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Brief Communication

An International Collaborative Animal Study of the Carcinogenicity of Mobile Phone Radiofrequency Radiation: Considerations for Preparation of a Global Project

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Radiofrequency radiation (RFR) was classified as a "possible" human carcinogen in 2011, which caused great public concern. A carcinogenicity study by the National Toxicology Program (NTP) found Code Division Multiple Access—and Global System for Mobile Communications—modulated mobile phone RFR to be carcinogenic to the brain and heart of male rats. As part of an investigation of mobile phone carcinogenesis, and to verify the NTP study results, a 5-year collaborative animal project was started in Korea and Japan in 2019. An international animal study of this type has two prerequisites: use of the same study protocol and the same RF-exposure system. This article discusses our experience in the design of this global study on radiofrequency electromagnetic fields (RF-EMFs). Bioelectromagnetics. 43:218–224, 2022. © 2022 The Authors. Bioelectromagnetics published by Wiley Periodicals LLC on behalf of Bioelectromagnetics Society.

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In 2011, radiofrequency radiation (RFR) was classified as a Group 2B "possible" human carcinogen by the International Agency on Cancer (IARC), an agency of the World Health Organization [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2013] based on limited evidence of carcinogenicity in humans and in experimental animals [Melnick, 2019]. Public concern is focused on the possible association between mobile-phone RFR and cancer.

The National Toxicology Program (NTP), a federal interagency group under the National Institutes of Health, conducted a large animal study to assess the carcinogenic effects of mobile phone RFR. Partial findings were revealed in May 2016 [Wyde et al., 2016], and the pilot study report [Wyde et al., 2018] and the final report [National Toxicology Program [NTP], 2018] were published in 2018. The NTP study found significantly high rates of malignant glioma in the brain and cardiac schwannoma of male, but not female, rats exposed to Code Division Multiple Access (CDMA)—and Global System for Mobile Communications (GSM)—modulated RFR (900 MHz).

There are always experimental uncertainties in in vivo studies of live animals, and definitive conclusions cannot be drawn from a single trial, regardless of its scale [Falcioni et al., 2018; Kim et al., 2020]. Moreover, animal studies with poor reproducibility cannot be considered objective scientific evidence [Voelkl et al., 2020]. Although the NTP study raised important issues regarding the carcinogenicity of mobile phone RFR, a validation study could not be conducted after the systems used for the animal studies had been removed. The NTP has been performing follow-up research with a smaller RFR-exposure chamber to investigate the effects of RFR on biological systems, including investigation of biomarkers and the role of stress in RFR carcinogenesis [National Toxicology Program [NTP], 2020]. While this follow-up study is worthwhile, further animal studies are essential to validate the carcinogenicity of RFR, even if the study conditions are not exactly the same. Since the NTP study findings were released, the need for further research to validate the NTP study has been cited continuously. The NTP and the International Commission on Non-Ionizing Radiation Protection (ICNIRP) have recommended further validation studies to clarify the NTP results [International Commission on Non-Ionizing Radiation Protection [ICNIRP], 2018], and at the Global Coordination of RF Communications on Research and Health Policy (GLORE) meeting in November 2016 (Yokohama, Japan), officials from Korea and Japan agreed to conduct a joint study to validate the results of the NTP study.

When international projects are conducted in two or more different countries, the study environments inevitably differ. There are two prerequisites for such studies on RFR: the RFR-exposure system and the study protocol must be the same in all participating countries. This international project is being conducted in two different cities: Daejeon, Korea (36.35°N 127.39°E) and Ichinomiya, Japan (35.30°N 136.80°E). Although their latitudes and longitudes differ, Korea and Japan are neighbors in northeast Asia, and in the same time zone. Researchers from both countries listed, and subsequently reviewed, the following eight basic protocol elements: target diseases; animal species (numbers of rats and study groups); details of the RF-exposure system including specific absorption rate (SAR); types of evaluations (in vivo observations and measurements); genotoxicity; anatomical pathology; statistical analysis; an international advisory committee (IAC); and standard protocols.

