Intermediate Frequency Magnetic Fields Did Not Have Genotoxic and Promotion Potentials in *in vitro* Tests

Satoshi Naksono[1], Masateru Ikehata[2], Masayuki Takahashi[1], Sachiko Yoshie[2], Tadashi Negishi[1]

[1]Central Research Institute of Electric Power Industry, 1646 Abiko, Abiko-city, Chiba 270-1194, Japan
e-mail:nakasono@criepi.denken.or.jp

[2]Railway Technical Research Institute, 2-8-38 Hikari, Kokubunji-city, Tokyo 185-8540, Japan
e-mail:ikehata@rtri.or.jp

Abstract

In recent years, the use of new electrical appliances, that use or emit intermediate frequency (IF; 300 Hz to 10 MHz) electromagnetic fields (EMFs), has been increasing. We have investigated genotoxic and promotion potentials of a vertical and sinusoidal IF MF of 0.91mT (rms) at 2 kHz, 1.1mT (rms) at 20 kHz and 0.11mT (rms) at 60 kHz. We used microbial mutagenicity tests, gene conversion tests, micronucleus tests or mouse lymphoma assay for genotoxicity, and Bhas42 promotion tests for promotion. The results indicate that the IF MFs did not have genotoxic nor promotion potentials in the experimental conditions.

1. Introduction

In contrast to extremely low frequency (ELF, related to power frequency; 50/60 Hz) and radio frequency (RF, related to mobile telephony; 0.8–2.45 GHz) electromagnetic fields (EMFs), the biological effects of intermediate frequency (IF; 300 Hz to 10 MHz) EMFs have not been studied extensively [1,2]. In recent years, the use of new electrical appliances, such as induction cookers, proximity readers and electrical article surveillance that use or emit IF EMFs (which also include both magnetic fields and electric fields), has been increasing. In Japan, the widespread use of household induction cookers has raised public concern regarding the health effects of IF MFs. For heating, the induction cookers generate approximately 20–90 kHz of MFs and the associated harmonic frequencies MFs. In addition, many industrial inverters have a switching frequency of several to tens of kHz. For example, an inverter with a basic frequency of approximately 2 kHz controls the main motors of the Shinkansen superexpress train in Japan [3]. According to the ICNIRP guidelines, the stray MFs from these devices are sufficiently low. However, there is lack of data to evaluate the biological effects of IF MFs.

The WHO Environmental Health Criteria (EHC) 238 monograph published at 2007 is for risk assessment of exposure to extremely low frequency electric and magnetic fields below 100 kHz [2]. However, the EHC monograph stated that the majority of studies have been conducted on power-frequency magnetic fields, which are 50Hz and 60Hz. Additionally, in "Recommendations for research", the EHC has stated "further research on intermediate frequencies (IF), usually taken as frequencies between 300 Hz and 100 kHz, is required." and "General requirements for constituting a sufficient IF database for health risk assessment include exposure assessment, epidemiological and human laboratory studies, and animal and cellular studies."

At present, there is no established mechanistic evidence linking MFs to carcinogenicity, however, the public considers these issues as having an immediate potential health impact. In this paper, we have examined the genotoxicity and promotion potentials of IF MFs (2 kHz, 20 kHz and 60 kHz) using bacterial cells for point mutations, using budding yeast cells for point mutations and gene conversions, using V79 cells for micronucleus formations, using L5178Y *tk*+/- 3.7.2c cells for point mutations and chromosomal aberrations, and using Bhas 42 cells for promotion potentials

2. Materials and Methods

2. 1 Exposure system

We constructed a Helmholtz type exposure system [4], which generates a vertical and sinusoidal IF MF in the frequency range of 2–60 kHz (Fig. 1). The exposure system was designed to generate 0.91mT rms at 2 kHz (34 times

978-1-4244-6051-9/11/$26.00 ©2011 IEEE

greater than the strength proposed in the ICNIRP guideline), 1.1mT rms at 20 kHz (41 times) and 0.11mT rms at 60 kHz (4 times), respectively. This system provided a large uniform IF MF environment (20 cm^3, field variation below 2.5%). The effects of vibration and sound in the incubation space, or generated from coils and electrical power supplies, were minimized using a water-jacketed incubator. The incubator was located in the center of the system. The incubation temperature was controlled at 37±0.4 ˚C for bacteria and mammal cells, and 30±0.4 ˚C for yeast, respectively.

