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Field Uniformity Assessment of a Reverberation Chamber for a Large-Scale Animal Study

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ABSTRACT An exposure system was designed and implemented for the purpose of joint large-scale animal experiments conducted in Korea and Japan using a reverberation chamber (RC) in both countries. Prior to the start of the animal experiments, the parameters S_{21}, electric field (E-field) uniformity, and Q factor were evaluated to confirm the performance of the developed chambers. The E-field uniformity, which is the most important performance factor of an RC, was evaluated at 900 MHz on 150 measurement values taken at 150 points in a loaded chamber with 80 live rats and in an empty chamber. The conditions of the loaded chamber with the rats are almost the same as the actual experimental environment and in which the rats, watering systems, animal bedding, cages, and cage racks were included. Two mean body weights of 330 and 470 g for the 80 rats were considered. The E-field uniformity was within the mean $\pm$ 1.0 dB and mean $\pm$ 2.3 dB under the empty and the rat-loaded conditions, respectively.

INDEX TERMS EMF exposure, animal study, reverberation chamber, field uniformity, 900 MHz.

I. INTRODUCTION

In May 2011, the WHO/International Agency for Research on Cancer (IARC) classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (i.e., Group 2B) based on an increased risk for glioma, which is a malignant type of brain cancer associated with wireless phone use. Group 2B is used as a label when there is limited evidence in humans and less-than-sufficient evidence in animals. Based on this classification, it is therefore necessary to secure mechanistic data that can explain the causal relationship between radiofrequency electromagnetic fields and carcinogenesis through animal experiments.

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Reverberation chambers (RCs) have been utilized as facilities suitable for long-term exposure of small animals to radio frequency (RF) electromagnetic waves because it allows a homogeneous and simultaneous exposure of a large number of free moving animals during their full lifetime [1]. The National Toxicology Program (NTP) of the National Institute of Environmental Health Science (NIEHS) conducted carcinogenesis and toxicity studies on the effects of cellular phone radiation of Code Division Multiple Access (CDMA) and Global System for Mobile (GSM) communication using rats and mice at 900 and 1900 MHz, respectively [2]–[5].

The US NTP reported the strongest evidence of carcinogenicity for male rats, where incidences of malignant schwannoma in the heart increased with both GSM and CDMA exposure [2]. If the animal experiment results cannot be reproduced, the research is considered to be unsupported by objective scientific knowledge. The US NTP, the Federal Office for Radiation Protection (Bundesamt für Strahlenschutz, BfS) in Germany, and ARPANSA of Australia have recommended further research for validation (reproducibility) and clarification of the US NTP study results (https://ntp.niehs.nih.gov/whatwestudy/topics/cellphones/index.html, https://www.bfs.de/EN/bfs/science-research/statements/emf/ntp-study/ntp-study-statement/ntp-statement.html, https://www.arpansa.gov.au/news/arpansa-reviews-animal-study-radiofrequency-exposure-and-health).

Therefore, research teams in Korea and Japan agreed to conduct joint research to increase the statistical power by combining the data for the purpose of validation of the US NTP research results, particularly on male rats exposed to RF radiation. A 900-MHz CDMA-modulated signal was selected for the RF exposure owing to its use in mobile communication services in Korea and Japan. RCs with the same structures were designed, manufactured, and installed in both countries.

This paper describes the exposure system based on an RC and reports the results of a field uniformity evaluation in the empty and loaded chambers. The field uniformity over the working volume of the chamber is essential to ensure the same exposure for all exposed animals independent of their movement or body orientation. In this study, the working volume is defined as the volume enclosed by the cages and cage racks. The electric field (E-field) distribution inside the working volume was measured for four cases, i.e., an empty chamber, a chamber loaded with an apparatus to house the animals, a chamber loaded with the apparatus and 80 rats with different average weights of 330 and 470 g, respectively.

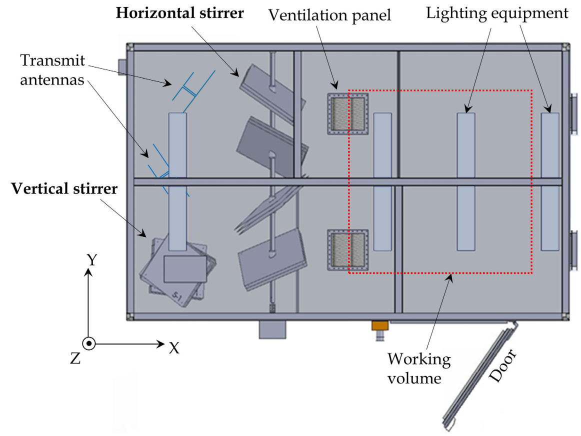
Because the two cases loaded with rats were almost identical to the actual animal experimental environments, the evaluation results of the E-field distribution under these conditions allowed an estimation of the field distortion and the corresponding SAR inhomogeneity of the exposed subjects.

