

# EVALUATION OF POSSIBLE BIOLOGICAL EFFECTS OF COMPLEX MAGNETIC FIELDS WITH STATIC AND TIME-VARYING COMPONENTS.

**Masateru Ikehata<sup>(1)</sup>, Tetsuya Nagai<sup>(2)</sup>, Yukihiisa Suzuki<sup>(3)</sup>, Masakazu Iwasaka<sup>(4)</sup>,**

**Masao Taki<sup>(5)</sup> and Takao Koana<sup>(6)</sup>.**

*(1) Railway Technical Research Institute, Kokubunji, Tokyo 185-8540, Japan, ikehata@rtri.or.jp*

*(2) Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan, tetsuya@com.eei.metro-u.ac.jp*

*(3) As (2) above, suzuki@eei.metro-u.ac.jp*

*(4) Chiba University, Chiba, Chiba 263-8522, Japan, iwasaka@faculty.chiba-u.jp*

*(5) As (2) above, taki@eei.metro-u.ac.jp*

*(6) Central Research Institute of Electric Power Industry, Komae, Tokyo 201-8511, Japan, koana@criepi.denken.or.jp*

## INTRODUCTION

In our environment, various types (e.g. frequency, field strength) of electric and magnetic fields (EMFs) exist and their distributions are complex in such as electrified train systems. However, there are few reports on biological effects of such complex EMFs exposure. At this point, our project aims to construct a strategy for the safety evaluation by exposure to complex EMFs. For plausible safety evaluation of complex EMFs, this study includes;

1. Clarification of MFs that are generated in our environment and estimation of the induced current using the numerical model of human whole body.
2. Development of exposure devices that can generate complex EMFs.
3. Estimation of the biological effects by exposure to complex EMFs.
4. Estimation of the indirect effects of EMFs on biological study. In this report, we focused on the mutagenic effects of complex EMFs and gene expression profile under complex EMFs as the first step of estimation on biological effects.

In this study, we focused on the mutagenic effects as the first step of estimation of biological effects by exposure to environmental complex EMFs. Mutagenicity of complex magnetic fields (MFs), especially with static and power frequency components was examined by two independent mutation assay systems. In addition, gene expression profiles in yeast cells were analyzed using cDNA microarray and real time PCR technique for exploring the possible effects of complex MFs.

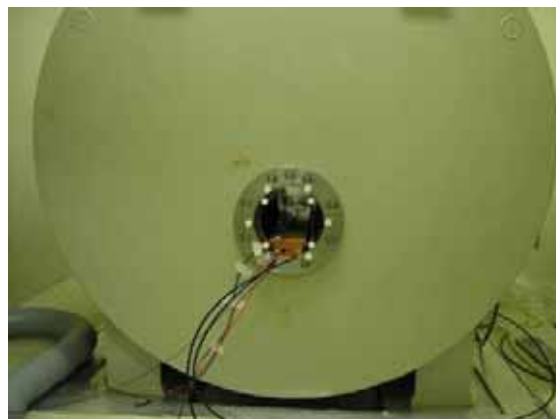
## MATERIALS AND METHODS

### Development of a exposure device that can generate complex MFs

For complex MFs exposure, a Helmholtz coil was made and located at the center of superconducting magnet (JS-500, Toshiba, Japan). The Helmholtz coil is able to generate 50Hz, 1mT time-varying MF under 5T static magnetic field. This exposure system was located in a constant temperature room and maintained exposure space at  $37 \pm 1$  or  $30 \pm 0.5$  °C. Figure 1 shows photo of the exposure device. Induced current in the test plate which contains agar and nutrient compounds for complex exposure condition was estimated by impedance method. In addition, the



Helmholtz' coil for complex exposure of DC and ELF magnetic fields



Settings for complex exposure experiments

Fig. 1 Exposure device for complex EMFs

This device can generate complex EMFs with 5T static magnetic field and 1mT, 50Hz sinusoidal magnetic field. Exposure space is 160mm × 100mm.

degree of displacement of surface agar by Lorentz force was estimated by numerical analysis.

#### Mutagenicity assay

To investigate the mutagenicity, we employed two mutation assays. One is the bacterial mutation assay (Ames test). In this assay, *Salmonella typhimurium* TA98, TA100, TA102 and *Escherichia coli* WP2 *uvrA* was used. These bacterial cells were exposed to complex MFs for 48 hours after plating onto glucose minimal media with trace of required amino acid (histidine and biotin for *S. typhimurium* strains and tryptophan for *E. coli* strain) at  $37 \pm 0.5$  °C. Control cells were incubated in conventional incubator. After 48 hours of incubation, number of prototroph mutant colony appearing on each plate was scored. The other mutation assay is yeast mutation assay using *Saccharomyces cerevisiae* XD83. For detecting point mutation frequency on *lys 1-1*, harvested cells were mixed with molten soft agar (0.6 % Bacto-agar, 0.5 % NaCl) and were poured onto low lysine synthetic complete plate. For detecting gene conversion frequency on *ARG4* allele (between *arg 4-4* and *arg 4-17*), cell suspension was poured on to low arginine synthetic complete plate. At least 6 plates were made for both condition and randomly divided into two groups. One group was exposed to complex MFs for 5 days at  $30 \pm 0.5$  °C. The other group was incubated as control. Number of colonies on each plate was scored as reverse mutant and the mutation frequency was calculated. Induced current in the test plate which contains agar and nutrient compounds for complex exposure condition was estimated by numerical analysis using impedance method.