The NTP study found evidence of carcinogenicity (Table 1). The three most common tumors in male rats were nervous system tumors: malignant glioma, a central nervous system tumor; schwannoma, a peripheral nervous system tumor; and pheochromocytoma, a neuroendocrine tumor. Therefore, our animal study is focusing on nervous system tumors. Malignant glioma is the first target. The level of carcinogenic evidence for this tumor was "equivocal"

TABLE 1. Summary of the Levels of Evidence Before and After Peer Review in the NTP Study

			Levels of evidence; before and after peer review	
Sex	Neoplastic lesions	Modulation mode	Before	After (revised)
M	Malignant schwannomas, heart	GSM	Some	Clear
		CMDA	Some	Clear
	Malignant glioma in brain	GSM	Equivocal	Some
		CMDA	Equivocal	Some
	Pheochromocytoma, adrenal gland	GSM	Equivocal	Some
		-	-	-
F	Malignant schwannomas, heart	GSM	No	Equivocal
		CMDA	Some	Equivocal

Abbreviations: CDMA = Code Division Multiple Access; GSM = Global System for Mobile Communications; NTP = National Toxicology Program.

in the initial NTP report; however, this changed to "some" in the final report. Malignant brain tumors include malignant glioma and glioblastoma multiforme, which is the most common primary brain malignancy; it is highly aggressive and tends to spread to the brain parenchyma [DeCordova et al., 2020]. The tumor is impossible to completely remove by surgery and the patient prognosis is extremely poor. Therefore, although the level of evidence was classified as "some" in the NTP study, it is important to validate that finding. Schwannoma of the heart is also being assessed. Cardiac tumors including both benign and malignant ones are extremely rare in humans [Li et al., 2021], while they are relatively common spontaneous tumors in rat species compared to human. Schwannomas are common tumors of the nerves and nerve sheaths; most are benign. In this study, all organs will be evaluated for evidence of carcinogenicity.

Harlan Sprague–Dawley rats are being used in our study, as in the NTP study (Envigo, Indianapolis, IN). Since evidence of carcinogenesis was found only in male rats in the NTP study, we are examining only male rats. The NTP study included both sham-exposed and RF-exposed groups, and used a historical control group in the absence of a cage–control group. Our international study includes cage–control, sham-exposed, and RF-exposed groups.

For their 2-year study, the number of male rats per CDMA exposure group was 90 after the interim study, i.e. more than the 50 recommended by the Organization for Economic Co-operation and Development (OECD) [2018]. In our study, 70 male rats are being used in each country, for a total of 140 rats, which should enhance the statistical significance of the study.

A 900-MHz CDMA RFR was selected for this study, as it is the most commonly used frequency and waveform in Korea and Japan. The duration of RFR exposure has been set to 18 h and 20 min per day, with a continuous cycle of 10 min on and 10 min off, as in the NTP study. In the NTP study, utero exposure was started on GD6 in the 28-day study and on GD5 in the 2-year study. On the other hand, in this study, utero exposure was started on GD5 in both the 28-day and 2-year studies. The NTP study used four different intensities based on the results of their pilot study. For the current international study, a single RF exposure intensity, a whole-body averaged specific absorption rate (WBA-SAR) of 4 W/kg, was selected. This exposure level corresponds to the operational adverse-health-effect threshold for an increase in body core temperature of 1 °C in human, to protect against adverse effects from acute exposure [International Commission on Non-Ionizing Radiation Protection [ICNIRP], 2020]. The ICNIRP limits the maximum permissible exposure to RFR (100 kHz to 300 GHz) for occupational workers and the general public to WBA-SAR values of 0.4 and 0.08 W/kg, respectively [International Commission on Non-Ionizing Radiation Protection [ICNIRP], 2020], based on the results of animal studies. Therefore, it is important to study whether RF exposure at a WBA-SAR of 4 W/kg is carcinogenic to animals. As the NTP study obtained significant results for RF exposure at a WBA-SAR of 6 W/kg, but not at 3 W/kg, an exposure level of 4 W/kg was considered optimal when using only one exposure condition. In this study, the exposure levels are being adjusted periodically to maintain the WBA-SAR as the animals gain weight. However, the WBA-SAR was found to be dependent on how many animals are exposed in the chamber in simulation because of the shadowing effect on wave propagation, caused by neighboring animals. Therefore, in this study, the WBA-SAR was calculated considering both the number of exposed rats and the weight of the rats. In the animal experiment, the E-field strength has been determined according to the number of survived rats

and their average weight [Jeon et al., 2021a,b; Ito et al., submitted].