II. MATERIALS AND METHODS

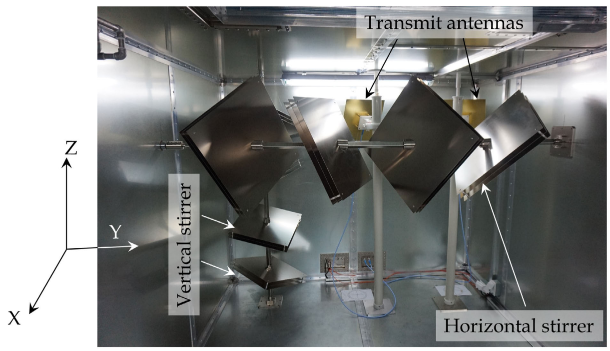
A. EXPOSURE SYSTEM

Environmental conditions such as the illumination, noise, ventilation, temperature, humidity, and size of the living space of the animals in the electromagnetic field (EMF)-exposed space of the chambers satisfy the requirements of the Good Laboratory Practice (GLP) facility. This section mainly describes the design results of RCs from an electromagnetic perspective.

Fig. 1 shows the top and interior views of the RC, both designed and installed. The two RCs for the exposure and sham groups were manufactured and installed by Korea Shield System (KSS), Ltd. at the Korea Institute of Toxicology (KIT) and DIMS Institute of Medical Science, Inc. (DIMS) in Korea and Japan, respectively. Two stirrers were built vertically and horizontally in each RC with internal dimensions of 2.5 × 4.0 × 2.0 m^3, and each stirrer is composed of four square flat metallic plates [6]. For a rich scattering of the fields, all plates were designed with different



(a) Top view (designed)



(b) Interior view (installed)

FIGURE 1. Structure of an RC.

orientations, and the plate sizes of the two stirrers are also different. Two standard gain antennas with a return loss of lower than −20 dB at 900 MHz were used as the transmit (Tx) antennas [7] and were arranged in different orientations facing the RC wall and screened by a horizontal stirrer such that the line-of-sight (LOS) propagation from the antenna to the working volume was minimized.

The position and polarization of Tx antennas in a well-implemented chamber generally have little effect on the E-field uniformity over the working volume if non-LOS conditions are established between each Tx antenna and the working volume. Nevertheless, several combinations of the polarization and locations of two Tx antennas in an empty chamber were tested, all of which showed a similar mean and SD for the E-field uniformity. Among them, the antenna arrangement providing the best results was selected.

A block diagram of the exposure system is shown in Fig. 2. The exposure system consists of an RC, a signal generator, a power meter, two power amplifiers, E-field monitoring equipment, a motor system, and a control computer. The E-field strength in the RC is monitored and controlled to remain at within ±3% of the target value throughout the exposure period. The control software manages and/or monitors the exposed E-field level, input power level, exposure schedule, stirrer rotation speeds, door sensor, lighting (on/off), temperature, and humidity. The rotation speeds of the two

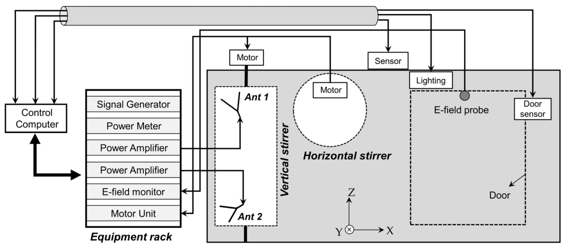


FIGURE 2. Block diagram of the exposure systems.

stirrers are optimized to obtain a uniform E-field distribution in an empty chamber, i.e., 5 revolutions per minute (RPM) and 3 RPM were determined for the vertical and horizontal stirrers, respectively. The chambers were designed to automatically stop the signal output and the stirrer rotation when the door opened for greater safety of the equipment operators.

The E4438C signal generator (Agilent Technologies) providing CDMA-IS95A modulation is used as the signal source. The signal characteristics are the same as those of a 900-MHz CDMA modulated signal used in a US NTP study; in addition, the bandwidth is 1.25 MHz at a center frequency of 900 MHz, and an uplink traffic channel is employed.

