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Effects of Long-Term and Multigeneration Exposure of *Caenorhabditis elegans* to 9.4 GHz Microwaves

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A large number of studies on the biological effects of microwaves are carried out using rodents and cells, but the conditions are difficult to control, and the irradiation period is short; the results obtained have always been controversial and difficult to reproduce. In this study, we expose nematodes to an electromagnetic environment for a long-term and multigeneration period to explore the possible biological effects. Wild-type N2 strains of *Caenorhabditis elegans* are exposed to 9.4 GHz microwaves at a specific adsorption rate of 4 W/kg for 10 h per day from L1 larvae to adults. Then, adult worms are washed off, and the laid eggs are kept to hatch L1 larvae, which are continuously exposed to microwaves until passing through 20 generations. The worms of the 10th, 15th, and 20th generations are collected for index detection. Interestingly, we found that the fecundity of *C. elegans* decreased significantly in the exposed group from the 15th generation. At the same time, we found that the growth of *C. elegans* decreased, motility decreased, and oxidative stress occurred in the exposed group from the 10th generation, which may play roles in the decreased spawning in worms. We preliminarily believe that the microwave energy received by worms leads to oxidative stress, which causes a decrease in the spawning rate, and the underlying mechanism needs to be further studied. Bioelectromagnetics. 43:336–346, 2022. © 2022 Bioelectromagnetics Society.

Keywords: 9.4 GHz microwave; *Caenorhabditis elegans*; fecundity; long-term multigeneration exposure; ROS

INTRODUCTION

With the rapid development of science and technology, people's daily lives can no longer be carried out normally without electronic and communication equipment. In the case of strict enforcement of relevant standards such as IEEE [2019] and ICNIRP guidelines (2020) [ICNIRP, 2020], the electromagnetic environment that people come into contact with in daily life is considered to be safe, but the possible health effects of long-term exposure are still unknown. Although the development of electromagnetic wave technology has been very fast, the present electromagnetic environment has not existed for a long time in people's daily lives. For example, mobile communication has only been in use no more than 50 years since the production of the first mobile phone in the United States. Therefore, little is known about the possible effects of electromagnetic radiation on future generations.

In 2011, the International Agency for Research on Cancer of the World Health Organization listed radiofrequency radiation as a class 2B human carcinogen [Baan et al., 2011], but there is a lack of experimental data on the direct relationship between radiofrequency radiation and human cancer. There are massive studies on the biological effects of electromagnetic radiation [Peng et al., 2021; Romeo et al., 2021; Zosangzuali et al., 2021], but there is also much controversy [Verbeek et al., 2021]. Some conclusions are even diametrically opposite, and many results are difficult to reproduce. The main reason is that the electromagnetic radiation energy is low, the damage is

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Conflict of interest: None.

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small, and it is easy to recover and not easy to detect. Therefore, it is necessary to find a new research model or experimental animal to explore the possible damaging effects of microwave exposure.

Since Sydney Brenner first used *Caenorhabditis elegans* (*C. elegans*) for scientific research in 1974 [Brenner, 1974], this nematode species has become an excellent model organism. Because of its small size, constant number of cells, short reproductive cycle, transparency, ease of observation, and environmental sensitivity, it has good prospects for application in the study of the biological effects of electromagnetic waves. Rodents or nerve cells are often used [Bayat et al., 2021; Dong et al., 2021], but the advantages of *C. elegans* are often ignored in this field. *C. elegans* can be exposed for a long period of time, and the conditions are easy to control. In this study, we kept *C. elegans* passaged in a 9.4 GHz microwave environment for 20 generations to study the possible biological effects of low-power electromagnetic radiation with a specific adsorption rate (SAR) of 4 W/kg. For nematodes, 9.4 GHz microwave could completely penetrate their body. The microwave energy was mainly concentrated on the surface of the culture medium, and the microwave absorption in the medium was low, which the nematodes could ignore. This model was very suitable for studying the biological effects of microwaves with a frequency above 6 GHz.

MATERIALS AND METHODS

Laboratory Animals and Irradiating Devices

Wild-type N2 strains of *C. elegans* were kindly provided by the American Caenorhabditis Genetics Center (CGC). *E. coli* OP50 (streptomycin$^+$) was kindly provided by Professor Mitani, Tokyo Women's Medical University, Japan. We built a nematode exposure system in a microwave darkroom, where the temperature and humidity remained constant. The humidity was 50%, and the temperature was 21 ± 0.5 ℃. The CM-H2DCFDA probe for reactive oxygen species (ROS) detection was purchased from Sigma (Shanghai, China).

Culture and Synchronization of *C. elegans*

The wild-type N2 strains of *C. elegans* were cultured in a 90 mm petri dish with nematode growth medium (NGM). Adult nematodes were collected and treated with 5 ml nematode lysate containing 400 µl 8% sodium hypochlorite, 80 µl 10 M NaOH, and 4.52 ml M9 buffer. The solution was shaken vigorously by hand for approximately 10 min. At the same time, the state of the lysate was observed. The lysate solution was centrifuged for 1 min at 1200 rpm to collect eggs and washed three times with M9 buffer. The eggs were inoculated on NGM agar plates without *Escherichia coli* OP50 for 16 h at 20 °C ($\pm$ 0.1 °C) to hatch L1 larvae. The synchronized L1 larvae were then collected and placed on 90 mm NGM agar plates with *E. coli* OP50 seeded. These nematodes were recorded as the F1 generation.

