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Effect of Power Frequency Magnetic Field on Chronic and Genetic Toxicities of Fruit Flies

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***Abstract*-- As a basic step in the study of the biological effect of power frequency magnetic field on creatures, some experiments were carried out to examine the chronic and genetic toxicities of fruit flies. Statistical analysis of the results showed that there is no obvious evidence that the exposure of fruit flies to power frequency magnetic field affected their longevity and mutation.**

***Keywords*—Power frequency magnetic field, Chronic and genetic toxicity, Fruit fly, Generalized Wilcoxon test, Goodness-of-fit test.**

I. Introduction

WITH the increase in transmission line current, number of electrical household appliances, etc., the possible biological effect of magnetic field has become one of environmental issues in society. In discussing the biological effect, medical terms of acute, chronic and genetic toxicities are used. They can be generally explained as follows in brief: (1) acute toxicity: short-term effect of something on creatures, to show a particular behavior, being attracted to it, escaping from it, etc. (2) chronic toxicity: long-term effect of something on creatures, shortening or prolonging longevity, etc. (3)genetic toxicity: genetic effect of something on creatures over different generations.

In this paper, the experimental results of effect of power frequency magnetic field on chronic and genetic toxicities of fruit flies are reported and several statistical analyses conducted on the results are discussed.

Note that magnetic field and magnetic flux density are treated as the same meaning here.

II. Selection of Specimens

The specimen selected was fruit flies, *Drosophila melanogaster*. The reasons for this selection are (1) the longevity of fruit flies is considerably short, (2) the number of specimens per single trial can be large, (3) fertility is strong and (4) it is easy to breed them. The development stages of the flies are shown in Fig.1. Emergence is completed within about ten days and the typical longevity under appropriate conditions is about 40 days. The fruit flies are those of highly purebred strains [1] and are well controlled from the egg stage.

Since the longevity of the flies is very much affected by the ambient temperature[2], an environmental chamber with an air conditioner was prepared as shown in Figs. 2 and 3. This chamber had an internal dimension of approximately 610 x 1500 x 860 mm. Typically, the air conditioner was used to keep the temperature inside the chamber between 25.8°C and 26.7°C. Six thermo-sensors arranged inside the chamber proved that the temperature distribution inside was quite uniform.

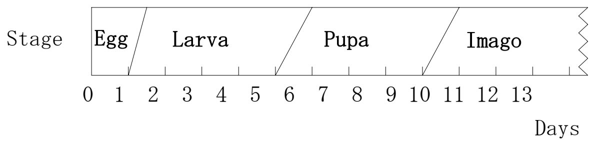

Fig. 1. Stage of development of fruit flies.

Fig. 2. Overview photograph of environmental chamber.

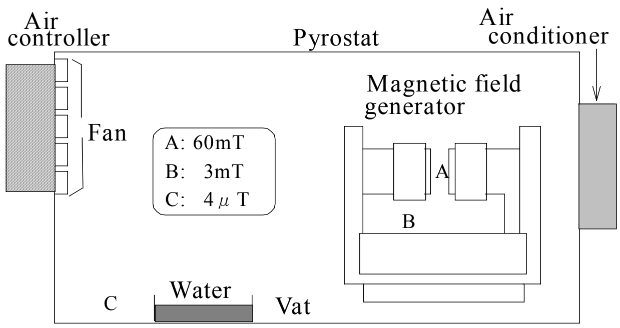

Fig. 3. Illustration of environmental chamber.

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III. Method of Experiment

A. Magnetic Field Levels

A magnetic field generator with an air gap in the magnetic circuit was installed in the chamber. The gap had a 75 x 75 x 70 mm space, where the breeding tubes described below were placed. Magnetic field was controlled by changing the input 60-Hz magnetizing current. A large reactive current required to obtain target magnetic field levels was compensated by large capacitors as indicated in Fig.4. Since the power supply was 100/200 volts, ac, the electric field produced by this generator was considered substantially zero.

Five magnetic field levels of 0.05μT, 0.3μT, 4μT, 3 mT, and 60 mT were selected in this experiment.

Filed levels of 0.05μT and 0.3μT were achieved in another chamber of the same size and the temperature control system with that shown in Fig. 3 without field generator in it. 0.05μT, which was obtained inside a shielding box installed in the chamber, was selected on the control.