The signal amplifiers were customized by TMD Technologies. The maximum output power of each amplifier is 400 W for the CDMA signal at 900 MHz, and two amplifiers were used for the exposure system. The output power of each amplifier is applied to the standard gain Tx antenna ($0.372 \times 0.372 \times 0.12$ m^3) through a 50-dB coupler. The forward power of the amplifier is measured using a power meter.

The E-field strength at a specific location close to the working volume inside the RC is monitored in real time using the FL7006 Kit laser field probe (AR RF/Microwave Instrumentation) to maintain the desired exposure value using a feedback control algorithm. The temperature and humidity are also monitored at ventilation outlets to maintain certain ranges. The lighting equipment is turned on/off every 12 h by the control software. An automatic watering system that does not affect the SAR distribution when rats drink the water was designed and manufactured in a structure similar to that of the US NTP [1].

B. MEASUREMENT METHOD

The probability density function (PDF) of the received signals in a non-LOS environment typically has a Rayleigh distribution. The S_{21} parameter was measured to check whether the received signal follows a Rayleigh distribution in empty chambers and loaded chambers with live rats. Two log periodic antennas (R&S HL040E, Rohde & Schwarz) were inserted in each RC. Outside the working volume of the chamber, one transmitting antenna was placed facing the vertical stirrer, and the other receiving antenna was placed facing the wall of the chamber to avoid making an LOS path. A total of 16,001 S_{21} samples were collected for 10 min.

The spatial E-field distribution was measured for a field uniformity evaluation under the empty and three different

(a) (b)

FIGURE 3. Chambers loaded with rats for measuring E-field distribution. (a) Apparatus+Rat$_{330g}$ and (b) Apparatus+Rat$_{470g}$.

loading conditions mentioned in Section I: a) an empty chamber, b) a chamber loaded with an apparatus (watering systems, animal bedding, cages, and cage racks) to house the animals, c) a chamber loaded with the apparatus and 80 rats with an average weight of 330 g, and d) a chamber loaded with the apparatus and 80 rats with an average weight of 470 g. The arrangement spacing between cages was maintained the same as that planned in actual animal experiments. When loaded with 80 rats, they were hosted in individual cages in the working volume of the RC. These four conditions are hereafter referred to as Empty, Apparatus, Apparatus+Rat$_{330g}$, and Apparatus+Rat$_{470g}$, respectively.

The measurement using live rats was approved by the Institutional Animal Care and Use Committee of KIT (Approval No.: IACUC 2004–0121).

Measurement locations for all four conditions on a three-dimensional grid were identical; in addition, the total number of measurement points was 150 ($5 \times 5 \times 6$) within the working volume ($1.5 \times 1.5 \times 1.3$ m^3). The distance between the measurement points was approximately 0.375 m in the x- and y-axes and 0.260 m in the z-axis (Fig. 4). A 900-MHz CDMA modulated signal with 10 W of power was applied to each of the two antennas. The three E-field components of E_x, E_y, and E_z were measured using a field probe FL7006 Kit at every 100 ms, and the probe-received data were averaged over a 1-min period at each location.

In this study, three types of E-field uniformities for the dataset measured at 150 points were employed: a) three individual standard deviations (SDs) of the three E-field components (E_x, E_y, and E_z), b) the SD of the total dataset consisting of 450 measurements made by combining the three individual components (E_x, E_y, and E_z), and c) the SD of the E-field strength (E_{total}), i.e., the root sum of the squares (RSS) of E_x, E_y, and E_z. The international standard regarding electromagnetic compatibility requires two E-field uniformities of a) and b) [8].

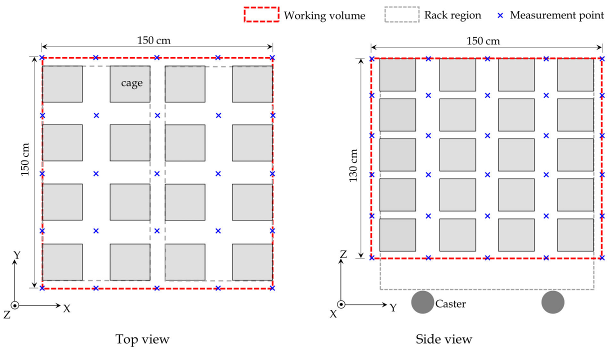


FIGURE 4. Measurement locations for E-field uniformity test.