Experimental Diagram

According to the above method, *C. elegans* were exposed from L1 larvae of the F1 generation. Continuous exposure to the adult larvae started at 8:00 am and ended at 6:00 pm every day. When the *C. elegans* adults laid a certain number of eggs, all adult worms were washed off. The hatched L1 larvae from the eggs of the F1 generation were continuously exposed to 9.4 GHz microwaves and were marked as the F2 generation. This same method was repeated, and worm exposures continued to the F20 generation (Fig. 1). The nematodes of the sham exposure group were placed in a small box made of copper mesh on the side of the exposure system, which was treated in the same way but without microwaves. *C. elegans* of generations F10, F15, and F20 of the exposure and sham groups were collected and tested to determine parameters including body length, motility, brood size, ROS levels, and lifespan.

Microwave Irradiation

The *C. elegans* specimens were irradiated by a 9.4 GHz continuous wave signal. To expose *C. elegans* to a relatively uniform electromagnetic field, the distance *h* between the antenna aperture and the nematode plane was selected as 67 cm, which is arger than the far-field distance of the antenna (53 cm). The configuration of the exposure system is shown in Figure 2, which is composed of a signal generator (AV1431, CETC, Qingdao, China), an amplifier (NTWPAS-6018020; RFlight, Shenzhen, China), a horn antenna (HD-100SGAH15 + N; Hengda Microwave, Xi'an, China), and a petri dish. The horn antenna was facing up, and a foam board was placed above it. A petri dish containing *C. elegans* was inverted on the foam board. The electric field was also measured and was approximately 70 V/m when the input power was 2.6 W, which is almost consistent with the calculation.

The electric field distribution of the exposure system is simulated and shown in Figure 3. The electric field at the petri dish position is relatively a plane wave, which ensures the uniformity of the

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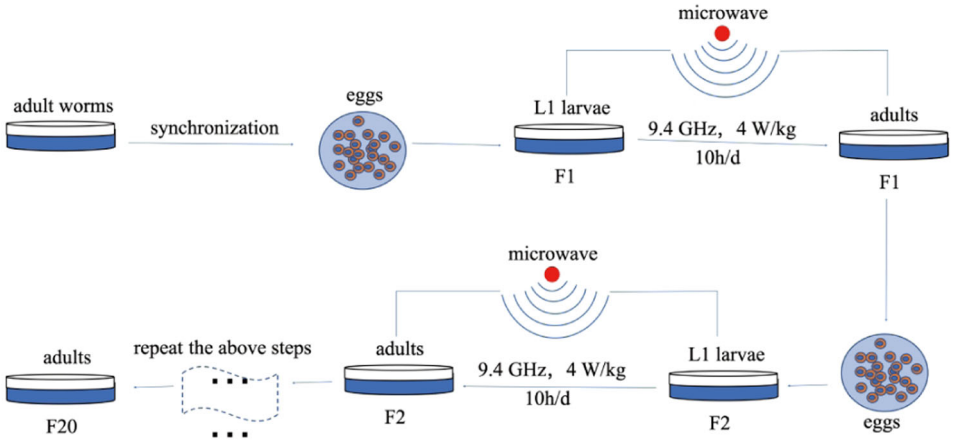


Fig. 1. Schematic diagram of experiment.

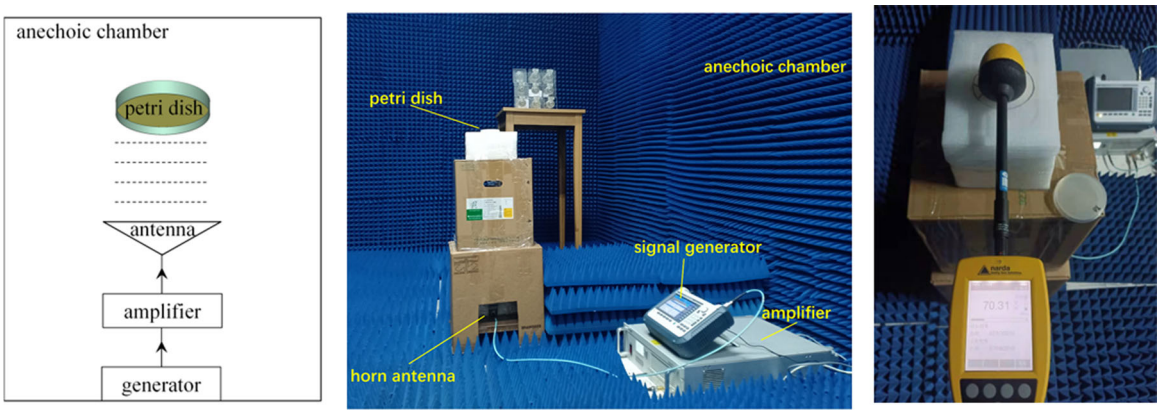


Fig. 2. Configuration of the radiation system and field measurement.