2. 2 Mutagenicity Tests

Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and two strains of *Escherichia coli* (WP2 *uvrA*, WP2 *uvrA*/pKM101) were chosen to examine a wide spectrum of point mutation, including AT base substitution, GC base substitution, suppressor mutation, and frame shift. Twelve plates were randomly divided into two groups; six plates were placed at the center of the MF exposure system while the other six were placed in a control incubator. Revertant colonies were scored after 48 hr of incubation at 37 ˚C [5].

2. 3 Gene Conversion Tests

The yeast strain, *Saccharomyces cerevisiae* XD83 (*MATa/ MATα, his4-519/his4-519, leu2-3/leu2-3, lys1-1/lys1-1, +/pet1, arg4-4/+, +/arg4-17, ade2-18/+*), was obtained from American Type Culture Collection (Manassas, VA, USA). The cells inoculated into plates and exposed to MFs for 48h at 30 ˚C. The rate of gene conversion was estimated by counting revertant colonies [6].

2. 4 Micronucleus Tests (MN)

The Chinese hamster V79 cells were used. The cells inoculated into T-175 flasks and exposed to MFs for 24h at 37 ˚C in 5% carbon dioxide. After the MF exposure for 24h, the cells were collected and inoculated into a chamber slide (Lab-TekII, 4well, Nalge-Nunc). Rates of micronucleus formation were determined as the proportion of binucleus cells with micronucleus to the total number of binucleus cells [7, 8].

2. 5 Mouse Lymphoma Assay (MLA)

The MLA can detect both point mutation (as large colony) and chromosomal aberration such as deletion (as small colony). A mouse lymphoma cell line, L5178Y *tk*+/- 3.7.2c, was used. The cells were exposed to the MFs for 24h at 37 ˚C in 5% carbon dioxide. After the exposure, the cells were inoculated into 96 well plate. After the plates were incubated for 10-14 days, the colonies were counted to evaluate the ratio of mutation frequency of point mutation, chromosomal aberration or total mutation between MF exposed and unexposed control groups [9, 10].

2. 6 Promotion Tests

The Bhas 42 cells, which derived from BALB/c 3T3 and transfected v-Ha *ras* gene, were used. The v-Ha *ras* gene is well known as an oncogene, and the cell could be used as initialized cell in 2-step transformation assay. The cells exposed to the IF-MFs for 10days at 37 ˚C in 5% carbon dioxide from day 4 to day 10. After the MF exposure, the cells were incubated for 7 days to make a focus by transformed cells. The number of focus was counted after 21 days total incubation [11, 12].

3. Results and Discussion

3.1 Genotoxicity

To examine the genotoxicity of the IF MFs (2 kHz, 20 kHz and 60 kHz), we used bacterial cells to assay for point mutations, budding yeast cells to assay for point mutations and gene conversions, V79 cells to assay for micronucleus formations, and L5178Y *tk*+/- 3.7.2c cells to assay for point mutations and chromosomal aberrations. In statistical analysis for all genotoxicity tests, neither significant nor reproducible difference was found between exposed and control groups (Table 1), even if each test replicated five times.

3.2 Promotion Potential

To examine the promotion potentials of IF MFs (2 kHz, 20 kHz and 60 kHz), we used Bhas 42 cells to assay for promotion potentials. In statistical analysis for promotion tests, neither significant nor reproducible difference was found between exposed and control groups (Table 1) , even if each test replicated five times.

Table 1. Effect of IF- MF on genotoxicity and promotion potentials in *in vitro* tests

Tests	Test Strain	Endpoints	Exposure Conditions		
			2kHz 0.91mT (5 times)	20kHz 1.1mT (5 times)	60kHz 0.11mT (5 times)
Bacterial Mutation Tests	*S.typhimurium*				
	TA98	Frameshift	0	0	1(↑)
	TA100	Base substitution (GC)	0	1(↑)	0
	TA1535	Base substitution (GC)	0	0	0
	TA1537	Frameshift	1(↑)	1(↑)	0
	E.coli				
	WP2 *uvrA*	Base substitution (AT)	1(↑)	0	0
	WP2 *uvrA* /pKM	Base substitution (AT)	0	0	0
Yeast Gene Conversion Tests	*S. cereviciae*				
	XD83	Gene Conversion	0	0	0
		Nucleotide Substitution	0	0	0
Micronucleus Formation Tests	derived from Chinese hamster				
	V79	MN formation	0	0	0
Mouse Lymphoma Assay	Mouse lymphoma cell line				
	L5178Y tk+/- 3.7.2c	S-MF	0	0	0
		L-MF	1(↑)	0	0
		T-MF	0	0	1(↓)
		%SC	0	0	0
Promotion Tests	derived from BALB/c 3T3				
	Bhas 42	Promotion	0	0	0

