Evaluation of Damage in DNA Molecules Caused by Very-Low-Frequency Magnetic Fields Using Bacterial Cells

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SUMMARY The effect of intermediate frequency magnetic fields or, very-low-frequency magnetic fields (VLFMF) on living biological cells was investigated using a highly sensitive mutagenesis assay method. A bacterial gene expression system for mutation repair (*umu* system) was used for the sensitive evaluation of damage in DNA molecules. *Salmonella typhimurium* TA1535 (pSK1002) were exposed to VLFMF (20 kHz and 600 μ T) in a specially designed magnetic field loading chamber. The experiment results showed the possibility of applying the *umu* assay for sensitive and effective evaluation of damage in DNA molecules. No effects from exposure to 20 kHz and 600 μ T magnetic fields in terms of damage in DNA molecules were observed.

key words: mutation repairing gene, *umu* operon, very-low-frequency magnetic field (VLFMF), *Salmonella typhimurium* TA1535 (pSK1002), *lacZ*, *Y*, β -galactosidase

1. Introduction

Recent public concern has focused on possible human health effects from exposure to magnetic fields produced by household appliances [1]. Many types of equipment with driving frequencies in an intermediate frequency magnetic fields (for which WHO defines a bandwidth of not less than 300 Hz and not more than 10 MHz), or, very-low-frequency magnetic fields are being used today. For instance, induction cooking appliances have come to be widely used in homes and restaurants. In 2004 it was estimated that there were over 650,000 induction cooking units in use in Japan. Moreover, the present 20 kHz excitation frequency of induction cooking appliances tends to produce higher frequency up to about 100 kHz in an effort to enhance performance. With induction cooking appliances in wider and wider use, the general citizen's opportunity for exposure to very-low-frequency magnetic fields (VLFMF) has increased.

In addition to induction cooking appliances, the video display terminal (VDT) which produces a magnetic field at VLFMF have been used since past 30 years. The effects of VLFMF on genesis has been much investigated using embryos of fowls and experimental animals such as mice and rats, reflecting the great interest of researchers in the risk

of exposure to a VDT magnetic field involved in VDT operation by pregnant women. As a result, both positive and negative effects of a VDT magnetic field on the human body have been reported, leaving no reasonably agreed results on this regard. On the other hand, a simulative action is the prevailing effect of an extremely-low-frequency electromagnetic field on the human body, whereas heat action is prevailing effect in the high frequency band. In these circumstances, many experiments have been conducted to verify the effects of an electromagnetic field on the human body for the extremely-low-frequency electromagnetic field and high frequency bands, and ICNIRP has established guideline values for protection in the extremely-low-frequency electromagnetic field and high-frequency bands [2]. However, experimental data are relatively poor for the very-low-frequency fields. In view of the lack of data on actions that directly affect the human body, the guideline values for the very-low-frequency band are determined by extrapolating relevant data from those for the extremely-low-frequency and high-frequency bands, which are positioned next to the VLFMF. As a consequence, a considerable data spread is inevitable for the values of different protection guidelines [3]. As described above, research on the effects of VLFMF on the human body has not moved ahead much in Japan and overseas. In particular, we find no research articles that mention genetic damage at a molecular or genetic level due to exposure to a VLFMF.

Therefore, the effect mechanism of VLFMF on human health has not yet been completely clarified, but general citizens are anxious about this effect. The key of this study is the use of living bacterial cells as a detection reporter for DNA injury. The genetic SOS repair response system for bacterial DNA molecule damage with the *umu* operon was used [4].

It is well known that mutation in DNA molecules is a direct cause of cancer development, and there is a close relationship between the oncogenesis of cells and the molecular damage of DNA [4]. Recently, genetic engineering has developed remarkably, and experimental techniques using DNA analysis are now applied to many fields of science. Therefore, gene level research on the impact of VLFMF exposure on living cells can also be done using molecular biological techniques. When the DNA of a bacterium is damaged, SOS repairing genetic systems such as a bacterial *umu* operon and a *recA* gene system which try to repair the damage rapidly, are expressed [4]. The gene ex-

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pression of genetic systems can be conveniently and quickly detected by the expression of reporter genes (such as *lacZ*, *lacY* or β -galactosidase), and the damage to the DNA can be evaluated quantitatively [4]. β -galactosidase generated by the gene expression of *lacZ*, *Y* is an enzyme for degradation of a disaccharide, lactose, and its enzymatic activity can be determined using X-gal which is a lactose derivatives and is turned to a blue-colored compound when degraded by β -galactosidase. Because of the coloring characteristics, β -galactosidase gene is used as a reporter gene to measure genetic expression strength targeted to be analyzed. Expression of the targeted genes, *umu* genes in this case which are co-expressed with the β -galactosidase gene, is measured by replacing *umu* gene expression with β -galactosidase gene expression.