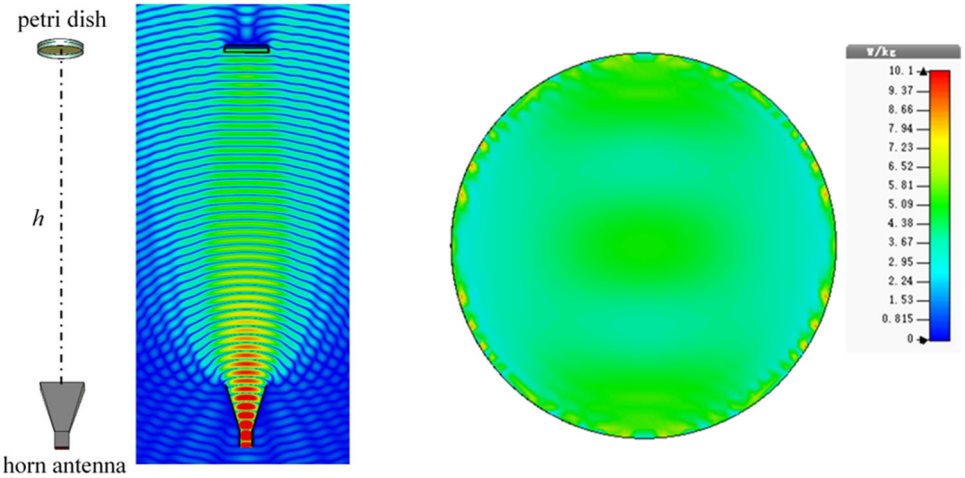


Fig. 3. Electric field and SAR distributions. SAR = specific adsorption rate.

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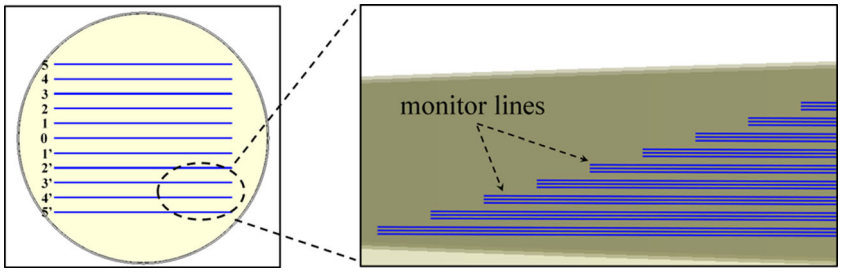


Fig. 4. SAR monitor line distribution. SAR = specific adsorption rate.

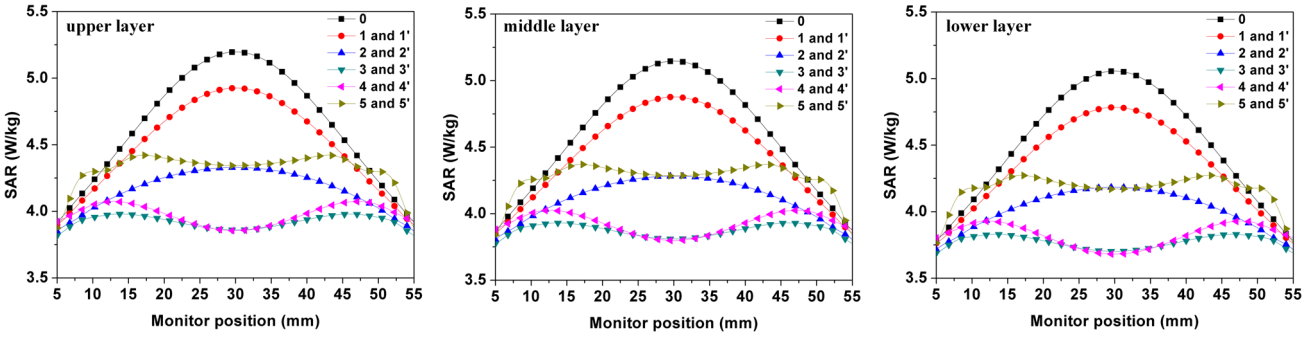


Fig. 5. SAR distribution of three layers in the nematode plane. SAR = specific adsorption rate.

electric field at the nematode plane. The SAR is also calculated based on the electric field simulation results and (1).

$$\mathrm{SAR} = \frac{\sigma E^2}{2\rho} \tag{1}$$

where σ is the conductivity of the culture medium (S/m), E is the electric field strength in the culture medium (V/m), and ρ is the density of the culture medium (kg/m^3). Because the dielectric properties of the culture medium vary with the frequency, the dielectric properties of the used culture medium were measured ($\sigma_{9.4GHz} = 15$ S/m, the relative permittivity $\xi_{9.4GHz} = 63$, and ρ is approximately 1000 kg/m^3). The SAR distribution at the nematode plane is shown in Figure 3.

To obtain the SAR inhomogeneity averaged over a volume with a height comparable to the nematode thickness, we added some monitoring lines (with symbols of 0–5 and 1′–5′ shown in Fig. 4) in the nematode domain. As the thickness of *C. elegans* is approximately 70 μm [Zhao and Jiang, 2010], the monitor lines were divided into three layers, namely, the upper layer, the middle layer, and the lower layer, as shown in Figure 4. The distance between each layer was 35 μm, and a local mesh function was used for simulation.

Figure 5 shows the monitored SAR distribution data in three layers with an injected continuous microwave power of 2.6 W in simulation. One can see that the SAR distribution trends are almost the same, but as the layer level decreases, the values drop slightly. According to the SAR distribution of the three layers, the mean SAR in the three layers is approximately 4.16 W/kg, and the standard deviation is approximately 0.33.