The numbers show the number of statistically significant results, and the arrows show the trend of the change. S-MF: mutation frequency for small colony, L-MF: mutation frequency for large colony, T-MF: total mutation frequency (S-MF+L-MF), %SC = (S-MF/T-MF) x100

These results indicate that the strong IF MFs used in this study did not have genotoxic potentials, which are related with the mechanisms of point mutation, gene conversion, micronucleus formation and chromosomal aberrations

in both prokaryotic and eukaryotic cells by using *in vitro* genotoxicity tests. The results also indicate that the strong IF MFs did not have promotion potentials by a sensitive *in vitro* promotion tests.

4 Conclusions

We have investigated the effect of the IF MHs of 0.91mT rms at 2 kHz (34 times greater than the strength proposed in the ICNIRP guideline), 1.1mT rms at 20 kHz (41 times) and 0.11mT rms at 60 kHz (4 times) on genotoxicity and promotion potential. These strong IF MFs did not have both genotoxicity and promotion potentials which relate with the mechanism of carcinogenesis.

5 References

1. Litvak, E., K.R. Foster and M.H. Repacholi,"Health and safety implications of exposure to electromagnetic fields in the frequency range 300 Hz to 10 MHz", Bioelectromagnetics 23 (2002) 68–82.

2. WHO - World Health Organization, "Extremely low frequency fields", Environmental Health Criteria, Vol. 238, WHO Press, Geneva, Switzerland (2007)

3. Watanabe, T., "Recent trend of inverter and its control method for electric car", QR RTRI 41 (2000) 5–10.

4. Nakasono, S., M. Ikehata, M. Dateki, S. Yoshie, T. Shigemitsu and T. Negishi, "Intermediate frequency magnetic fields do not have mutagenic, co-mutagenic or gene conversion potentials in microbial genotoxicity tests", Mutation Research, 649 (2008) 187–200.

5. Ames, B.N., J. McCann and E. Yamazaki, "Methods for detecting carcinogens and mutagens with the *Salmonella*/ mammalian microsome mutagenicity test", Mutat. Res. 31 (1975) 347–363.

6. Dixon, M.L., and R.K. "Mortimer, A yeast screening system for simultaneously monitoring multiple genetic endpoints", Mutat. Res. 161 (1986) 49–64.

7. Kirsch-Volders, M., T.Sofuni, M. Aardema, SAlbertini, D. Eastmond, M. Fenech, , M. Ishidate, E. Lorge, H. Norppa, J. Surralles, W. von der Hude, and A. Wakata. "Report from the *In Vitro* Micronucleus Assay", Working Group. Environ. Mol. Mutagen., 35 (2000) 167-172.

8. Hayashi, M., T. Sofuni and M. Ishidate, "An application of acridine orange fluorescent staining to the micronucleus test", Mutation Res., 120 (1983) 241-247.

9. Clive, D., and J. Spector, "Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells", Mutation Research 31 (1975) 17-29.

10. Oberly, T., D. Yount and M. Garriott, "A comparison of the soft agar and microtitre methodologies for the L5178Y TK+/- mouse lymphoma assay", Mutation Research 388 (1997) 59-66.

11. Sasaki, K., H. Mizusawa and M. Ishidate, "Isolation and characterization of ras-transfected BALB/3T3 clone showing morphological transformation by 12-O-tetradecanoyl-phorbol-13-acetate", Japanese Journal of Cancer Research, 79 (1988) 921-930.

12. Sasaki, K., H. Mizusawa, M. Ishidate and N. Tanaka, "Transformation of ras transfected BALB 3T3 clone (Bhas 42) by promoters: Application for screening and specificity of promoters", Toxicology in Vitro, 4 (1990) 657-659.