The paragraph below explains the reasons for its adoption. This study has focused on damage to DNAs at molecular and genetic levels, unlike from other similar studies using cells on the effects of a magnetic field on the human body. It has not been scientifically clarified how mutation is induced at a genetic level in the very-low-frequency magnetic fields. This study adopts a simplified *umu*-assay to detect genetic damage to DNA molecules that might have been caused by exposure to a very-low-frequency magnetic field, using the SOS repair response of bacteria.

Umu-assay using bacteria is an established mutagen test to evaluate the mutagenic capacity of bacteria against genes, and is an official testing method widely used worldwide as a method for testing water supplies and sewage, and for evaluating the safety of drainage. This method has the important advantage of enabling a quantitative evaluation in comparison with other risks by detecting the biological effects and evaluating the safety of VLFMF.

Moreover, mutation in an electromagnetic field is a probability event with a very low frequency band, thereby requiring the handling of a large number of biont to detect it. For example, more than one million mice must be examined, which would be impossible in terms of the scale of experimental animals and costs. Meanwhile, the *umu*-assay allows us to detect mutations due to bacterial cells existing at more than 100 million in 1 ml of bacteria-containing liquid, so that we can easily increase their population.

In this study, we exposed the bacterium *Salmonella typhimurium* TA1535 (pSK1002) (a derivative strain whose pathogenicity was deleted) to VLFMF, and the expression intensity of the activity of β -galactosidase, which is located downstream of the *umu* SOS gene operon, was examined using a previously developed *umu* assay method with a non-exposure control. In this paper, the results of the *umu* assay of the damage to DNA molecules caused by VLFMF exposure are reported.

The DNA structure is the same for prokaryotic cells and eukaryotic cells. Therefore, the knowledge of this study may apply to evaluate the human DNA molecule damage.

2. VLFMF Exposure Equipment

Figure 1 shows the VLFMF exposure equipment used in this study. It consists of Helmholtz coils for canceling the earth's magnetic field, a coil for generating the VLFMF exposure, and a device for controlling the temperature of a bacterial culture liquid in a micro tube.

2.1 Earth's Magnetic Field Canceling Coils

It was highly probable that the effects of a DC magnetic field (earth magnetic field) and a 20 kHz AC magnetic field work in a superposed manner. Accordingly, we cancelled earth magnetic field in the study to examine only the effects of independent exposure to an AC magnetic field. Two square coils were placed facing each other 40 cm apart with a common axis to constitute a Helmholtz coil. Earth magnetic-field canceling coils were installed in the VLFMF exposure equipment. The coils were installed using square frames 80 cm on a side, each having 15 turns. The vertical and horizontal components of the earth's magnetic field were canceled using two sets of Helmholtz coils, one for generating the VLFMF exposure and the other as a non exposure control. The coil bobbin was made of wood, and the volt nuts were made of brass. A magnetometer probe for measuring the earth's magnetic field was placed at the center of the Helmholtz coils. The magnetometer was a type of flux gate and its minimum resolution was a 10 nT. Direct current

Fig. 1 Experimental system for testing bacterial DNA damage by VLFMF exposure.

Fig. 2 Incubator that sets up microtube and microtube where bacterial culture liquid is put.

was passed through the Helmholtz coils. The direct current intensity was adjusted in order to minimize the earth's magnetic field according to the reading of the magnetometer. The residual earth magnetic field strength of the vertical and horizontal components was less than 10 nT. The uniformity of both the vertical and horizontal components of the residual earth magnetic field strength, which the whole bacterial culture liquid on the micro plate was exposed to was within 1%.