Detection of the Surface Temperature of NGM Medium

The temperature changes on the surface of NMG medium where *C. elegans* live were detected in real-time with a nonmetallic fiber-optic probe (Lumasense Technology, Santa Clara, CA). Three equally spaced (15 mm) and radially distributed holes were drilled for temperature probe insertion to detect real-time temperature changes in the nematode domain during microwave irradiation. The temperature data were recorded for 2.5 h before irradiation, 17.5 h during irradiation, and 1 h after irradiation.

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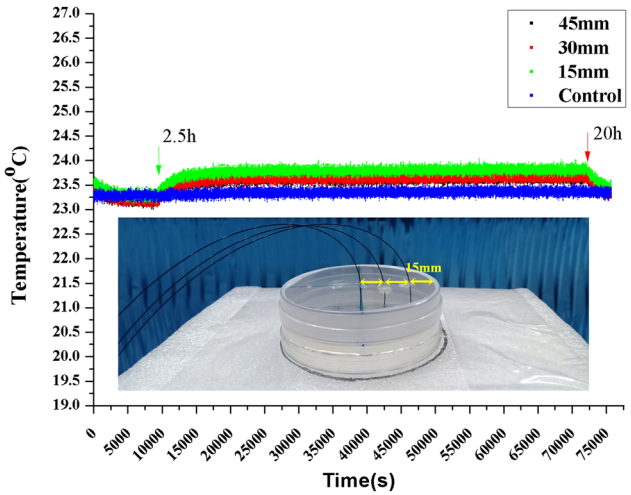


Fig. 6. The temperature distribution in the nematode plane. The green arrow is the start time point of microwave exposure, and the red arrow is the end time point of microwave exposure.

Detection of Body Length

The *C. elegans* body length of generations F10, F15, and F20 was measured after long-term irradiation by low-power microwaves. Ten worms were randomly selected from each group and imaged using a Nikon SMZ-1500 stereoscopic zoom microscope (Nikon, Yokohama, Japan) combined with NIS-Elements F4.0 software (Nikon). The magnification was set to ×30. The images were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD) to determine the body length of *C. elegans*. We used the following parameters: length and create trace feature (Thresh = 3, Smooth = 0, Speed = 3, Noise = 3), with pixels as the unit of length in Image-Pro Plus 6.0.

Detection of Locomotory Capacity

To detect the locomotory capacity of *C. elegans*, a body bending assay was performed. In this experiment, 10 worms were randomly selected by a special pick needle and placed onto a new NGM plate without *E. coli* OP50. After 2 min of adaptation, the worms were tracked using a Nikon SMZ-1500 stereoscopic zoom microscope and NIS-Elements F4.0 software. The number of body bends, termed locomotory capacity, was manually counted over a 30-s period by reviewing the video.

Reproduction Assay

Nine worms in the L4 larval period of each group were selected and transferred onto three new 35 mm NGM plates with *E. coli* OP50, and there were three nematodes in each dish. The number of eggs laid by the worms was counted every day until no more progeny were produced. Each experiment was repeated at least three times.

Survival Curve After Microwave Radiation

One hundred worms were selected and transferred onto a new NGM plate from each group for lifespan analysis. The survival, death, and loss count of the worms were recorded starting on Day 10. The experiment was repeated at least three times.

ROS Level Detection

ROS levels in *C. elegans* were detected by the CM-H2DCFDA probe on Days 0, 1, 3, 7, and 12 after microwave irradiation [Miyakawa et al., 2001; Dawe et al., 2006]. Ten worms were randomly selected from each group and transferred to 1.5 ml Eppendorf tubes. Worms were incubated in M9 buffer containing 10 μM CM-H2DCFDA probe for 20 min in the dark and then washed three times with M9 buffer. Next, worms were treated with 2 μl 5 mM levamisole. Fluorescence microscopy images were photographed using an Olympus IX71 inverted microscope (Olympus, Tokyo, Japan) combined with NIS-Elements F4.0 software. The fluorescence intensity was observed by fluorescence microscopy under an excitation light wavelength of 488 nm and emission light wavelength of 510 nm. The fluorescence intensity was quantified using ImageJ (National Institutes of Health, Bethesda, MD).

Statistical Analysis

All experiments were repeated at least three times, and the values are expressed as the mean ± standard deviation (Mean ± SD). One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were used to analyze significant differences between the groups. $P < 0.05$ indicates a significant difference, and $P < 0.01$ indicates a very significant difference.

RESULTS

Effects of Pulsed 9.4GHz High Peak Power Microwave Radiation on the Environmental Temperature of Feeding *C. elegans*

During the irradiation period, the temperature of the culture medium surface of *C. elegans* was detected in real-time. The results showed that the surface temperature increased by approximately 0.375 ℃ during irradiation (Fig. 6).

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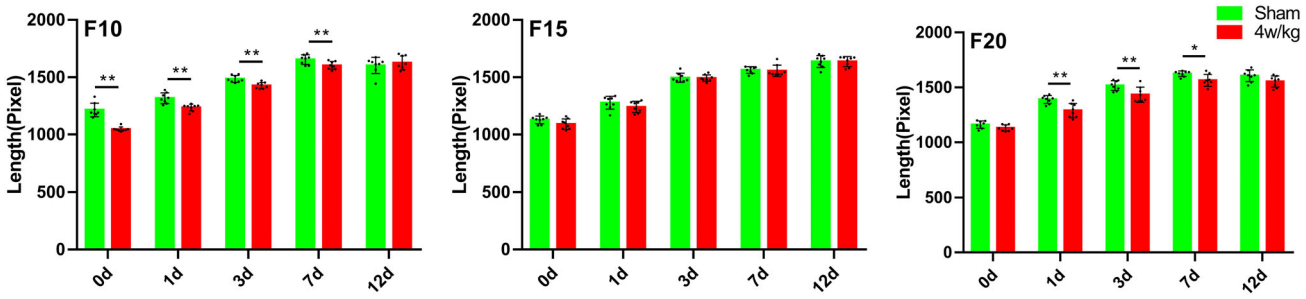