Fig. 5 is the front view of magnetic field distribution in the environmental chamber. 60 mT was achieved in the air gap in the magnetic circuit as shown in Figs. 6, which was considered extraordinarily strong compared to those usually experienced in the daily life. Field level near the generator was 3 mT, corresponding to the level obtained in a very limited space such as electric power facilities. 4μT is the minimum value available in the environmental chamber with the coil in it, which is equivalent to a field level measured under high voltage overhead transmission lines. Locations of 4μT, 3 mT and 60 mT are corresponding to A, B, and C in Fig. 3, respectively.

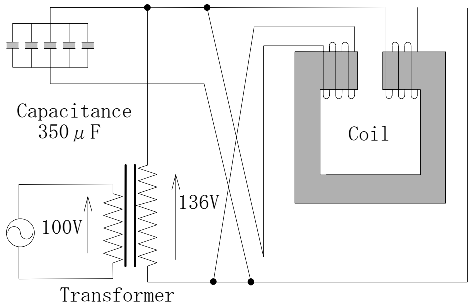

Fig. 4. Electric circuit diagram of the environmental chamber.

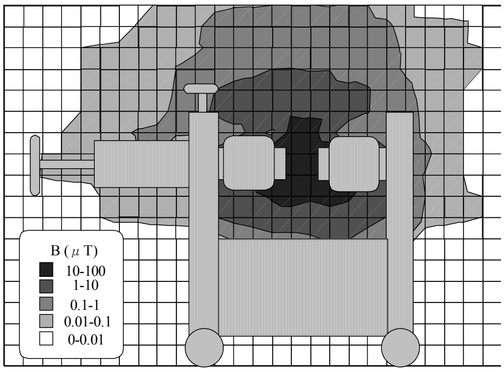

Fig. 5. Magnetic field distribution in the environmental chamber.

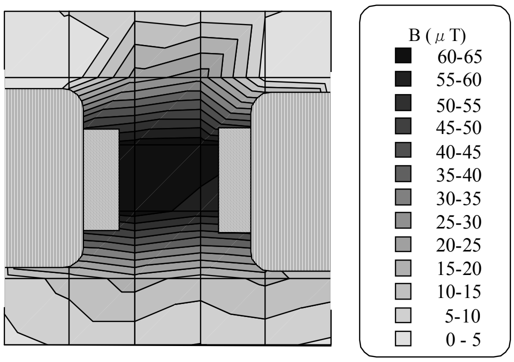

Fig. 6. Magnetic field distribution in the air gap.

B. Chronic Toxicity

A transparent plastic tube as illustrated in Fig. 7, was used for exposure of specimens to magnetic field, whose space was sufficient for the life of 30-40 fruit flies. The tubes were laid horizontally during the experiment at the designated locations in the chamber.

80 specimens of male or female distributed to each of four tubes by 20 pieces were subjected to a given magnetic field at one time. Exposure of specimens to magnetic field started 3 days after emergence and continued throughout their life except 15 minutes per day, which was required for counting and removing dead specimens and for feeding.

Fig. 8 is an example of arrangement of the breeding tubes, where male and female tubes were arranged alternately in order to give more uniform magnetic field in the air gap of the chamber by rotating tubes daily in case of 60 mT level.

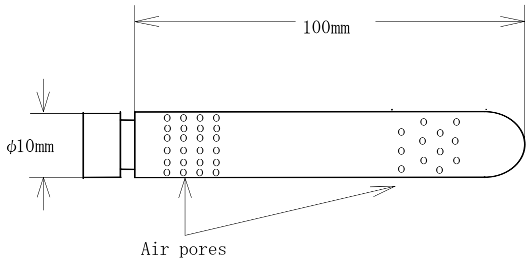

Fig. 7. Tube used for exposure to magnetic field.

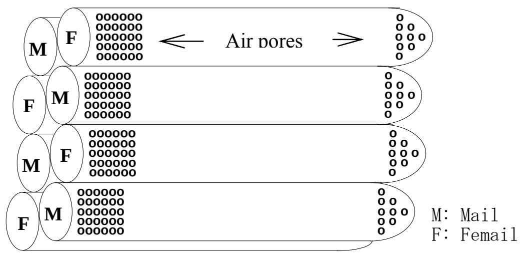

Fig. 8. Example of arrangement of breeding tubes.