#### Gene expression profiling

DNA microarray technique was used to determine the cellular response. Yeast Chip which includes 5,876 cDNA out of 6,400 ORFs of *S. cerevisiae* (DNA chip research Inc., Japan,) was used. *S. cerevisiae* 4388 (wildtype) was used in this experiment. Cells were incubated overnight in YPD medium and 1/1000 volume of the cell suspension was re-suspended to 25ml fresh YPD medium in 25ml of media bottle. The culture bottle capped tightly and incubated with/without complex MFs. RNA isolation, cDNA preparation and hybridization were performed by the protocols that provided by the manufacturer with slight modification. The data obtained from each spot were normalized and ratios of gene expression in exposed cells compared to that of control cells were calculated. Clustering analysis was performed with selected genes related to several cellular processes (TCA cycle, DNA damage response, oxidative stress response, protein synthesis and other stress response which was annotated by the *Saccharomyces* genome database (<http://genome-www.stanford.edu/Saccharomyces/>)) using Cluster and TreeView software (Stanford Univ., USA). At least two independent experiments were performed under every experimental condition.

Real-time RT-PCR was used to investigate the expression of *PCK1* and *IDP2* genes that was induced by exposure to strong static magnetic field [3]. Primers and probe for *PCK1*, *IDP2* and *ACT1* were designed using the Primer Express<sup>TM</sup> software (Applied Biosystems). *ACT1* and 18S ribosomal RNA were used as internal control genes. RT-PCR was performed using TaqMan<sup>®</sup> One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems) according to the manufacturer's instructions. PCR products were continuously measured with an ABI PRISM<sup>®</sup> 7000 Sequence Detection System (Applied Biosystems). The relative amount of *PCK1* and *IDP2* transcript were normalized to the amount of *ACT1* or 18S rRNA transcript using the  $2^{-\Delta\Delta CT}$  method.

## RESULTS AND DISCUSSION

### Mutagenicity of complex MFs

In bacterial mutation assay, there is no difference of the number of prototroph reverse mutant between control and complex MFs exposed group in all of the tester strains (Fig. 2). This result shows that complex MFs (static, 5T and 50Hz, 1mT) do not induce point mutation in bacterial tester strains. On the other hand, it was observed that gene conversion/recombination frequency on *ARG4* allele in *S. cerevisiae* XD83 increased slightly by exposure to complex MFs while point mutation frequency was not affected on the *LYS1* allele (Fig. 3). This result shows that complex MFs slightly induce recombination between homologous gene locus rather than induction of point mutation.

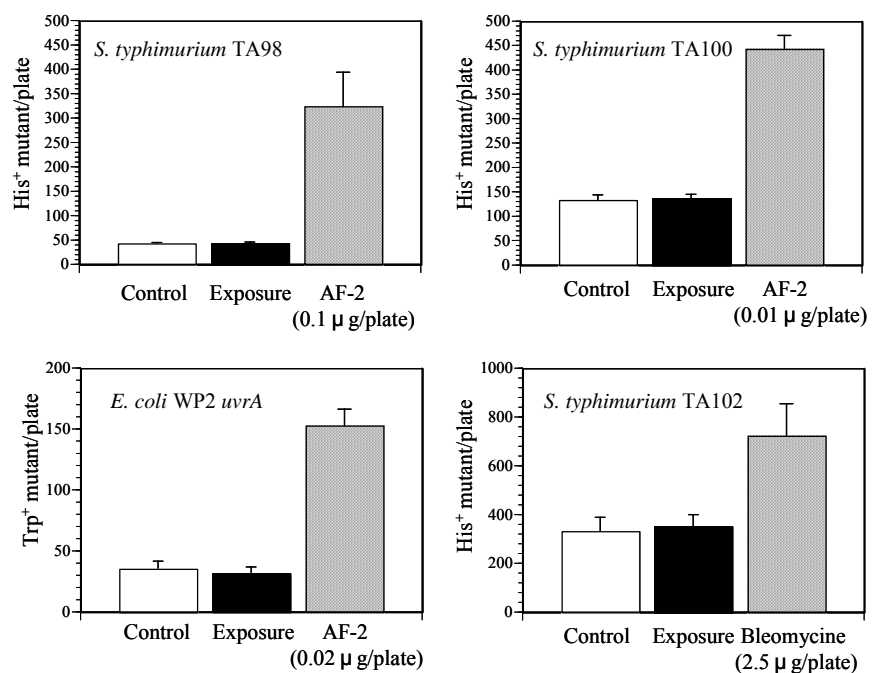


Fig. 2 Results of bacterial mutation assay

Data are given as mean with standard deviation from at least four independent tests with quadruplicate plates.