The quality factor Q of the RC can be calculated using

$$Q = \frac{2\pi V \langle |E|^2 \rangle}{P_{in}\mu_{tx}\eta\lambda}, \quad (1)$$

where V is the volume of the RC, $<|E|^2>$ is the spatial-average of the squared value of the E-field strength, μ_{tx} is the efficiency factor of the Tx antenna, η is the wave impedance of free space, and λ is the wavelength.

According to [9], for the RC to be effective, its Q must be large compared the threshold, Q_{thr}. Here, Q_{thr} is given by

$$Q_{thr} = \left(\frac{4}{3}\pi\right)^{2/3} \frac{3V^{1/3}}{2\lambda}, \quad (2)$$

For the designed RC ($2.5 \times 4.0 \times 2.0$ m^3) used in this study, Q_{thr} is 32 at 900 MHz.

III. RESULTS

The complex scattering parameter, S_{21}, was collected at 37.5 ms intervals and the total number of S_{21} samples was 16,001. Fig. 5 shows the histogram and corresponding theoretical PDF of the Rayleigh distribution for the two Apparatus+Rat conditions. Each theoretical PDF was obtained using (3). Note that the histogram is expressed in terms of density and not frequency. The bin width of each histogram is 0.001 and the total area of all bars is equal to 1.

$$\mathrm{PDF}(x) = \frac{x}{\sigma^2} \cdot \exp\left(-\frac{x^2}{2\sigma^2}\right), \quad (3)$$

where x represents the magnitude of the received signal S_{21}, and σ is the scale parameter of the distribution.

The mean of the $|S_{21}|$ data is approximately 1.2533σ [10]. For RC1, it was 0.0321 and 0.0315 for Apparatus+Rat_{330g} and Apparatus+Rat_{470g}, respectively. An extremely similar mean of $|S_{21}|$ (0.0315 and 0.0330) was observed for RC2. As shown in Fig. 5, the received signal follows a Rayleigh distribution well under each condition.

The spatial E-field data for each condition were normalized to the mean. Fig. 6 shows the log-scaled distributions for E_{total} in the working volume under the Empty and Apparatus+Rat_{470g} conditions. The maximum and minimum E_{total} of an empty chamber were within the mean $\pm$ 1.5 dB (Fig. 6 (a)); however, it was observed that the field distributions are more distorted in the loaded chamber (Fig. 6 (b)).

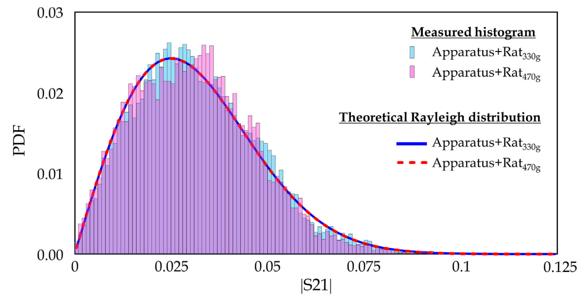


FIGURE 5. Measured $|S_{21}|$ and rayleigh distribution (RC1).

Table 1 shows the E-field uniformity of RC1 under the empty and loaded conditions. The SDs of a), b), and c) represent the three E-field uniformities mentioned in Section II. The arithmetic mean of E_{total} for a total input power of 1 W supplied to the two Tx antennas was 33.3, 27.7, 14.2, and 13.3 V/m, respectively, for Empty, Apparatus, Apparatus+Rat_{330g}, and Apparatus+Rat_{470g}, which demonstrates a field reduction in the working volume resulting from the load effects.

The mean and SD for the body weights of the 80 rats were 331.3 and 17.06 g for Rat_{330g} and 468.8 and 36.27 g for Rat_{470g}. It was observed that the SD increased as the amount of the load increased, indicating degraded spatial field uniformities. Compared to the empty condition, the field uniformities and for the loaded conditions worsened, but there were no significant differences between the two conditions of Apparatus+Rat_{330g} and Apparatus+Rat_{470g}.

The field uniformity of RC2 was also measured under the Empty, Apparatus+Rat_{330g}, and Apparatus+Rat_{470g} conditions, and the results were almost the same as those of RC1. In addition, the mean of E_{total} was 33.3, 14.5, and 13.6 V/m, and the SD of E_{total} was 0.51, 1.08, and 1.09 dB, respectively.