C. Genetic Toxicity

The effect of power frequency magnetic field on mutation rate of fruit flies was investigated. Many kinds of mutation have been observed in fruit flies such as color of eye, shape of wing, shape of leg, shape of feeler, and so on [2]. Since wings and feeler may be physically damaged during the processes of the experiment, attention was paid to only the color of eyes in the present study. And, fruit flies with white eyes, which were sex-linked recessive inheritance, were chosen whereas mutated fruit flies have red eyes. Under natural condition, it is said that the mutation rate is several hundred-thousandth according to literature[2].

Just after emergence, male and female flies were separated. Then, 100 male or female specimens distributed to each of four tubes in the arrangement as shown in Fig.8 were exposed to magnetic field for five days. The said timing and duration of exposure were decided because: (1) Egg, larva and pupa are unstable from the biological point of view. Therefore, even if mutation rate changes due to exposure of fruit flies to magnetic field in these states, it cannot be concluded that it is caused by magnetic field; (2) Fertility of fruit flies increases in this period after emergence; (3) Considering the longevity, 5-day long for fruit flies corresponds to 10 years for human being. It seems that 5-day exposure is enough long for fruit flies.

After exposure to magnetic field, 100 male and 100 female flies were put into a glass breeding tube together, as shown in Fig. 9. The breeding tubes had been laid horizontally during experiment. A cotton plug was so designed that fresh air could be supplied into the inside space of the breeding tube. The tubes were replaced with new ones every five days.

Approximately five thousand flies hatched from 100 male and 100 female. When eggs hatched, the number of newborn flies was counted and existence of flies with red eyes, due to mutation, was confirmed. Then the same procedure as above was repeated.

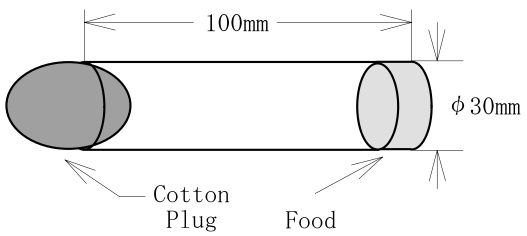

Fig. 9 Breeding tube.

IV. Statistical Analysis of Experimental Results

A. Chronic Toxicity

Figs. 10 and 11 show mortality for 80 male and 80 female specimens, respectively, exposed to three magnetic field levels of 0.3μT, 3mT and 60mT. Although there seems no significant difference in mortality among the three groups of magnetic fields, statistical approach is necessary for more quantitative discussion. Since the distribution of mortality shown in Figs. 10 or 11 does not follow any normal distribution, Kaplan-Meier product limit method and generalized Wilcoxon test [3], [4] are utilized in this study.

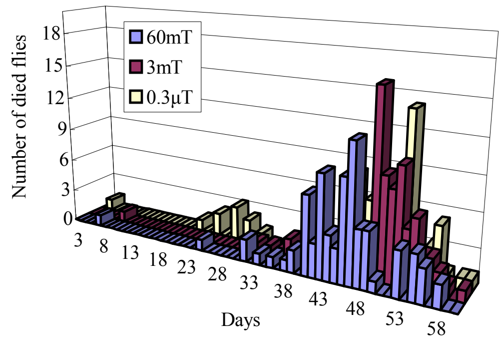

Fig.10. Mortality of male fruit flies.

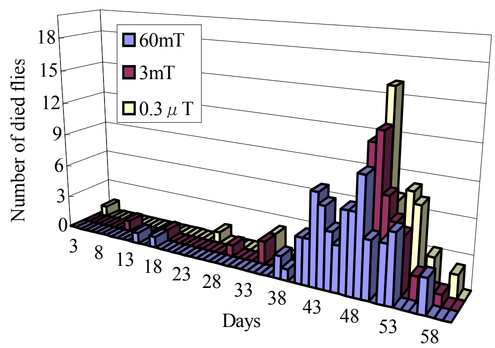

Fig. 11. Mortality of female fruit flies

Kaplan-Maier product limit method is useful to obtain survival curves and the calculation procedure is as follows:

1) All the data, whose number is n, are sorted in the order of mortality;
2) Survival rate, p_i at the time of t_i when the i-th fruit fly dies are defined as

$$p_i = \frac{n-i}{n-i+1} \tag{1}$$

3) Cumulative survival rate at the time of ti, P_i and standard error of cumulative survival rate, $SE(P_i)$ are given by

$$P_i = \prod_{j=1}^{i} p_j \tag{2}$$

$$SE(P_i) = \sqrt{P_i^2 \sum_{j=1}^{i} \frac{1}{(n-j)(n-j+1)}} \tag{3}$$

4) Survival curve is drawn.