As part of the basic requirements for the international study, an RF-exposure system was designed and an exposure-assessment protocol was prepared. A reverberation chamber (RC) was custom-designed by the Electronics and Telecommunications Research Institute (ETRI, Daejeon, Korea) and Nagoya Institute of Tech nology (NIT, Nagoya, Japan). Compared to the system used in the NTP study, the volume of the RC is almost similar, while the uniform area is slightly larger (Table 2). The system was manufactured by Korea Shield System (KSS, Cheongju, Korea). Details of the RF-exposure system have been reported previously [Jeon et al., 2021a,b]. Briefly, the system has two stirrers for each RC (KSS),

TABLE 2. Comparison of Experimental Design Between the NTP Study and the International Project

	National Toxicology Program	International
Study	Single institutional	Multi-institutional
Study phases	Three-phase study 1. 5-day pilot studies 2. 28-day prechronic toxicology studies 3. 2-year toxicology and carcinogenicity studies	Two-phase study 1. 28-day toxicology study 2. 2-year carcinogenicity study
Institutions	Illinois Institute of Technology (IIT) Research institute Chicago, USA: 41.88/-87.62	Korea Institute of Toxicology (Korea) DIMS Institute of Medical Science, Inc. (Japan) Daejeon, Korea: 36.35/127.39 Ichinomiya, Japan: 35.18/136.91
Reverberation chamber	Custom-designed 21 RCs (14 RC for rats) IT'IS foundation, Switzerland External dimension: $2200(W) \times 3700(L) \times 2600(H)\ mm^3$ Uniform area: $1500 \times 1500 \times 1500\ mm^3$	Custom-designed 4 RCs (two for each institution) Korea Shield System Ltd., Korea External dimension: $2536(W) \times 4052(L) \times 2052(H)\ mm^3$ Uniform area: $1500 \times 1500 \times 1300\ mm^3$
RF Frequency	900 MHz and 1,900 MHz	900 MHz
RF Modulation	CDMA- and GSM-modulated cell phone RFR	CDMA-modulated cell phone RFR
RF Exposure Intensity	Time-averaged Averaged weight-based SAR 0 (sham control), 1.5, 3, and 6 W/kg	Time-averaged both weight-and number of surviving rat-based SAR 0 (sham control), and 4 W/kg
Animal	Sprague–Dawley (HSD: Sprague–Dawley SD), rat and B6C3F1 mouse female and male	Sprague–Dawley (HSD: Sprague–Dawley SD) Rat only Male only
Number of rats per group in 2-year study	90	140; 70 (Korea) and 70 (Japan)
Number of rats in each stage of the 2-year study	F1 core study: 90 male and female GSM-exposed F1 core study: 90 male and female CDMA-exposed F1 interim study: 10 male and female F1 genetic toxicity: 5 male and female	140; F1 core study: 70 male CDMA-exposed (each country) F1 interim genetic toxicity: 5 (each country)
Experimental groups	Four groups Sham- and RF-exposed (three different ones)	Three groups Sham-, RF-exposed (one), and cage-control

Abbreviations: WBA SAR = whole-body averaged specific absorption rate; RFR = radiofrequency radiation.

