Science of the Total Environment 896 (2023) 165211

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Defined exposure of honey bee colonies to simulated radiofrequency electromagnetic fields (RF-EMF): Negative effects on the homing ability, but not on brood development or longevity

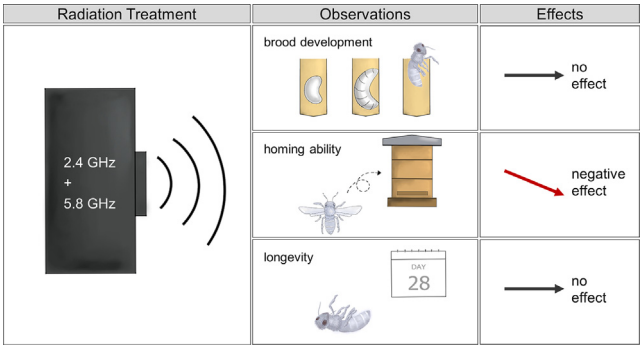
Manuel Treder [a,*], Marcus Müller [b], Larissa Fellner [a], Kirsten Traynor [a], Peter Rosenkranz [a]

[a] State Institute of Bee Research, University of Hohenheim, Stuttgart, Germany
[b] Communications Engineering Lab, Karlsruhe Institute of Technology, Karlsruhe, Germany

HIGHLIGHTS

- RF-EMF exposure significantly reduced homing success of honey bees.
- These effects on homing behavior were only evident after long-term, not short-term, irradiation.
- Honey bee brood development and longevity was not affected by RF-EMF.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Henner Hollert

Keywords:
Electromagnetic radiation
RF-EMF exposure
Honey bees
Apis mellifera
Homing ability

ABSTRACT

Urbanization and the increasing use of wireless technologies lead to higher emission rates of radiofrequency electromagnetic fields (RF-EMF) in populated areas. This anthropogenic electromagnetic radiation is a form of environmental pollution and a potential stressor on bees or other flying insects. Cities often have a high density of wireless devices operating on microwave frequencies, which generate electromagnetic frequencies e.g. in the 2.4 and 5.8 GHz bands commonly used by the wireless technologies. To date the effects of nonionizing electromagnetic radiation on the vitality and behavior of insects are poorly understood. In our experiment we used honey bees as model organisms and analyzed the effects of defined exposures to 2.4 and 5.8 GHz on brood development, longevity and homing ability under field conditions. To generate this radiation, we used a high-quality radiation source which generates a consistent, definable and realistic electromagnetic radiation, engineered for this experiment by the Communications Engineering Lab (CEL) at the Karlsruhe Institute of Technology. Our results show significant effects of long-term exposures on the homing ability of foraging honey bees, but no effects on brood development and adult worker longevity. Using this novel and high-quality technical set-up, this interdisciplinary work provides new data on the effects of these widely used frequencies on important fitness parameters of free-flying honey bees.

1. Introduction

More than 90 % of the world's population has access to mobile broadband networks, and the number of people actively using the internet has continued to grow in recent years. This usage is not evenly distributed; in urban areas the percentage of individuals using the internet is almost

* Corresponding author.
E-mail address: Manuel.Treder@uni-hohenheim.de (M. Treder).

http://dx.doi.org/10.1016/j.scitotenv.2023.165211
Received 13 March 2023; Received in revised form 24 June 2023; Accepted 27 June 2023
Available online 28 June 2023
0048-9697/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

twice as high as in rural areas (International Telecommunication Union, 2021). Wireless communication technologies generate a form of nonionizing radiation called radiofrequency electromagnetic fields (RF-EMF) (Malkemper et al., 2018) which are widely utilized for a broad number of technologies including mobile phone networks, Bluetooth and Wi-Fi. Typically measured in the unit of Hertz, RF-EMF range from 100 kHz up to 300 GHz coupled with different wavelengths (ICNIRP, International Commission on Non-Ionizing Radiation Protection, 2020). The commonly used Wi-Fi (IEEE802.11-Standard) is responsible for more than a half of all data traffic and typically utilizes the 2.4 or 5 GHz bands (Khorov et al., 2020). Due to the increasing use of these technologies, these fields have become ubiquitous in Europe (Sagar et al., 2018). However, these technologies and the possible threats to human health and wildlife are intensely debated as there are still large knowledge gaps about the acute or long-term effects, including the almost unknown effects on the diversity or abundance of invertebrates and vertebrates, such as bees or birds (Bandara and Carpenter, 2018; Malkemper et al., 2018; Russell, 2018; Sutherland et al., 2018; Vanbergen et al., 2019). Part of the challenge is that the effects of the tested electromagnetic fields (EMF) depend strongly on their frequency range, radiation intensity, or the tested organism, and therefore the results of one study do not automatically provide insight into possible effects on other organisms, especially at other frequencies or exposure scenarios.