Figs. 12 and 13 are survival curves for male and female specimens, respectively, which are obtained from data shown in Figs. 9 and 10. Generalized Wilcoxon test is effective in order to know if there is a significant difference between two survival curves. In this test, W and $Var\ W$ defined by (4) and (5) are calculated.

$$W = \sum U_{ij} \quad (4)$$

$$VarW = \frac{n_1 n_2 \sum_{i=1}^{n_1+n_2} U_i^2}{(n_1+n_2)(n_1+n_2-1)} \quad (5)$$

where n_1 and n_2 are number of specimens of groups 1 and 2, respectively. x_i and y_i are lifetime of specimens of groups 1 and 2. $U_{ij} = -1$ when $x_i < y_i$, $U_{ij} = 0$ for $x_i = y_i$, $U_{ij} = 1$ for $x_i > y_i$. Here,

$$Z = \frac{|W|}{\sqrt{VarW}} \quad (6)$$

follows the normal distribution with means of 0 and standard deviation of unity.

Generalized Wilcoxon test was applied to the survival curves shown in Figs.12 and 13. A null hypothesis is that there is no significant difference in survival curves obtained under different magnetic filed levels. The hypothesis will be rejected if Z defined by (6) is larger than 1.96 when a significant level of 5% is adopted. The calculation results of Z are summarized in Table I. Since Z does not exceed 1.96 in any case, the hypothesis is not rejected, which means that it can not be said that there is a significant difference in survival curves obtained under any two magnetic field levels.

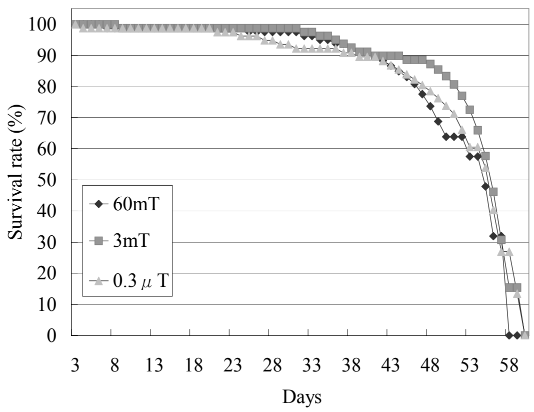

Fig. 12. Survival rate of male fruit flies.

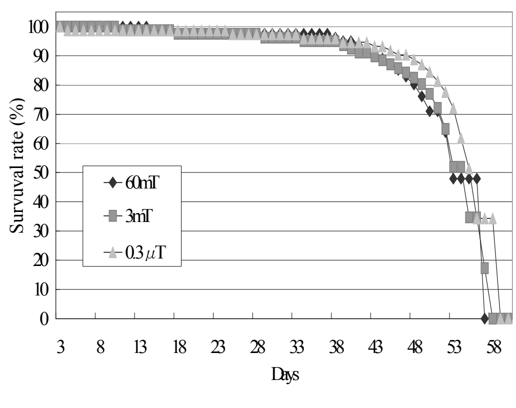

Fig. 13. Survival rate of female fruit flies.

TABLE I
RESULTS OF GENERALIZED WILCOXON TEST, Z.

	Male	Female
60 mT －3 mT	0.01	1.37
60 mT －0.3 μT	0.67	0.73
3 mT －0.3 μT	1.08	0.50

Furthermore, Table II indicates the average longevity of 60 male fruit flies and 60 female fruit flies exposed to 4 levels of magnetic field. In order to evaluate the difference in average longevity between 0.05μT and other magnetic field levels, a statistical test was conducted considering χ^2 distribution, which is given by

$$T = \sum_{i=1}^{k} \frac{(n_i - e_i)^2}{e_i} \quad (7)$$

where T is the test statistic, n_i is the observed frequency, and e_i is the expected frequency, which is assumed to be the same as the average longevity at 0.05μT. In this case, the number of degree of freedom, k is 3. The null hypothesis is that there is no difference in longevity of fruit flies between 0.05μT and other magnetic field levels exposed. The calculation result of (7) is shown in Table III with significant levels of 1 % and 5 %. Since the obtained test statistics on both male and female are below these significant levels, the hypothesis can not be rejected.

