

Rusovan et al. (1992)

- Increased in the nerve regeneration rate (100 μ T)

Maaroufi et al. (2011)

- Significantly increase of the lipid peroxidation in the cerebellum (6.25 μ T)

Win-shwe et al. (2015)

- Upregulation of memory function-related genes such as NMDA receptors and their signal transduction pathway molecules in the hippocampus during organogenesis and adolescent periods and these changes are transient and recovery after termination of exposure without histopathological changes (3800 μ T)

de Kleijn et al. (2016)

- Increased numbers of neutrophils and CD4+ lymphocytes (10 μ T)
- Significantly lower in POMC expression and plasma adrenocorticotrophic hormone (10 μ T)

Kumari et al. (2017b)

- Mild impairment of learning and memory performance in Morris swim task and the passive avoidance task (120 μ T, peak)
- Increased mRNA expression of cytokine TNF α (120 μ T, peak)

Figure 2. Adverse effects of IF-EMF in animal models in vivo. The magnetic flux density (μ T) was rms value, but if it was a peak value, it was indicated as 'peak'. TNF α : tumor necrosis factor- α ; IL-6: interleukin 6; IL-1 β : interleukin 1 beta; G: gestation; PD: postnatal day; CNP: 2',3'-cyclic nucleotide 3'-phosphodiesterase; AChE: acetylcholine esterase; NGF: nerve growth factor; NMDA: N-methyl-D-aspartate; POMC: proopiomelanocortin.

the brain, were also reported. However, the health effects of IF-EMF exposure were not independently reproduced and were not dependent on the degree of IF-EMF exposure. Therefore, we concluded that IF-EMF exposure within ICNIRP limits (ICNIRP reference levels: 27 μ T for the general public and 100 μ T for occupational exposure) did not produce any harmful effects on animals. Of course, more research needs to be done using the latest biomedical tools; however, a comprehensive review of IF-EMF studies, especially animal studies, will provide a valuable basis for further risk analysis of IF-EMF in humans.

Author contributions

H.-J. Lee: conceptualization, investigation, and writing-original draft preparation. H. Jin: writing-original draft preparation, visualization, investigation, reviewing, and editing. Y.H. Ahn: conceptualization. N. Kim: conceptualization and supervision. J.K. Pack: conceptualization. H.-D. Choi: writing-reviewing and supervision. Y.-S. Lee: conceptualization, writing-original draft preparation, writing-reviewing and editing, and supervision. All authors read and approved the final manuscript.

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