a standard gain antenna [Telecommunications Industry Association [TIA], 1989], an E4438C signal generator (Agilent Technologies, Santa Clara, CA) operating in CDMA-IS95A mode, a custom-made signal amplifier (TMD Technologies, Hayes, UK), and four 20-W fluorescent lamps that were mounted on the ceiling. We used the fluorescent lights instead of the incandescent lights used in the NTP study. This is to provide the same lighting environment for the RF exposure group, the sham group, and the cage control group in our study. All connections of the fluorescent lamps were RF-filtered. The RF E-field was measured to check the radiation of RF energy from the fluorescent lamps, and a difference was not found in RF radiation for the on/off of the fluorescent lamps. The illuminance is set to 150–300 lux and the light source is 0.85 m from the floor, according to the standard guidelines of the Institutional Animal Care and Use Committee (IAACUC). KSS installed two chambers at the Korea Institute of Toxicology (KIT) in Daejeon, Korea, and two at the DIMS Institute of Medical Sciences (DIMS) in Ichinomiya, Japan. Both institutes are in compliance with the OECD Principles of Good Laboratory Practice (GLP) [Organisation for Economic Co-operation and Development [OECD], 1998]. The chamber systems were verified by ETRI and NIT before the 28-day study began, and before the 2-year study. Although the chamber systems are the same, the size and shape of the rooms that house them differ (Table 2).

To maintain a stable study environment, the temperature (23 ± 3 °C), humidity ($50 \pm 15\%$), light cycle (12 h light/12 h dark), light intensity (150–300 lux), air changes (10–20/h), noise (<60 dBA), ammonia concentration (<20 ppm), and air pressure (1–5 mm H_2O) in the RCs are set according to the GLP guidelines [Organisation for Economic Co-operation and Development [OECD], 1998] in both countries. The rats are housed in polycarbonate cages ($235 \times 260 \times 210\ mm^3$), as in the NTP (Kyeryong Science, Daejeon, Korea). The choke, rack, and automatic water supply system were designed (Kyeryong Science) to minimize the influence of RF radiation on the rats [Capstick et al., 2017]. The choke was modified according to cage spacing, and to avoid concentrated RF radiation around the mouth when the rat sucked on the end of the stainless auto drain system. Local municipal tap water is supplied to the animals ad libitum in both countries, after being irradiated by ultraviolet light. Food is prepared for the prenatal (PMI Nutrition International, New York, NY) and postweaning (Envigo) phases. The same bedding and environmental enrichments are being used in both institutions (Tapvei, Harjumaa, Estonia).

Bodyweight and food consumption are being monitored. The amounts of food provided to each animal are measured, along with residual food. Body temperatures were monitored during the 28-day toxicity study, but are not being monitored during the 2-year lifelong study, as in the NTP study. Temperatures were measured rectally using a device designed for rats (AD-1687; A&D, Tokyo, Japan) as quickly as possible after the second exposure (15:00). The temperatures of the sham-exposed and cage–control groups were measured after those of the RF-exposed group. Clinical signs, mortality, moribundity, general appearance, and behavioral changes are recorded (date/time and duration). The physical condition of each animal is established by a veterinarian or experienced technician. Mortality and morbidity observations are made twice daily, except during the acclimation period. Additional observations are conducted on the day of necropsy. To evaluate genotoxicity, the Comet assay, which evaluates DNA damage in the prefrontal cortex, hippocampus, cerebellum, liver, and blood leukocytes, and the bone marrow micronucleus assay, which evaluates cytogenetic damage, are performed at the 14-week interim evaluation. A pathological evaluation is also performed. After all of the slides have been analyzed, they are sent to the test facilities of KIT and DIMS for pathology peer review. In the 28-day study, data analysis is performed in a nonblinded manner. However, in the 2-year study, an initial pathology review is performed in a nonblinded manner by KIT and DIMS. If pathology peer review is required after the initial pathology review, it is also performed in a nonblind manner by the pathology peer review committee. For any observed RF exposure-related findings, the data are reviewed in a blinded manner by the pathology working group that consists of specific-organ specialists and pathologists. The RF-exposed, sham-exposed, and cage–control groups are being compared statistically. The final report will include all details required by the applicable regulatory agency, including the test system, study procedures, results, discussion, and conclusions.