TABLE II
AVERAGE LONGEVITY AND ITS STANDARD DEVIATION OF FRUIT FLIES AT SEVERAL MAGNETIC FIELD LEVELS.

	Male		Female	
Magnetic field	Average longevity, day	Standard deviation, day	Average longevity, day	Standard deviation, day
60 mT	44.21	7.58	35.80	7.05
3 mT	45.38	7.22	37.17	6.87
4μT	43.32	6.97	35.41	7.31
0.05μT	43.62	6.58	36.83	6.70

TABLE III
RESULT OF TEST STATICS REGARDING LONGEVITY OF FRUIT FLIES UNDER MAGNETIC FIELD

	Test statistic, T	1% significant level	5% significant level
Male	0.079	11.34	7.81
Female	0.087	11.34	7.81

B. Genetic Toxicity

Fig. 14 shows cumulative number of specimens tested under five magnetic field levels. The results obtained so far are summarized in Table IV. Since the experiment under the magnetic field level of 0.05μT started lately, the number of specimens is smaller than others. Number of fruit flies with red eyes, which are caused by mutation, is 0 to 4 under any magnetic field level. The mutation rate seems almost the same as that under natural conditions.

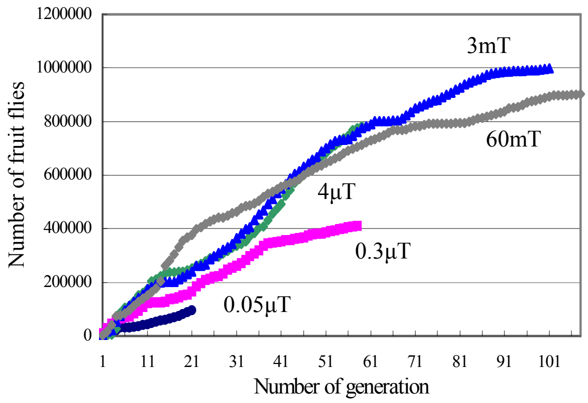

Fig.14. Cumulative number of specimen fruit flies tested under magnetic field levels.

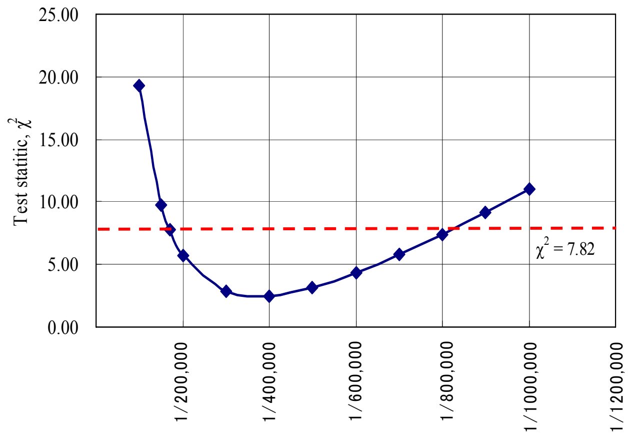

Fig.15. Results of goodness-of-fit test.

TABLE IV
NUMBER OF FRUIT FLIES WITH RED EYES (MUTATION) APPEARED AFTER EXPOSURE TO MAGNETIC FIELD.

	White eyes	Red eyes (Mutation)	Total
60 mT	906,974	2	906,976
3 mT	1,002,000	4	1,002,004
4 μT	783,199	1	783,200
0.3 μT	418,085	0	418,085
0.05 μT	142,345	0	142,345
Total	3,252,603	7	3,252,610

In order to evaluate the results statistically, a goodness-of-fit test [5] and interval estimation [6] were selected.