Fig. 7. Effects of long-term and multigeneration exposure to 9.4 GHz microwave radiation at an SAR of 4 W/kg on the growth and development of *Caenorhabditis elegans* (*$P < 0.05$; **$P < 0.01$; F10, F15, and F20 represent the 10th, 15th, and 20th generations of *C. elegans*, respectively, $n = 8$).

Effects of 9.4 GHz Microwave Irradiation at an SAR of 4 W/kg on the Growth and Development of *C. elegans*

In this long-term and multigeneration exposure model, F10, F15, and F20 nematodes were collected to detect their growth and development. The body length of *C. elegans* was observed at 0, 1, 3, 7, and 12 days after microwave irradiation. We found that the body length of *C. elegans* in the F10 exposure group was significantly lower than that in the sham exposure group at 0, 1, 3, and 7 days after irradiation. In the F15 generation, the body length of *C. elegans* in the exposed group also decreased to some degree at 0, 1, 3, and 7 days after irradiation. Similarly, the body length of the exposed group in the F20 generation was significantly lower than that in the sham exposure group at 1, 3, and 7 days after irradiation. However, in the adult stage at 12 days after irradiation, there was no significant difference in body length between the two groups (Fig. 7).

Effects of 9.4 GHz Microwave Irradiation at an SAR of 4 W/kg on the Locomotory Capacity of *C. elegans*

The movement state and locomotory capacity of *C. elegans* were detected by body bending assay, with more bends indicating more activity. We counted the number of body bends of F10, F15, and F20 nematodes at 0, 1, 3, 7, and 12 days after microwave irradiation. On the day after exposure, the number of body bends of the 15th generation nematodes in the exposure group was significantly higher than that in the sham exposure group. However, on the 1st day of the F10 generation and the 3rd day of the F15 generation, the locomotory capacity of *C. elegans* in the exposure group was significantly lower than that in the sham exposure group. On the 12th day, due to the obvious aging of *C. elegans*, the locomotory capacity in each group was very low, and there was no significant difference among the groups (Fig. 8). The results here show that long-term exposure to 9.4 GHz microwave irradiation at an SAR of 4 W/kg might have a certain effect on the locomotory capacity of *C. elegans*.

Effects of 9.4 GHz Microwave Irradiation at an SAR of 4 W/kg on Egg Production of *C. elegans*

The fecundity of *C. elegans* is mainly reflected by the amount of egg production. To study the effect of continuous microwave radiation at an SAR of

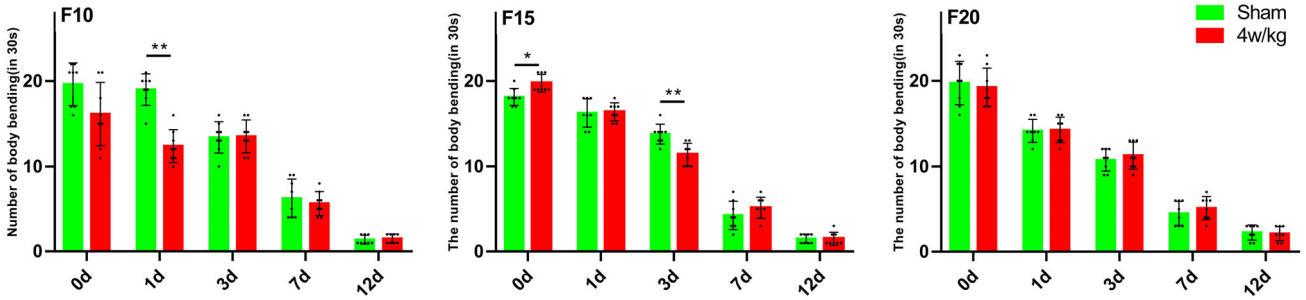


Fig. 8. Effects of long-term and multigeneration exposure to 9.4 GHz microwave radiation at an SAR of 4 W/kg on body bending of *Caenorhabditis elegans* (*$P < 0.05$; **$P < 0.01$; F10, F15, and F20 represent the 10th, 15th, and 20th generations of *C. elegans*, respectively, $n = 8$).

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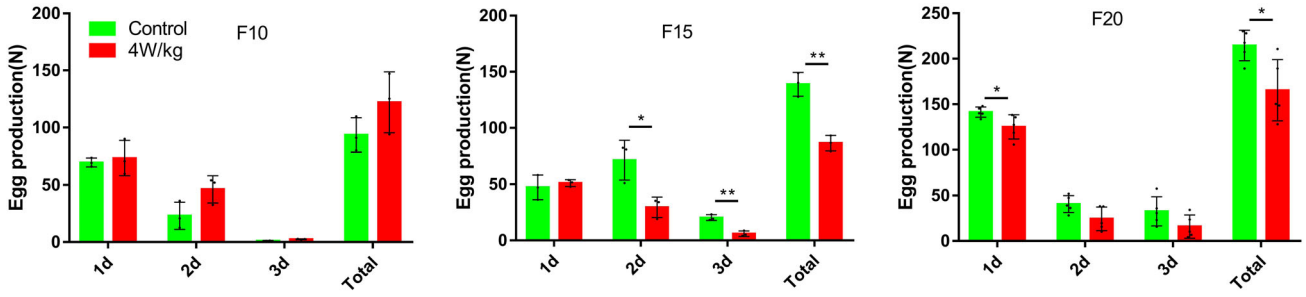


Fig. 9. Effects of long-term and multigeneration exposure to 9.4 GHz microwave radiation at an SAR of 4 W/kg on egg production of *Caenorhabditis elegans* (*$P < 0.05$; **$P < 0.01$; F10, F15, and F20 represent the 10th, 15th, and 20th generations of *C. elegans*, respectively, $n = 3$).