Fig. 7 shows the cumulative distribution function (CDF) of E_{total} normalized to the mean. All 150 E_{total} values were within the mean $\pm$1.5 dB under the empty condition, whereas those under the loading conditions were wider. A grey dotted rectangle in the graph represents the deviation of the 10 to 90 percentiles, indicating that Empty, Apparatus, and the two Apparatus+Rat conditions fall within the mean $\pm$0.5 dB, $\pm$1.0 dB, and $\pm$1.5 dB, respectively.

The CDFs for Apparatus+Rat_{330g} and Apparatus+Rat_{470g} were very similar. This suggests that the whole-body average (WBA) SAR levels for 80 rats exposed within the working volume also vary within a similar deviation from its mean despite the change in the weight of the exposure group.

The E-field strength at a few points outside the working volume was investigated under the Apparatus condition. It is extremely important to find a location close to the mean E_{total} because the E-field strength monitored at that location in real time using the field probe is assumed to be the exposure level. The location right above the working volume was selected and showed an E-field strength with a difference of only 3.8% from the mean E_{total}.

Table 2 shows the Q factor calculated from the 150 measured E-field values for RC1 and RC2. The value is extremely

TABLE 1. E-field uniformity (RC1).

Condition / Item		Empty		Loaded					
				Apparatus[1]		Apparatus+Rat$_{330g}$		Apparatus+Rat$_{470g}$	
		E_{mean} (V/m/W)	SD (dB)	E_{mean} (V/m/W)	SD (dB)	E_{mean} (V/m/W)	SD (dB)	E_{mean} (V/m/W)	SD (dB)
a)	E_x	17.7	0.79	13.7	1.16	6.8	1.55	6.4	1.61
	E_y	18.3	0.72	15.8	1.49	8.3	1.20	7.7	1.42
	E_z	18.8	0.92	15.3	1.11	7.5	2.24	7.0	2.24
b)	E_x, E_y, E_z	18.3	**0.83**	14.9	**1.36**	7.5	**1.82**	7.0	**1.87**
c)	E_{total}	33.3	**0.48**	27.7	**0.78**	14.2	**1.10**	13.3	**1.11**

[1] Apparatus includes watering systems, animal bedding, cages, and cage racks.

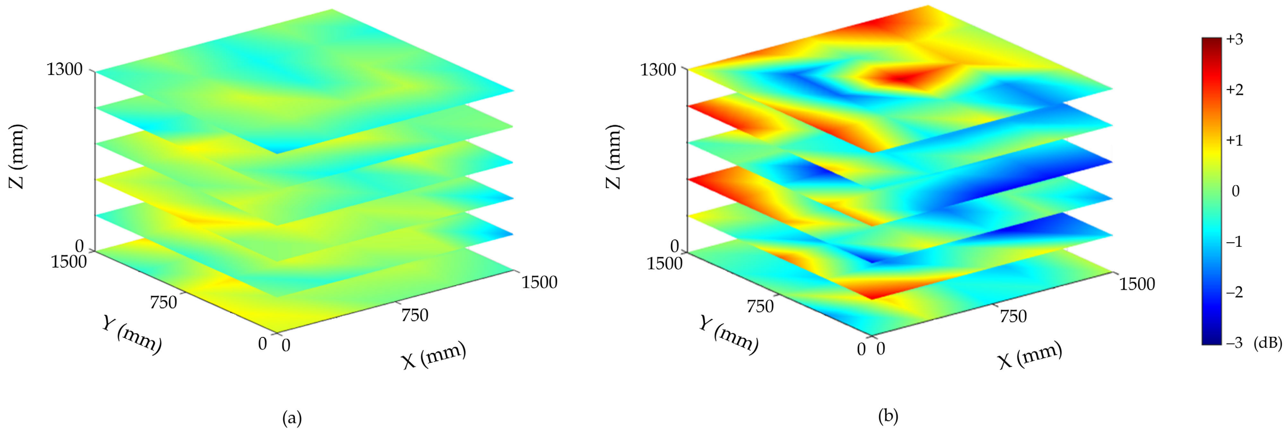


FIGURE 6. Three-dimensional E-field distribution (RC1). (a) Empty and (b) Apparatus+Rat$_{470g}$.

TABLE 2. Q factor of RCs.

Condition	RC1	RC2
Empty	1116	1113
Apparatus+Rat$_{330g}$	204	215
Apparatus+Rat$_{470g}$	181	188

similar between the two chambers and much higher than that of Q_{thr}, which was given in (2), even for the case using live 470-g rats.