2.2 Temperature Control Device of Incubator Bottle

Warm water was circulated from a controlled water bath by a pump through a silicon tube (10 mm inside diameter) connected to an incubator bottle of acrylic resin, $23 \times 17 \times 7.5 \text{ cm}^3$ as shown in Fig. 2(a) with a space allowing the insertion of the micro tube. The temperature of the bacterial culture liquid in the micro tube was kept at a constant 37°C . The bacterial culture liquid was filled in a 1.5 ml micro tube, 1.1 cm in diameter and 3.4 cm length as shown in Fig. 2(b). The temperature of the water bath was set at 37°C and a 20 kHz VLFMF of $600 \mu\text{T}$ was applied for 8 hours; the temperature change in this period was 0.1 degree or less. We carried out this experiment in order to examine the thermal environment of incubator bottle under the condition of operating the coil for long time. The temperature distribution was thus uniformly controlled within the range of measurement error. We measured temperature changes of inside the microtube and the incubation temperature. The temperature differences of inside the microtube and the incubation temperature were less than 0.1°C . The rise in the temperature of culture by the eddy current loss can be ignored.

2.3 Helmholtz Coil for VLFMF Exposure

The rectangular coils were 18.5 cm wide and 19.5 cm long, each having 10 turns. The coil bobbins were made of bakelite resin, and metal was not used. Two rectangular coils were placed facing each other 9.3 cm apart to constitute a Helmholtz coil for generating VLFMF exposure, as shown in Fig. 3. The coil for generating VLFMF exposure was installed within the earth magnetic field canceling coil. The

Fig. 3 Helmholtz coil to generate VLFMF exposure.

Fig. 4 Contour plot of magnetic field uniformity of the coils for generating VLFMF in the x - y plane ($Z=0$).

exciting 20 kHz sinusoidal waveform was generated from a function generator. It was amplified by power amplifier, and was supplied to the Helmholtz coil. The voltage used was 100 Volts, and a current of 13.1 amperes pass through the coil.

The magnetic fields were applied perpendicular to the axis of the micro tube. The magnetic flux densities were measured from the pickup coil (50 turns of 0.1 mm ϕ enameled copper wire) wound around the bobbin of acrylic resin 5 mm in diameter. The pickup coil sensor provides an output voltage proportional to the strength of the magnetic field of parallel component in longitudinal direction of the sensor. The electric fields also couple to the pickup coil sensor, but an output voltage due to the electric fields was smaller than that of magnetic fields.

Figure 4 shows the contour plot of magnetic field distribution of the Helmholtz coils for generating VLFMF obtained by the measurement in the lattice point at 0.5 cm intervals of the x - y plane ($Z=0$). The origin in the coordinate system is a center of the Helmholtz coil for generating VLFMF.

The numerical values give the percent deviation of the axial field (y direction) from the value of the field at the center of the coil system. The uniformity of VLFMF in the x - y plane $Z=\pm 2 \text{ cm}$ were almost same as that $Z=0 \text{ cm}$. Therefore, the magnetic field along Z axes was almost uniform from $Z=-2 \text{ cm}$ to $+2 \text{ cm}$. The range of magnetic field

(20 kHz and 600 μ T) applied to the whole bacterial culture liquid in the incubator bottle was less than 2%.

3. Protocol of *umu* Assay Method

3.1 Development of the Protocol to Detect Damage in DNA Using a Mutagenic Reagent and VLFMF

DNA damage detection bacterium *Salmonella typhimurium* TA1535 (pSK1002) which has lactose metabolic genes (*lacZ*, *Y*) as the reporter genes with an SOS DNA repairing *umu* operon was purchased from a pharmaceutical company. Figure 5 shows the genetic constitution for *umu* assay of mutagenesis. *S. typhimurium* TA1535 (pSK1002) is a bacteria that has *umuC*, *D* and *lacZ*, and *Y*. *UmuC*, *D* is an ultra-mutant genetic operon which repairs injured DNA. *LexA* is a repressor protein which represses the genetic expression of *umuC*, *D* in normal DNA. Operon is that genes coding for functionally related proteins are clustered along the DNA. A typical operon consists of a group of structural genes (*umuD*, *C*, and *lacY*, *Z*) that code for enzymes involved in a metabolic pathway. These genes are located contiguously on a stretch of DNA and are under the control of one promoter (a short segment of DNA to which the RNA polymerase binds to initiate transcription). The promoter is controlled by various regulatory elements that respond to environmental cues. One common method of regulation is carried out by a regulator protein that binds to the operator region, which is another short segment of DNA found between the promoter and the structural genes. The regulator protein can either block transcription, in which case it is referred to as a repressor protein; or as an activator protein it can stimulate transcription. The protocol of the detection method of damage in DNA by magnetic field exposure using the *umu* assay method is as follows.