4 W/kg on fecundity, we counted the number of eggs laid per day and the total number of eggs laid by *C. elegans* of the F10, F15, and F20 generations. The results indicated that the number of eggs laid by *C. elegans* in the exposed group decreased significantly from the F15 generation. In the F15 generation, nematode oviposition in the exposed group decreased significantly on the 2nd and 3rd days, and the total fecundity in the exposed group was significantly lower than that in the control group. In the F20 generation, the number of eggs laid by *C. elegans* in the exposed group decreased significantly from the 1st day and decreased on the 2nd and 3rd days, and the total fecundity decreased significantly compared with that in the control group. The results showed that the fecundity of *C. elegans* decreased significantly from the F15 generation after continuous microwave exposure to 9.4 GHz at an SAR of 4 W/kg, suggesting that long-term electromagnetic exposure might affect the reproductive function of *C. elegans* (Fig. 9).

Effects of 9.4 GHz Microwave Irradiation at an SAR of 4 W/kg on ROS Levels in *C. elegans*

The level of reactive oxygen species is a direct index of the oxidative stress state of *C. elegans*. In general, oxidation levels increase with the aging of *C. elegans*. In this study, we detected ROS in *C. elegans* on Days 0, 1, 3, 7, and 12 after irradiation. In the F15 generation, the level of ROS in the exposure group increased significantly on Days 1, 3, and 7 after irradiation. However, in the F10 generation, the level of ROS decreased significantly on the 7th day after exposure, and the specific reasons need to be verified by further experiments. In the F20 generation, although there was no significant difference between the two groups, the ROS levels also increased on the 3rd day after irradiation (Fig. 10). The results showed that the ROS levels increased significantly from the F15 generation on Days 1, 3, and 7 after continuous microwave exposure to 9.4 GHz at an SAR of 4 W/kg, suggesting that long-term electromagnetic exposure might induce oxidative stress in *C. elegans.*

Effects of 9.4 GHz Microwave Irradiation at an SAR of 4 W/kg on the Lifespan of *C. elegans*

The lifespan of *C. elegans* was observed from Day 10 after irradiation until all *C. elegans* died, and then the survival curve was drawn. The results showed that all specimens died within 23 days, and there were no significant differences between the two groups in the three generations. In summary, long-term exposure of 9.4 GHz microwave irradiation at an SAR of 4 W/kg had no significant effect on the lifespan of *C. elegans* (Fig. 11).

DISCUSSION

Due to the wide application of electromagnetic wave technology, health hazards from exposure have generated concern [Repacholi, 2001; Hao et al., 2018; de Seze et al., 2020]. However, except for the thermal effect of electromagnetic radiation, no definite biological (nonthermal) effect has been found at present. The main reason is that the energy of electromagnetic waves is low and is not enough to destroy biomolecules; the damage effect from microwaves is mild, which is not easy to observe and difficult to reproduce. Some studies have found that radiofrequency electromagnetic radiation could cause changes in animal behavior, and most of these reports suggested that the nervous system was the main target [Belpomme et al., 2018; Russell, 2018; Magiera and Solecka, 2019]. The authors believed that electromagnetic radiation under certain conditions could lead to a decline in learning and memory and emotional changes in animals. However, the above research results were vague, and there was no good explanation for the underlying mechanism