To ensure successful performance of this global project, an international advisory committee (IAC) was formed and eleven international experts, including two each from Korea and Japan, were invited as members of IAC. The project leaders and active researchers from the two countries are not members of the IAC, but they organize the IAC meetings and actively participate.

As there have been no previous international animal studies of radiofrequency electromagnetic fields (RF-EMFs), no study guidelines exist. Because this project is being conducted in two different countries, a detailed description of the study protocols is important. For the

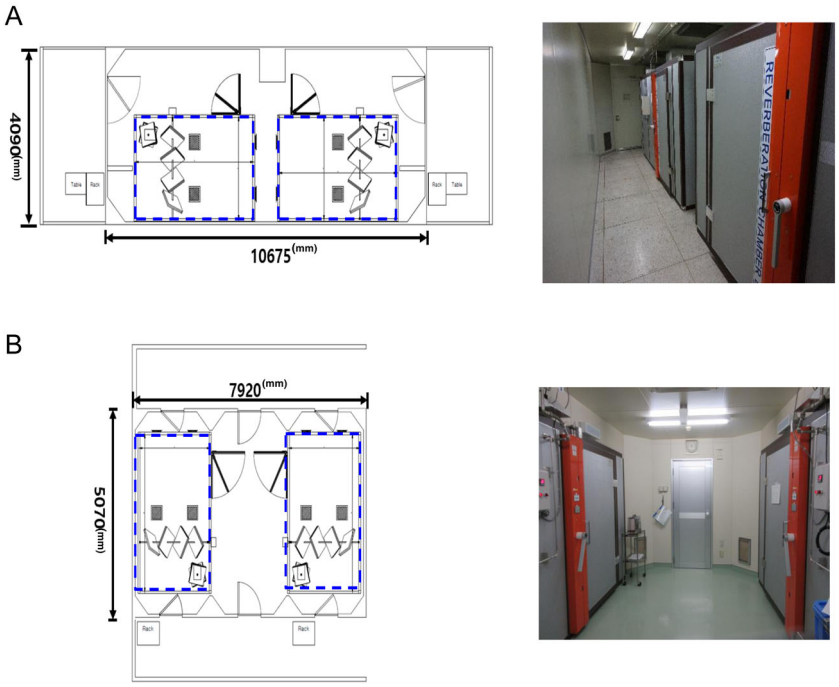

Fig. 1. Comparison of the RF exposure system including reverberation chambers. The GLP room for the chambers in KIT (**A**) and DIMS (**B**). The dimension of RC chamber is the same in both countries ($2500 \times 4000 \times 2000\ mm^3$). DIMS = DIMS Institute of Medical Sciences; GLP = good laboratory practice; KIT = Korea Institute of Toxicology; RC = reverberation chamber; RF = radiofrequency.

28-day toxicity and 2-year carcinogenicity studies, standard protocols were drawn up according to OECD guidelines (Nos. 451, 489, and 474) and GLP guidelines. The animal-welfare procedures conform to the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research (ILAR).

The International Validation Project of the NTP Study on the Carcinogenesis of Mobile-Phone RFR was launched in 2019 and involves two studies, as stated above: a 28-day toxicity study and a 2-year carcinogenicity study. The trials are being conducted simultaneously in Korea and Japan (Fig. 1).

While this project is much smaller than the NTP study, it includes more animals in each group (Table 2). While the NTP study used several thousand rats and mice, this study is using only 225 male rats per country. As there is only one RF exposure condition (4 W/kg), we are not examining the dose-response and are instead focusing on the carcinogenicity of CDMA-modulated RFR at 4 W/kg.

As this study is being conducted in two different countries, there are differences in the geomagnetic field and local water supply. Although the ventilation systems of the two institutes also differ, both are in accordance with the GLP and OECD guidelines (Table 2).

As mentioned above, an international animal study of this nature must use the same study protocol and RF-exposure system. Close communication between researchers is also important. Herein, we have discussed our experience in designing this international animal study of the effects of RF-EMF, and described the prerequisites (minimum study requirements).

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