S. typhimurium TA1535 (pSK1002) was incubated for 16 hours in an LB culture medium (at 37 degree on a rotating shaker), and in a TG culture medium for 2 hours (at 37 degree on a rotating shaker) [5]. An aliquot (1.5 ml) of bacterial culture was divided into micro tubes. 0.1 M phosphate buffer (pH 7.4) was added. The culture was exposed

Promotor: Site where RNA polymerase binds to begin transcription.

Operator: Site where repressor protein binds to block transcription.

Repressor: protein that shuts off operon. If bound to operator, it prevents RNA polymerase from initiating transcription.

lacZ, *Y*: genes which produces β -galactosidase (one kind of enzyme).

Fig. 5 Genetic constitution for *umu* assay of mutagenesis.

to VLFMF (600 μ T) for 2 hours (a control culture was not exposed to VLFMF). The optical absorbance of the bacterial culture at a wavelength of 600 nm (OD_{600}) was measured.

3.2 Measurement of Activities of β -galactosidase

The final evaluation of the DNA damage by the *umu* test was based on the β -galactosidase activity of *S. typhimurium* TA1535 (pSK1002), and the measurement was carried out under the following procedures. An aliquot (1.5 ml) of bacterial culture was divided into test tubes. 2.7 ml Z buffer solution was added [5]. An aliquot (75 μ l) of 0.1% SDS and an aliquot (15 μ l) of chloroform were added. The mixture was shaken vigorously, then kept at 28 degree for 3 minutes without shaking. 2-nitrophenyl- β -D-galactopyranoside (4 μ g/ml) was added, and the tube was kept at 28 degree for 15 minutes. An aliquot (2 ml) of Na_2CO_3 (1.0 M) was added to stop the reaction. The OD_{420} and OD_{550} , optical densities measured optical absorbance at wavelengths of 420 nm and 550 nm, respectively. The degree of damage in DNA molecules was quantitatively evaluated as expressed β -galactosidase activity calculated using the following equation [4].

Activity of β -galactosidase

$$= 1000(OD_{420} - 1.75 \cdot OD_{550}) / (t \cdot v \cdot OD_{600}). \quad (1)$$

where,

t : reaction time (15 minutes),

v : dilution rate (0.1).

To confirm the effectiveness of the test protocol developed in this study for the DNA damage, furofuramide (AF2), known as a strong mutagen which induces damage in DNA molecules, was used as a positive control and a standard mutagenic substance.

4. The Confirmation of the DNA Damage Assay System (A Positive Control Test of *umu* Assay)

S. typhimurium TA1535 (pSK1002) is a bacterium that has *umuC*, *D* and *lacZ*, *Y*. If DNA-destroying substances are present, *S. typhimurium* TA1535 (pSK1002) generates β -galactosidase by the gene expression of *lacZ*, *Y*. The principle of this process is shown in Fig. 6. First, when mutagenic substances damage the DNA in *S. typhimurium* TA1535 (pSK1002), not injured site of the single strand DNA send the SOS signal, and *recA* gene is expressed and produced RecA protein. RecA protein binds to the not injured site of the single strand DNA. Then the bound RecA protein decomposes LexA protein, which is a repressor for the *umu* operon. Because the operator is not blocked, RNA polymerase can bind to the promoter and begin transcription. After the repressor detaches from the *umu* operator region, the *umuC*, *D* genes begin to repair the damaged DNA. Finally, the *lacZ*, *Y* genes which are placed downstream of *umuC*, *D* are also expressed to produce β -galactosidase. β -galactosidase is enzyme hydrolyzes the bond between the

Fig. 6 The principle of β -galactosidase production by damaging a part of the DNA.

two sugars, glucose and galactose. It is coded for by the gene *lacZ*. Therefore, the degree of damage to the DNA can be evaluated quantitatively, by detecting the activities of β -galactosidase. This is called the *umu* assay method and was used in this study.

Positive controls are used to check if the genetic system still functions normally following genetic damage because of any mutagen, and also to check its sensibility to detect mutagens. Accordingly, they are not designed specially for carcinogenicity tests. However, they say we can judge if something is "significantly different" in an *umu*-assay if approximately twice the difference is found compared to controls.

