

## Principal Research Results

# Evaluation of Biological Effects of Intermediate Frequency Magnetic Fields – Micronucleus formation and chick embryo development –

## Background

The utilization of intermediate frequency (IF; 300Hz to 10MHz) electromagnetic fields (EMFs) is widely spreading as novel electric devices in both domestic and occupational fields. In contrast to extremely low frequency and radio frequency EMFs, the biological effects of IF EMFs have not been studied very well. Especially, genotoxic/carcinogenic and reproductive/developmental effects are the subject of most concern by the device users. These toxicological issues should be clarified by reliable toxicological tests.

## Objectives

The aims of this research are to evaluate the effect of the IF magnetic fields (MFs) on genotoxicity by micronucleus assay (Fig.1) and on the chick embryonic development.

## Principal Results

### 1. Evaluation of genotoxicity

The Chinese hamster V79 cell was chosen to estimate the effects of the IF MFs exposure (0.91mTrms (146 times greater than ICNIRP guideline \*1) at 2kHz, 1.1mTrms (178 times) at 20kHz or 0.11mTrms (18times) at 60kHz) on micronucleus formation. The V79 cell was exposed to MFs at 37°C in 5% carbon dioxide. Rates of micronucleus formation were determined as the proportion of binucleus cells with micronucleus to the total number of binucleus cells. In statistical analysis (n=3, 5 replicates, 3 exposure condition plus control), neither significant nor reproducible difference was found in the micronucleus formation rates between MF exposed and each IF MF exposure condition (Table 1).

### 2. Evaluation of co-genotoxicity

The effects of the IF MFs on DNA damage repair caused by mitomycin C (MMC), which can induce micronucleus formation, were also estimated. The V79 cells were exposed to MMC and each IF MFs simultaneously for 24h. In statistical analysis (n=3, 5 replicates, 3 exposure condition plus control), two cases of 2kHz MF exposure with MMC showed significant differences, however the differences were very small and in opposite directions to each other. Neither significant nor reproducible difference was found between the rates for all MF exposure conditions. These results indicated that the strong IF MFs used in this study did not induce micronucleus formation and did not affect DNA damage by MMC or DNA damage repair system in mammalian cells (Table 1).

### 3. Developmental toxicity evaluation with chick embryos at organ growth period

Chick embryos were exposed to 20 kHz sinusoidal, vertical MF at 1.1 mT(rms) from the first to the 11th day of embryogenesis continuously. Embryos pretreated with a chemical teratogen were also exposed to the same MF to examine if the exposure could modify the teratogen induced embryotoxicity. Incidence of embryotoxicity including embryonic death and morphological abnormalities was examined under a stereoscopic microscope. Statistical evaluation revealed that there have been no adverse effects of the MF exposure on all the developmental endpoints. Results indicate that, under the present experimental conditions, the MF exposures did not produce any significant adverse effects on the chick embryo development. Lack of progressive effects by the field on the teratogenicity induced by a known teratogen was also demonstrated (Fig.2, Fig.3).

## Future Developments

Other genotoxicity evaluation by mouse lymphoma assay and reproductive/developmental toxicity of rodents will be carried out for the accumulation of further scientific knowledge.

### Main Researchers:

Satoshi Nakasono, Ph.D.

Senior Research Scientist, EMF Environment Sector, Environmental Science Research Laboratory

Izumi Nishimura, Ph.D.,

Senior Research Scientist, EMF Environment Sector, Environmental Science Research Laboratory

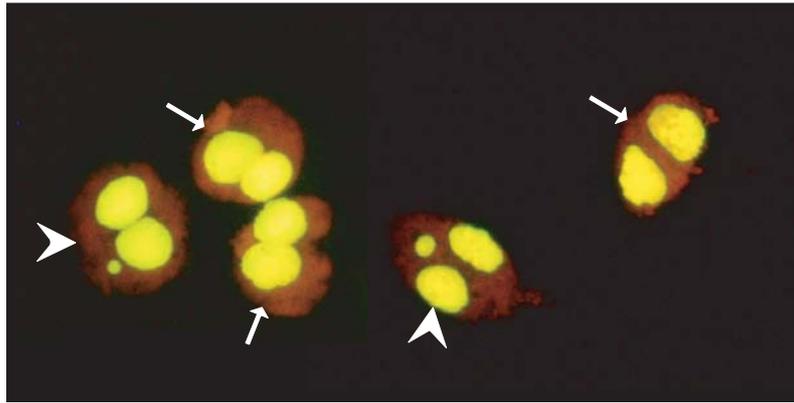
### References

I. S.Nakasono *et.al.*, 2007, "Effect of Intermediate Frequency Magnetic Fields on Micronucleus Formation in Mammalian Cell Line," CRIEPI Report V06007 (in Japanese)