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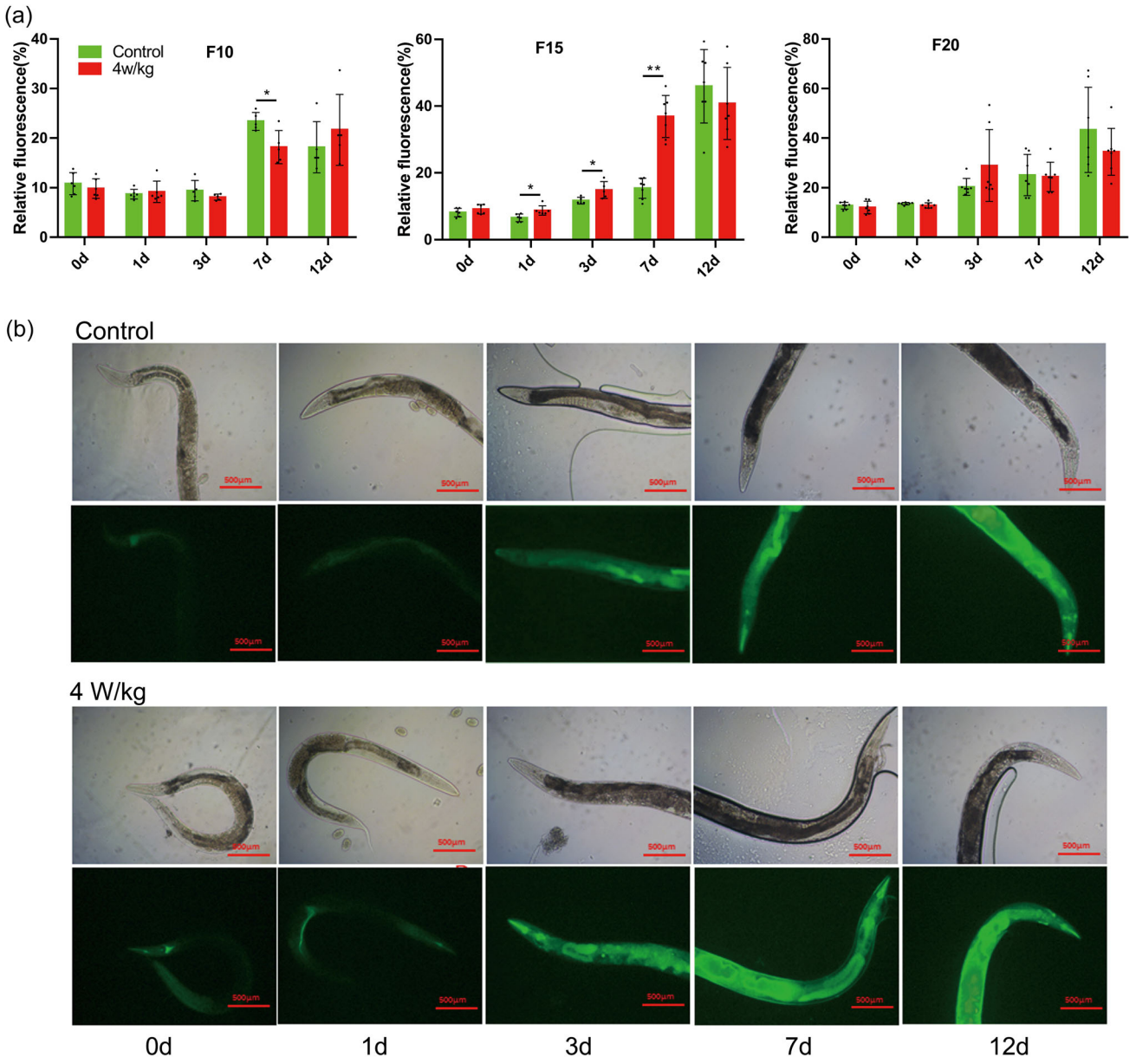


Fig. 10. (**a**) Effects of long-term and multigeneration exposure to 9.4 GHz microwave radiation at an SAR of 4 W/kg on ROS production of *Caenorhabditis elegans* (*$P < 0.05$; **$P < 0.01$; F10, F15, and F20 represent the 10th, 15th, and 20th generations of *C. elegans*, respectively, $n = 6$). (**b**) Graphs of ROS levels in *C. elegans* of the F15 generation as detected by the CM-H2DCFDA probe on Days 0, 1, 3, 7, and 12 after microwave irradiation. ROS = reactive oxygen species; SAR = specific adsorption rate.

[Manna and Ghosh, 2016; Kocaman et al., 2018]. We believe that there were some shortcomings in the design of the above studies, such as that the materials used could not be exposed for a long period of time and could not be exposed for multiple generations. There were many influencing factors for the rodents and cells, which might have affected the stability of the results. For example, it is well known that water can absorb microwaves. When cells are used for microwave research, the absorption of microwaves by culture medium will greatly affect the effect on cells. *C. elegans* [*C. elegans* Sequencing Consortium, 1998; Culetto and Sattelle, 2000] have good application prospects in the study of the biological effects of electromagnetic waves because of their small size, constant number of cells, short

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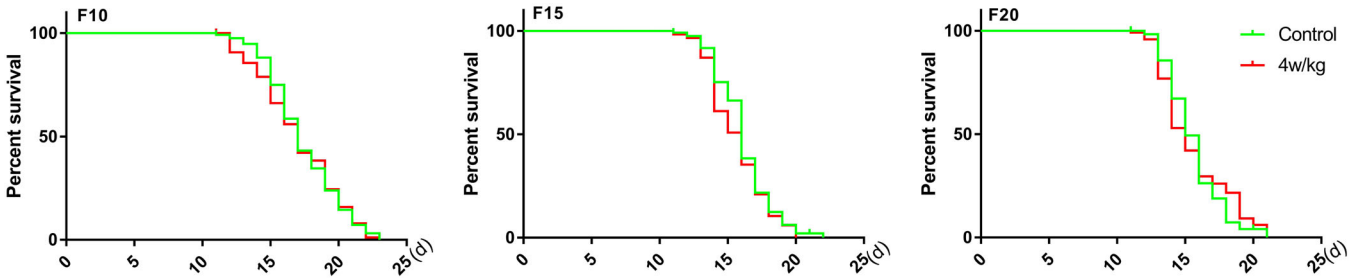


Fig. 11. Effects of long-term and multigeneration exposure to 9.4 GHz microwave radiation at an SAR of 4 W/kg on the lifespan of *Caenorhabditis elegans* (F10, F15, and F20 represent the 10th, 15th, and 20th generations of *C. elegans*, respectively, $n = 100$).

reproductive cycle, transparency, ease of observation, and sensitivity to changes in the environment.