Lesions in DNA molecules were manifested using furylfuramide (AF2), a well-known mutagen as a positive control, and the gene mutation was evaluated by the *umu* assay protocol which was established in this study for measuring the effect of VLFMF. The effectiveness of this test method was thereby confirmed. The protocol of the positive controls is omitted [7]. The OD₆₀₀, OD₄₂₀ and OD₅₅₀ measurement was carried out every 2 hours. The experimental procedure after this step was the same as that used in the measurement of the activity of β -galactosidase in the previously mentioned *umu* assay method, and the activity was calculated by Eq. (1).

Fig. 7 The hourly time change in the activity of β -galactosidase.

Figure 7 shows the activities of β -galactosidase measured with results of a positive control tests and without AF-2. It is clearly recognized that the value of the activity of β -galactosidase increases as AF-2 concentration rises. The activity of β -galactosidase rises for 2 hours, and it is almost constant after 2 hours. The data of activity of β -galactosidase after two hours except in case of AF2 concentration 0.9 μ g/ml were given to official approval by using the Thompson rejection test. From these results, we decided on an exposure period of 2 hours.

5. The Measurement of Possible Induction DNA Damage from Exposure to VLFMF

In this study, using the *umu* gene expression system, we report on the results of evaluations of damage to DNA molecules in bacterial cells caused by VLFMF exposure. A liquid bacterial culture of *S.typhimurium* TA1535 (pSK1002) was exposed to a VLFMF of 600 μ T. The magnetic field measurements were made for several induction cooking appliances. A maximum magnetic field strength 20 kHz, and 16 μ T was observed in front of a induction cooking appliance [8]. So, VLFMF of 600 μ T was 37.5 times as great as measurement strength.

The data listed in Table 1 were measured under the condition where the earth magnetic field was eliminated. The applied VLFMF frequency was 20 kHz and the period of exposure was 2 hours. According to the study of Miyakoshi et al., an experiment with exposure of MeWo cells derived from human melanoma to a 50 Hz and 400 mT magnetic field proved that the frequency of inducing mutations for HPRT genes increased in proportion to the density of the induction current in an AC magnetic field. In our experiment, we also paid attention to the induction current and set the density at 600 μ T so that an induction current similar to that derived under the above conditions (50 Hz and 400 mT) can be obtained [5].

Using the *umu* assay method, the DNA damage was quantitatively evaluated. The incubation temperature of the bacteria was set at 37°C. Table 1 shows the results (each for the 4 times measurement) of the β -galactosidase activity of the bacterial culture liquid from VLFMF exposure for 2 hours and of the control bacterial culture liquid without

Table 1 Experimental results of damage caused to DNA molecule by exposure to VLFMF.

I			II		
Sample number	Activity of β -galactosidase		Sample number	Activity of β -galactosidase	
	Control	Exposure		Control	Exposure
1	253.50	273.11	1	260.70	208.33
2	269.32	261.08	2	259.76	230.35
3	RD	256.77	3	264.61	193.51
4	257.83	283.15	4	248.54	254.68
5	261.56	271.39	5	239.38	RD
6	276.48	265.50	6	244.40	224.71
7	282.00	290.69	7	263.60	215.54
8	263.35	286.90	8	236.06	203.88
Average	266.29	273.57	Average	252.13	218.71
Dispersion	89.43	133.86	Dispersion	114.04	348.31
SD	9.46	11.57	SD	10.68	18.66
significance	X		significance	O	

III			IV		
Sample number	Activity of β -galactosidase		Sample number	Activity of β -galactosidase	
	Control	Exposure		Control	Exposure
1	355.47	RD	1	262.78	RD
2	393.29	342.34	2	RD	300.75
3	323.70	324.36	3	321.84	300.95
4	391.93	350.19	4	328.24	299.81
5	372.40	333.98	5	348.78	280.23
6	337.74	364.46	6	309.25	274.51
7	352.94	354.37	7	290.73	276.42
8	397.51	372.24	8	301.89	288.97
Average	365.62	348.85	Average	309.07	288.81
Dispersion	664.85	240.95	Dispersion	662.56	120.38
SD	25.78	15.52	SD	25.74	10.97
significance	X		significance	X	

SD = Standard deviation, RD = Rejected data by Thompson rejection test.

VLFMF exposure. There were 8 samples per measurement. The OD₆₀₀, OD₄₂₀ and OD₅₅₀ of the liquid bacterial culture from VLFMF exposure for 2 hours, and of the liquid bacterial culture without VLFMF exposure were also measured. The activity of β -glycosidase was obtained from Eq. (1).