I. Nishimura and T. Negishi, 2007, "Developmental toxicity evaluation of intermediate frequency magnetic field exposure in chick embryos - Effects of 20 kHz, sinusoidal field exposure in the early 11 days of embryonic development,-" CRIEPI Report V06006 (in Japanese)

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\* 1 : The reference level is 00625mTrms for MFs exposure to public by the International Commission on Non-Ionizing Radiation Protection (ICNIRP).



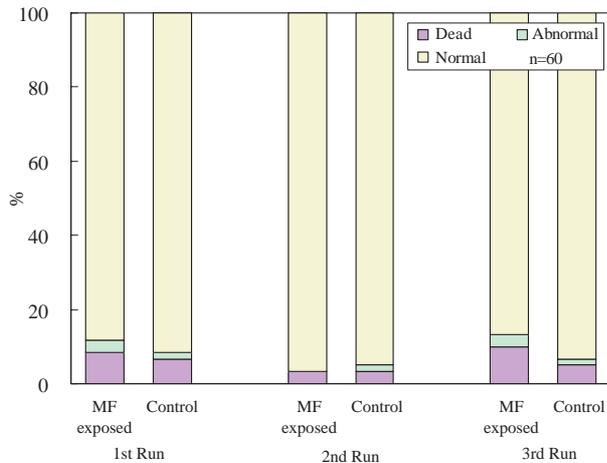
**Fig.1** Fluorescent microscopic photograph:  
Normal binucleus cells and binucleus cells with micronucleus

- Red fluorescence shows cytoplasm and yellow-green fluorescence shows nucleus.
- Division of cytoplasm was inhibited by a chemical. And normal cells can be shown as binucleus cells (→). The binucleus cells with micronucleus (▶) were found as genotoxic influenced cells.

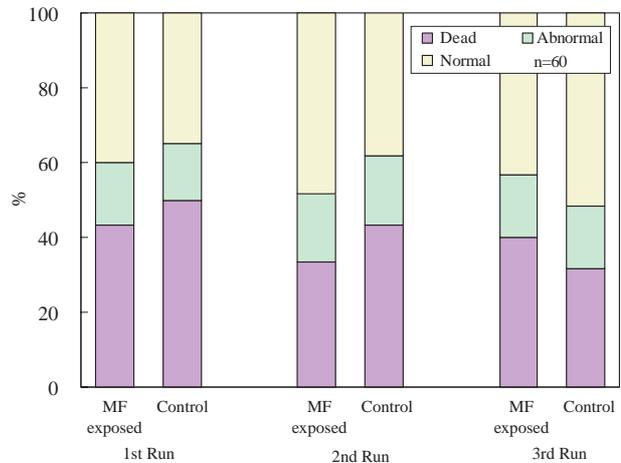
**Table 1** Results of micronucleus tests

Conditions	Exposure Conditions			
	Control	2kHz 0.91mTrms	20kHz 1.1mTrms	60kHz 0.11mTrms
no mutagen	0/7	0/5	0/5	0/5
with mutagen (MMC)	0/7	2/5 (↑ ↓)	1/5 (↓)	0/5

- Denominator shows the number of tests, and numerator shows the number of statistically significant results ( $P < 0.05$ ) by Student's t-tests. The arrow shows the direction of change (↑ :UP, ↓ :DOWN). Some significant results were obtained, however since the trend of differences was inconsistent and lacked reproducibility, no firm evidence regarding the impact of MF exposure was secured.



**Fig.2** Chick embryotoxicity in MF-exposure experiments



**Fig.3** Chick embryotoxicity in teratogen+MF-exposure experiments

Results from MF exposure experiments in triplicate revealed that the 20 kHz, sinusoidal MF at 1.1 mT(rms) did not induce embryotoxicity in the intact chick and did not modify teratogen-induced embryotoxicity.