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Absence of reproductive and developmental toxicity in rats following exposure to a 20-kHz or 60-kHz magnetic field

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ABSTRACT

The use of intermediate frequency (IF) magnetic fields (MFs) in occupational equipment and domestic appliances, such as inductive heating cookers, is increasing. The WHO indicated a lack of scientific evidence needed to assess the health risk of exposure to IF MFs. Male and female rats (24/group) were exposed to a 20 kHz, 0.2 mT(rms) or 60 kHz, 0.1 mT(rms) sinusoidal MF for 22 h/day from 14 days prior to and during mating. Copulated females were exposed until gestation day 7 and sacrificed thereafter. Mated males were sacrificed to examine MF exposure effects on spermatogenesis. Reproductive examinations were blinded, and experiments were duplicated per frequency to ensure reproducibility. No statistically significant, exposure-related changes were found in the estrous cycle, copulation and fertility indices, numbers of corpora lutea and implantation sites, or pre- and postimplantation loss. No reproducible changes were observed in sperm count, motility, or morphological abnormality, or in the weights of testes and epididymides after MF exposure. No significant abnormalities were observed in gross pathology or histopathology of the uterus, ovary, testis, and epididymis in the MF- or sham-exposed groups. MF exposure during the preimplantation period was not toxic to fertility or early embryogenesis under the experimental conditions.

1. Introduction

In 2007, the World Health Organization (WHO) published Environmental Health Criteria (EHC) 238 after conducting a risk assessment of magnetic field (MF) exposures at frequencies of <100 kHz (WHO, 2007). The EHC 238 indicated that there was a lack of studies on primary hazard identification and quantitative risk evaluation of intermediate frequency (IF) MFs with frequencies from 300 Hz to 100 kHz (Litvak et al., 2002; WHO, 2007).

A popular source of IF MFs is video display terminals (VDTs) that emit weak MFs of several 10 μT (peak-to-peak: pp) at the most, and typically of approximately 20 kHz, in a complex sawtooth waveform. Although a few epidemiological studies have suggested an elevated risk of spontaneous abortion (Goldhaber et al., 1988; Lindbohm et al., 1992), a pooled analysis indicated that there were no increased risks for miscarriage, birth of children with malformations, or low-body-weight fetuses (Parazzini et al., 1993; WHO, 1998).

Increased resorption or fetal loss during early pregnancy was also reported in earlier animal studies on IF MF exposure. Frölen et al. (1993) found such changes in CBA/S mice exposed to a 20 kHz, 15 μT(pp) MF in a sawtooth wave form (VDT-like MF) on postconception days 1–19. Resorption did not occur in pregnant mice exposed only to MFs after postconception day 7, suggesting that preimplantation exposure was associated with these resorptions. However, an additional study by the same research group (Svedenstål and Johanson, 1995) failed to replicate the increased fetal losses, but reported a significant increase in the percentage of dead fetuses and decreases in the body weight and length of living fetuses exposed to MF during the preimplantation period (days 1.0–5.5 postconception). For the most part, subsequent rodent studies have shown no reproductive or developmental effects as a result of exposure to weak intensity VDT-MFs (Brent, 1999a,b; Huuskonen et al., 1998a; Robert, 1999).

Other than VDTs, instruments such as magnetic resonance imaging machines, induction heaters, and welding machines have been reported to be the sources of relatively high-intensity IF MF emissions (Decat et al., 2006; Floderus et al., 2002; Stuchly and Lecuyer, 1985). Other new sources of IF MFs include inductively coupled power transmission (IPT or wireless/contactless power transfer) for industrial material handling machines or home appliances, and commercial and domestic induction heating (IH) cookers or ovens (ICNIRP, 2008).

An IPT system commonly employs a 10–50 kHz MF with a sinusoidal waveform to transfer electric power efficiently through the

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large air gap between a pair of coils (Boys et al., 2000; Budhia et al., 2009). IH cooking hobs generate vertical, space-varying IF MFs at a fundamental frequency such as 20 or 60 kHz, and the associated harmonic frequencies are used together for cooking (Fujita et al., 2007; Yamazaki et al., 2004). These MFs are emitted from a hob and are converted to magnetically induced current in a pan that produces heat for cooking. Given that the location of the hob is close to the abdomen and reproductive organs of the cook, the potential for reproductive and developmental effects is a concern.

We have previously demonstrated that MFs of up to 1.1 mT(rms), 20 kHz were not associated with teratogenicity in a chick embryo model (Nishimura et al., 2009). In another previous study in rodents (Nishimura et al., 2011), we exposed pregnant rats to IF MFs at 20 kHz in 0.2 mT(rms) and at 60 kHz in 0.1 mT(rms) during organogenesis for teratological evaluation. The present study in rats was performed in conjunction with the rat teratology study to cover an exposure period from pre-mating to implantation.

The aim of this study was to determine whether exposure to IF MFs has any toxic effects on fertility and/or early embryonic development. In accordance with our rat teratology study, we chose the same exposure intensity, which was a feasible facility-maximum strength at the respective frequencies for hazard identification: 0.2 mT(rms) at 20 kHz or 0.1 mT(rms) at 60 kHz. The frequencies of IH cookers that are typically employed are 20 and 60 kHz; 60 kHz is the third harmonic of the 20 kHz frequency and has never been examined for reproductive and developmental toxicity during the preimplantation period. The sinusoidal waveform was chosen because of its clear definition and simple reproducibility. Experiments were performed in duplicate for each frequency to confirm the reproducibility of the outcomes. All examinations after necropsy were conducted in a blind manner by an independent, GLP-licensed laboratory to ensure quality assurance.