In this study, Poisson's distribution has been taken because (1) number of specimens is very large, (2) the mutation rate is very small, and (3) mutation is an independent phenomenon. Chi-square (χ^2) goodness-of-fit test was carried out, which is widely used to determine if the difference between observed and expected frequencies is due to contingency or not. A null hypothesis is that the mutation rate obtained under magnetic field in this experiment is the same as that under natural conditions. Since no mutation has been observed yet at 0.05μT, where the specimen size is still rather small, the rate is assumed one out of several hundreds thousand specimens based on the literature [7]. In this case, the number of degree of freedom is 3, disregarding the level of 0.05μT. The test statistic to be calculated is expressed by

$$\chi^2 = \sum_{i=1}^{3} \frac{(y_i - \lambda_0 \times n_i)^2}{\lambda_0 \times n_i} \qquad (8)$$

where y_i is number of mutated specimen under the exposure of magnetic field level i, n_i number of total specimen under the magnetic field level i and λ_0 the mutation rate under natural conditions. Considering the case of the significant level of 5%, the hypothesis is not rejected if the value of χ^2 is less than 7.82. The calculation results of (8), varying the mutation rate, are shown in Fig. 15. It is concluded that it can not be said that mutation rate obtained under magnetic field exposure is different from that under natural conditions, if the mutation rate under natural conditions lies between approximately 1/170,000 and 1/800,000.

Another statistical approach utilizing interval estimation of expected frequency of mutation was also made. Assuming Poisson's distribution, the expected number of mutated specimens with significant level of 5% is given by

$$0.025 < \sum_{x=0}^{X} \frac{m^x e^{-m}}{x!} < 0.975 \qquad (9)$$

where m is the expected frequency of mutation and x is number of mutated specimens observed in the experiment. By solving this inequality numerically, the range of integer m can be obtained. The results are summarized in Table V. Expected frequency was calculated by multiplying the total number of specimens by the assumed mutation rate. In cases of A and B, the mutation rate under natural conditions is assumed 1/400,000 and 1/200,000, respectively. Since the expected frequency stands in the range calculated by interval estimation, it is possible to conclude that the mutation rate obtained in the experiments seems the same as that under assumed natural conditions.

TABLE V
RESULTS OF INTERVAL ESTIMATION.

		Results of interval estimation		Expected frequency
		Lower	Upper	
60 mT	A	0	5	2.27
	B	4	14	4.53
3 mT	A	0	5	2.51
	B	4	16	5.01
4 μT	A	0	4	1.96
	B	3	13	3.92

Note) Assumed mutation rate under natural conditions, A : 1/400,000, B : 200,000

Odds ratio was also calculated together with its 95% confidence interval[8] as an epidemiological approach. Since no mutation occurred so far at magnetic field levels of 0.3μT and 0.05μT, 4μT was selected as the control level in this analysis. The calculation results are summarized in Table VI. The odds ratio is larger than unit in both cases. But the lowest value of 95% confidence interval is smaller than unit, suggesting that there is not a significant difference in mutation ratio for both magnetic fields.

TABLE VI
ODDS RATIO AND 95% CONFIDENCE INTERVAL FOR ODDS RATIO.

	Odds ratio	95% confidence interval
60 mT	1.73	0.16 – 19.0
3 mT	3.13	0.35 – 30.0

In order to obtain the final conclusion on the genetic effect of power frequency magnetic field on fruit flies, further experiment to accumulate more data are needed since the mutation rate under natural conditions is very small.

V. CONCLUSIONS

Effect of power frequency magnetic field on longevity of fruit flies was studied experimentally. Genetic effect of magnetic field on fruit flies was also investigated. The results obtained by statistical analyses are as follows:

(1) By applying Kaplan-Meier product limit method and generalized Wilcoxon test to the experimental results, it was concluded that it cannot be said that magnetic field affects the longevity of fruit flies.

(2) Experimental results on mutation were analyzed by a goodness-of-fit test, interval estimation and odds ratio. It is possible to conclude at present that the mutation rate seemed not to be affected by magnetic field.

VI. ACKNOWLEDGMENT

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VIII. BIOGRAPHIES

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In1984, he joined NGK INSULATORS, LTD. He first served as a design engineer of insulators. Then, he worked as an application engineer of NGK-LOCKE, INC., USA from 1989 to 1994. And he worked as a manager of NGK-LOCKE POLYMER INSULATORS, INC., USA from 1997 to 2002. He is now a manager of High Voltage Laboratory of Power Business Group. And, he is also a graduate student of Graduate School of Engineering, Nagoya Institute of Technology. He is a member of the Institute of Electrical Engineers of Japan

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Prof. Naito is a Life Fellow and a Distinguished Lecturer of IEEE, a Distinguished Member of CIGRE and a Life Member of the Institute of Electrical Engineers of Japan.