### Effect on gene expression

In analysis using cDNA microarray, we observed no significant change of gene expression profile in exposed cells compared with control. This result shows cells did not have any response to exposure to complex MFs.

To confirm the result above, expression of *PCK1* and *IDP2* was determined by real-time RT-PCR. Fig. 4 shows the result in complex MFs exposure with our previous data (static magnetic field of 5, 8, 14T). This result shows complex MFs did not change the expression profile of both genes. Our previous result shows that threshold of induction of *PCK1* will exist between 5 and 8T. These results suggest simultaneous exposure of 50Hz, 1mT MF did not modulate the effect of static 5T MF on *PCK1* gene expression.

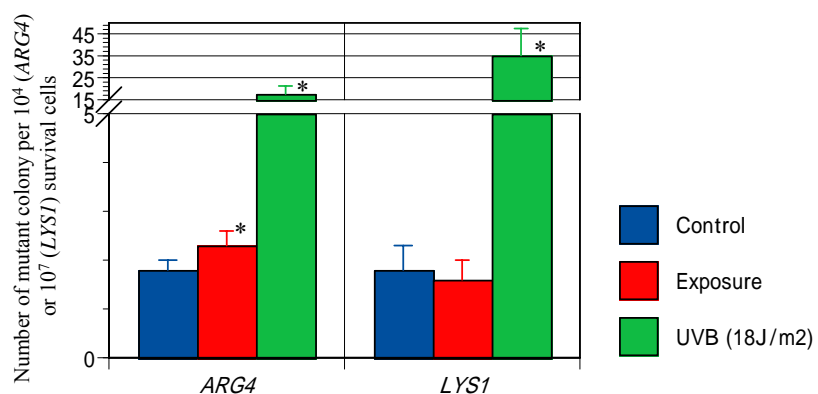


Fig. 3 Results of yeast mutation assay

Data are given as mean with standard deviation from at least seven independent tests with quadruplicate plates.

\*  $p < .05$ , compared with control

### Discussion

In this study, we observed that complex MFs exposure caused slight induction of recombination in *S. cerevisiae* cells but did not induce point mutation in *S. cerevisiae* and four tester strains of *S. typhimurium* and *E. coli*. On the other hand, gene expression profiles in *S. cerevisiae* cells were not changed by exposure to complex MFs. These results are consistent with our previous study for strong static MFs [1,2], thus, we infer that the mutagenicity observed in this study is mainly caused by exposure to strong static magnetic field. However, it is interesting that the extent of mutagenicity is slight weaker than that by exposure to a 5T static MF alone. On the other hand, we already have observed no effects on genome wide gene expression profile in yeast cells after exposure to 5T static MF for 24h [3].

In this study, although 50Hz, 1mT MF simultaneously exposed with static 5T MF, there is no significant effects on gene expression profile. Thus, there is no synergistic effect with static MF and 50Hz MF on gene expression profile in yeast. Depend on our previous data, we infer very low level of oxidative stress that is generated by exposure to strong static magnetic fields causes an increase of mutation frequency especially recombination, however this does not induce any response in cells including any gene expression. Results in this study suggest that there is little synergistic effect to combine static and 50Hz MFs in mutation frequency and also gene expression profile.

In future studies, different combination of field strength and frequencies including intermediate frequency of complex MFs will be investigated for estimation the effects of complex MFs in our environment. In addition, calculation of induced current using the human whole body model in the complex MFs is underway. This calculation will lead to establishing the consistency between experimental exposure condition and environmental exposure. It is necessary to understand environmental circumstances and its risk assessment.

#### ACKNOWLEDGMENT

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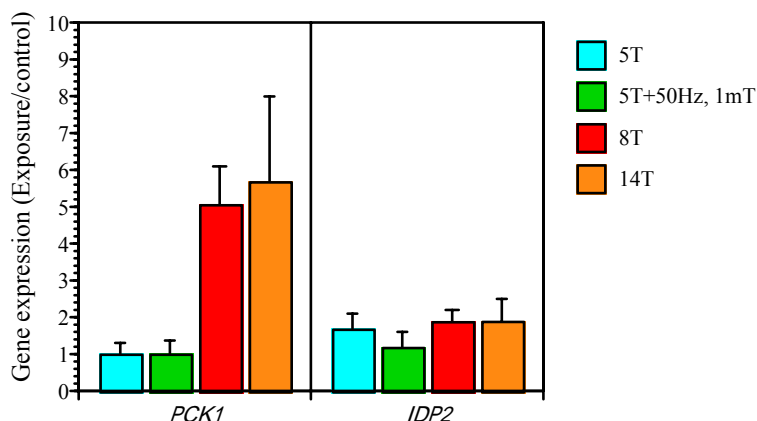


Fig. 4 Results of real-time RT-PCR