2. Materials and methods

2.1. Animal husbandry

The Institutional Animal Experiment Committee of the Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry, approved all of the animal experimental procedures of this study in accordance with the Committee's guidelines under the Japanese Law Concerning the Protection and Management of Animals. This study was comprised of 4 independent, but identical experiments, 2 with the 20 kHz MF exposure and 2 with the 60 kHz MF exposure. The same protocol was used in all experiments, with an exception regarding mating time in 1 experiment that is described in Section 2.4 (Mating procedures and the post-mating period).

A total of 53 male and 53 female 8-week-old SPF/VAF, Crl:CD(SD) rats were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). The rats were quarantined for 1 week in the barrier system of an experimental animal facility where the background MFs were extremely low (<0.001 μT(rms) at 20 and 60 kHz, and 0.03 μT(rms) at 50 Hz). Room air was HEPA-filtered, and room temperature and humidity were maintained at 23 ± 2 °C and 50 ± 20%, respectively. The light cycle was 12/12 h (0700–1900 on). The rats were housed in polycarbonate cages (23 cm W × 33 cm D × 17 cm H; Tokiwa Kagaku Kikai Co., Ltd., Tokyo, Japan) and fed with pellets (NMF, 15 kGy irradiated, Oriental Bioservice, Kyoto, Japan) and water (distilled, autoclaved, 10 ppm chlorine, adjusted to pH 3 by 1 N HCl) *ad libitum*. Commercial wood chips (White flake, 30 kGy irradiated, Charles River Laboratories Japan Inc.) were used.

On the last day of the quarantine period, the male and female rats were weighed and randomly assigned to either the MF-exposed ($n = 24$) or sham-exposed ($n = 24$) groups. They were moved to dedicated MF exposure facilities on the morning of the first day of exposure.

2.2. Magnetic field exposure facilities

The MF exposure facility was explicitly described in a previous paper that also included a photograph (Nishimura et al., 2011). A pair of exposure facilities, one for 20 kHz and another for 60 kHz, were built in an area where the background 50 Hz MF was extremely low at <0.02 μT(rms). Both facilities were identical with respect to appearance, layout, structure, and other room conditions, and were located 37.4 m apart to minimize stray IF MFs during the exposures. When the exposure coils were activated to produce the facility-maximum MFs, the animals in the sham-exposed facility received less than 0.001 μT(rms) MF at 20 or 60 kHz and 0.03 μT(rms) at 50 Hz. The geomagnetic field at the location was about 48.3 μT.

Merritt-type (Merritt et al., 1983), 4 square coils were layered horizontally to produce extremely uniform, vertical MFs in a large cubic space (100 × 100 × 100 cm) located within the coils. In the designated exposure space, 3 wooden racks were equipped to accommodate up to 36 rat cages: 12 on each rack in a 3-row by 4-column layout. The electric currents used to produce the MFs were maintained within a range of the target values ±0.2% during exposure. MFs were calibrated before and after experiments based on 51 spot measurements (17 spots × 3 racks) within the exposure space. The MF variability within the exposure spaces was less than 3% in both coil systems (Yamazaki et al., 2007). Harmonic components were negligible, at 1/100–1/1000 of each primary frequency.

The level of ultrasound noise that may have been created by the vibration of the coils during MF exposure was measured up to 100 kHz with a microphone (UC-29), amplifiers (NH-05A, NA-42, Rion Co., Ltd, Tokyo, Japan), and a handmade brass shielding box with a clay cap. The signals obtained were analyzed with an FFT analyzer (R9211A, Advantest Co., Tokyo, Japan). Given that both the 20 and 60 kHz MF exposures strongly interfered with the measurement instruments, directly measuring the noise levels at these primary exposure frequencies was not feasible. Although the possible noise frequencies were theoretically secondary and tertiary harmonic frequencies, the FFT analysis revealed no specific peaks of noise at these frequencies. The recorded background noise level was very low: 16–29 dB, at 40 kHz under 20 kHz, 0.2 mT(rms) MF exposure in the animal exposure space.

In addition, the optical wave microphone method (Sonoda, 1996) was applied to measure sound levels during the 20 and 60 kHz MF exposures using a laser beam and photodiode instead of an electric microphone. A laser (MINI-635-30C40WL, Blue Sky Research, Milpitas, CA, USA), a photodiode (S5821), an amplifier (Type 6030, Aco Co., Ltd, Tokyo, Japan), and an FFT analyzer (CF-7200, Ono Sokki Co., Ltd, Kanagawa, Japan) were used. The sound levels for both MF exposures were found to be less than 60 dB up to 100 kHz, which was lower than the background noise levels due to air conditioning.

Coil vibrations were measured using a laser Doppler-vibration sensor (PDV 100, Polytech Japan, Kanagawa, Japan). The background vibrating velocity was 5 μm/s, and the shifts (0.0398–0.119 nm) and acceleration (0.628–1.884 m/s^2) as a result of MF exposures were negligible. Field-associated heat from the coil bobbins was not appreciable. The room temperature was continuously measured (QFA-2060, Siemens AG, Berlin and Munich, Germany) and did not change during the MF exposures.

2.3. Exposure and the pre-mating period

In the exposure facilities, female rats were kept in groups of 2 per cage, whereas male rats were housed individually during the