A 2016 study in the United States, in which rodents were exposed to radiofrequency radiation for two years, found that cell phone radiation may be related to cardiac carcinogenesis and adrenal tumors in male rats [Wyde et al., 2016; Wyde et al., 2018a,b,c]. Otherwise, some studies have pointed out that there were some shortcomings limiting the reliability of the results [Melnick, 2020]. Nevertheless, with *C. elegans*, many conditions can be well controlled and easily observed. A study found that the growth and lifespan of *C. elegans* were not affected by radiofrequency electromagnetic fields [Fasseas et al., 2015], and another review considered that radiofrequency electromagnetic fields tended to promote nematode growth, which was mainly due to thermal effects [Cucurachi et al., 2013]. Gao exposed nematodes to a nonthermal radiofrequency field for a prolonged time (1750 MHz, 60 h, 3 W/kg), and the results showed that there were no significant changes in reproductive function [Gao et al., 2016]. Bojjawar et al. [2006] found a result of decreased fertility of *C. elegans* after nanopulse electromagnetic exposure but without heating effects; the authors suggested that nanopulses interfered with fertilization or development by an as-yet undetermined mechanism. In this study, we established an artificial electromagnetic environment in which *C. elegans* lived for a long period of time and across 20 generations.

In our study, the nematodes were exposed to 9.4 GHz electromagnetic fields at an SAR of 4 W/kg from the beginning state of the L1 larvae. When the nematodes reached L4 larvae beginning to lay eggs, the adults were washed off, and the L1 larvae were continuously exposed, allowing passage generation under the electromagnetic field. *C. elegans* of the F10, F15, and F20 generations were selected for observation. The most interesting finding of this study was that the number of eggs laid by *C. elegans* began to decrease significantly from the F15 generation after long-term exposure to 9.4 GHz electromagnetic fields at an SAR of 4 W/kg. At the same time, the body length and motility of *C. elegans* decreased to varying degrees. The ROS level of *C. elegans* increased significantly in the F15 generation exposure group, but there was no significant difference in the lifespan between the two groups. Therefore, we preliminarily judged that long-term and multigeneration electromagnetic exposure could induce oxidative pressure in *C. elegans*, interfere with and disrupt development and exercise ability, and ultimately lead to a decrease in fecundity (Fig. 12).

Although nematodes are excellent model organisms, there have been few studies on their biological effects due to electromagnetic radiation

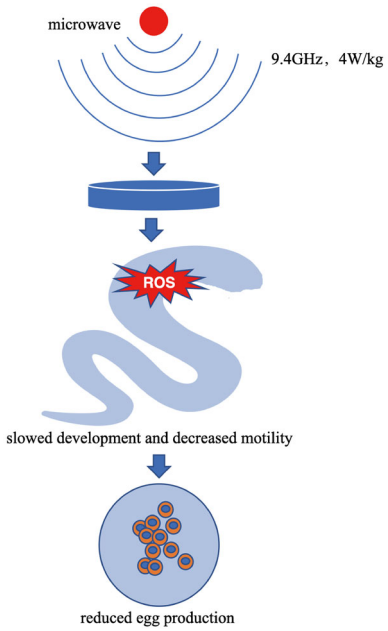


Fig. 12. A schematic diagram of the decrease in nematode oviposition caused by microwave energy.

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[Bojjawar et al., 2006; Cucurachi et al., 2013; Fasseas et al., 2015; Shi et al., 2015; Gao et al., 2016; Sun et al., 2018, 2019]. A study in the United States used rodents to carry out a long-term radiofrequency exposure experiment, which took 2 years to expose animals for just one generation [Wyde, 2018b,c]. Nevertheless, *C. elegans* is easy to control, its life cycle is short, and exposure for 20 generations can be completed in 2 months. The interesting experimental results of this study were also readily reproduced. We thought that *C. elegans* in an electromagnetic environment for a long period of time would continuously absorb energy from microwave radiation, which was beneficial to *C. elegans* in the beginning when the absorbed energy amount was very low. However, excessive microwave energy causes metabolic disorders in *C. elegans*, which induce oxidative stress and eventually lead to a decrease in the number of eggs laid. Magnetically functionalized "ironoxide-clad" nematodes can be effectively separated and moved using an external magnetic field. Surface-functionalized nematodes exhibited preserved viability and reproduction [Minullina et al., 2011]. In theory, the coverage of metal nanoparticles can shield external electromagnetic waves and reduce the direct exposure of nematodes to external electromagnetic waves. However, the coverage of metal nanoparticles may also lead to a local concentration of the external electromagnetic field distribution, resulting in a higher temperature rise at the concentrated part. Due to the effect of heat transfer, whether the temperature rise will affect nematodes is worthy of attention and discussion. In addition, we will use metabolomics, transcriptome, and proteome techniques to determine the signaling pathway that plays a regulatory role in egg production.

CONCLUSIONS

Long-term and multigeneration exposure to 9.4 GHz continuous microwaves at an SAR of 4 W/kg might affect nematode oviposition from the F15 generation. The growth and development of *C. elegans* slowed, their motility decreased, and oxidative stress appeared from the 10th generation, but their lifespan was not affected. We preliminarily suggest that the microwave energy received by worms ultimately led to oxidative stress, which induced a decrease in egg production. Next, we will use metabolomics, transcriptome, and proteome techniques to determine the signaling pathway that plays a regulatory role in egg production.

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