Table 3 lists the contributions to the uncertainty of the measured E-field level at the E-field monitoring location. The values of all contributions except the field control were determined from the manufacture-provided specifications. An uncertainty of 0.26 dB for the field control was used because the monitored field level was set to remain within ±3% of the target level. As a result, the combined standard uncertainty for the E-field measurement was 0.63 dB.

IV. DISCUSSION

For a successful international joint animal study, it is one of the basic requirements for each country to use the same exposure system. Two RCs, one for the exposed group and the other for the sham control group were installed by KSS, Ltd. in both countries based on the same design drawings considering the given GLP facilities of Korea and Japan. The distance between animals is an important factor because it affects the deviation in the WBA SAR between the exposed subjects despite the uniformity of the exposed field distribution. The larger the spacing, the better the SAR uniformity; however, the acceptable number of rats decreases [11]. The resultant working volume of the implemented RC can accommodate up to 80 rats hosted in individual cages with distances of 0.350, 0.310, and 0.235 m in the x-, y- and z-axes, respectively, between the center of the cages.

The US NTP study reported that 112 rats were exposed in an RC. The field measurement was conducted at 216 points (6 × 6 × 6) within a volume of 1.5 × 1.5 × 1.5 m^3 under the Empty condition, and the SD of E_{total} and E_x, E_y, E_z was 0.59 and 0.84 dB, respectively at 900 MHz, indicating a performance similar to that of the present study. The field uniformity of a chamber loaded with rat phantoms with only 32 measurement points easily accessible at the cage tops was analyzed, and a field uniformity with a mean of ±2.8 dB was reported [1].

In this study, the E-field uniformity was evaluated for measurements taken at 150 points in a loaded chamber with 80 live rats as well as in an empty chamber. The E-field uniformity was within ±1.0 dB of the mean under the empty condition but was degraded to ±2.3 dB of the mean under the loading conditions. The signal received in the two chambers followed a Rayleigh distribution well, and the Q factor

TABLE 3. Measurement uncertainty of monitored E-field strength.

Item	Uncertainty (dB)	Distribution	Divisor	Standard Uncertainty (dB)
Field amplitude accuracy	0.8	Normal	2	0.4
Isotropy	0.5	Rectangular	$\sqrt{3}$	0.29
Dynamic range linearity	0.5	Rectangular	$\sqrt{3}$	0.29
Field control	0.26	Normal	1	0.26
Combined standard uncertainty				**0.63**

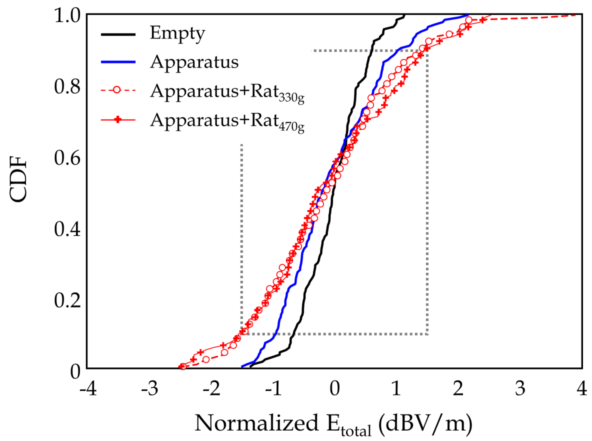


FIGURE 7. CDF for E_{total} (RC1).

calculated with the average measured E-field distribution was much larger than the threshold value.

A specific location outside the working volume where the measured E-field strength represented the mean E_{total} of the working volume was carefully selected. The E-field strength at this location is currently being measured, monitored, and maintained at the desired value using a feedback control algorithm throughout our long-term animal experiments.

V. CONCLUSION

An RC is considered a suitable facility for large-scale EMF exposure experiments on small animals such as rodents because it allows free movement and a simultaneous exposure to a spatially homogeneous field. However, an increase in the lossy materials, i.e., the number of subjects, degrades the performance of the field uniformity and decreases the field level.

There are no standard requirements for an RC for animal experiments other than an electromagnetic compatibility (EMC) test. The evaluated E-field uniformity, S_{21}, and Q factor in this study fully satisfy the requirements for an EMC test.

This international study, jointly conducted by Korea and Japan for validating the US NTP research results, requires a practically constant exposure level over the lifetime of the animals within individual cages. Therefore, the locations of the exposed subjects are being rotated periodically throughout the exposure period to minimize the differences in exposure level by location.

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