A Thompson rejection test was performed for each of the eight sample data. Therefore, we judged whether the eight sample data could be used and if the data at the time of exceeding a significant level was rejected. The data adopted by the Thompson rejection test was equalized, and we ascertained whether there was a significant difference between the magnetic field exposure and control samples using a *t*-test at 5% (rate of rejection) of significant levels. The rejected data were shown as "RD" in Table 1. As a result of performing the *t*-test on the sample data for the 4 times, the significant difference were not pegged in three times measurement (I, III, IV) increasing and decreasing of the β -galactosidase activity by magnetic field impression of 20 kHz, and 600 μ T at 37°C. On the other hand, a significant difference was found in one time measurement (II).

Although, we raised the temperature inside the incubator bottle, no significant difference was detected between the incubator temperature of 37°C and 42°C from the exposure experiments to examine the relationship between temperature changes and DNA damage level. We may, therefore, assume no change to the DNA damage level due to a temperature change of within only 0.1°C.

These results show that the possibility of a weak relation between exposure to VLFMF and damage to DNA

cannot be denied.

However, the reference level according to the *umu* assay method stays around twice and thus it cannot be determined that there are significant differences in damage to DNA due to exposure to VLFMF.

6. Discussion

To examine the effects of a very-low-frequency electromagnetic field on the human body, several experiments using embryos of fowls and experimental animals such as mice and rats by simulating an electromagnetic field generated on a VDT with saw-tooth waves have been conducted. In our review, we found approximately some reports in which probability of survival (POS) and teratogenesis of a fowl's embryos are used as valuation indicators. However, there is no basis for believing that the results agree. Meanwhile, about several experiments have been reported, in which teratogenes and embryonic development of pregnant mice are selected as indicators. Most of these experiments reported that no specific effect of exposure to an electromagnetic field was detected, but some of them insisted that the number of teratogenetic cases increased when pregnant mice are exposed to a magnetic field after ARA-C (a teratogenic substance) was administered. Whereas, other experiments with embryonic development of pregnant rats as the core indicator reported that no effects of exposure to an electromagnetic field were detected. Accordingly, we have not yet reached an ultimate judgment on the effects of a VDT electromagnetic field on the human body, with both positive and negative results reported together. Although equipment generating a VLFMF is being increasingly used, only a few surveys that directly treat frequency as primary research subject have been conducted [9]. In the VLFMF, neither simulative action nor heating action is preminent, leaving prevailing the effects on the human body of the VLFMF unclear with regard to previous experimental results. In addition, very little is known about the influence of on genetic damage due to exposure to a VLFMF. It is clear that information of this type is essential to an understanding of the mechanism of effects of VLFMF on the human body. We could find only one research on genetic damage (mutation) at a molecular or genetic level due to exposure to extremely-low-frequency (50 Hz and 400 mT) magnetic field [5].

According to Miyakoshi's investigation, mutant frequency of HPRT gene was proportional to the induced current.

In our study, 20 kHz and 600 μ T was almost same as 50 Hz and 400 mT in terms of $f \times B$. However, no significant differences between exposure to 20 kHz and 600 μ T magnetic fields and control were observed in the *umu* gene expression. This may be attributed to the radius of Schale for culture. The induced current density in culture is shown following equation.

$$J = \sigma \pi f B r \quad (2)$$

where,

σ : conductivity of culture
 f : magnetic field frequency
 B : magnetic flux densities
 r : radius of Schale

The induced current is proportional to not only $f \times B$ but also r of Schale for culture. The radius r of Schale used by experiment of Miyakoshi was approximately 10 times larger than that of microtube using in our study. Then current induced by 50 Hz and 400 mT magnetic field was 10 times larger than that of our experiment.

As research on the effects of a VLFMF on the human body, has just started, more detailed studies will be required in the future at different levels such as animal, cell [10], molecule, and gene to clarify biological effects.

7. Conclusions

The *umu* assay method was applied to evaluate the direct impact of exposure to a VLFMF on DNA damage. The experimental results showed that the *umu* assay method could be effectively applied to the evaluation of damage in DNA molecules caused by exposure to a VLFMF. In view of the present results, no effects from exposure to 20 kHz and 600 μ T magnetic fields in terms of damage in DNA molecules were observed. It is not possible from present knowledge to make definitive statement about the safety or hazard associated with exposure to VLFMF.

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