Whether electric and magnetic fields can in principle be perceived by different species has been examined in various studies. For example, evidence has shown that migratory birds perceive these fields (Engels et al., 2014), bumble bees use their mechanosensitive hairs to detect electric fields (Sutton et al., 2016), *Drosophila* responds to geomagnetic fields (Bae et al., 2016), whereas honey bees also perceive them (Lambinet et al., 2017; Liang et al., 2016). Further research focusing on effects of EMF on insects used different frequency ranges and different exposure scenarios. We can roughly distinguish here between studies on low frequency electromagnetic radiation, as generated by overhead powerlines, and radiofrequency (high-frequency) electromagnetic radiation.

Negative effects on honey bees of extremely low frequency EMF has been shown in various studies using Helmholtz Coils, such as in Shepherd et al. (2018), who confirmed negative impacts of 50 Hz EMFs on learning ability measured via the proboscis extension response and for the flight-, foraging- and feeding-behavior. In a more recent work, Shepherd et al. (2019) demonstrated that 50 Hz EMF treated bees are more aggressive toward colony intruders. Laboratory studies using similar 50 Hz frequencies resulted in inconsistent effects on various behavioral traits (Migdał et al., 2021; Migdał et al., 2022). In the field, the study of Molina-Montenegro et al. (2023) describes the effects of low frequencies emitted by transmission towers. This includes the induction of physiological stress and reduced flower visitation rates of honey bees, impacts on plant diversity and abundance, and reduced seed production of California poppy.

We know from simulation studies, that higher frequencies also lead to a higher energy absorption rate in honey bees (Thielens et al., 2020; Toribio et al., 2022). Due to these increased absorption rates, higher frequencies may also lead to stronger effects. What are the actual effects of such high-frequency or so-called radiofrequency EMF (RF-EMF) on honey bees or other insects? There are some field studies showing effects of RF-EMF on different insects or pollinators including honey bees. The correlational study of Lázaro et al. (2016) conducted at different island locations highlights negative effects of mobile telecommunication antennas on wild pollinator abundance and composition, though interestingly the severity of the effect depended on the nesting behavior of the insects; above ground species were more strongly impacted than bees that nest underground. Other studies using extant mobile phone stations noted negative effects on the number of leaving and returning worker bees per minute in close proximity to the towers (Taye et al., 2017) or no effects on fecundity and number of offspring (Vijver et al., 2014). Using the alternative technical setup of individual mobile phones or signal generators, other negative influences of the 900 MHz band have been documented, including altering the communication signal of worker piping, disregulating the development of queen pupae, decreasing catalase activity and the lipid peroxidation levels, or causing possible DNA damages (Favre, 2011; Odemer and Odemer, 2019; Vilić et al., 2017). As seen with low frequencies, there are some indications that 2.4 GHz alter short-term memory (Lopatina et al., 2019), but not longevity under laboratory conditions (Westerdahl and Gary, 1981). Unfortunately, only a few studies can be found that examine effects of the Wi-Fi frequencies.

A detailed review (Vanbergen et al., 2019) outlined the accumulated evidence of negative impacts and current knowledge gaps of EMF on pollinators, highlighting that many impacts are unresolved and that the methods used in future studies urgently need to be improved. Recommendations included increasing the number of repetitions, use of appropriate controls, realistic exposure scenarios, and field realistic conditions. In particular, the authors demand that radiation sources are exactly described to allow replication of such studies.

In light of this review, our current interdisciplinary experiment implemented these recommendations and we present the effects of realistic 2.4 and 5.8 GHz (Wi-Fi) exposure scenarios on important fitness parameters of free-flying honey bees using a definable and high-quality radiation simulator. The radiation simulator was designed specifically for this experiment by the Communications Engineering Lab (CEL) at the Karlsruhe Institute of Technology. We investigated if exposure to the selected RF-EMF affects the development of honey bee worker brood, homing success, and adult worker longevity.

2. Materials & methods

2.1. Field site & experimental setup

The experiments were performed from July through September 2020 and again in July through October 2021 at the State Institute of Bee Research, University of Hohenheim, Germany.

For our experimental setup we used 16 queenright colonies, established in April 2020 and headed by newly reared sister queens in 6-frame MiniPlus Styrofoam hives. All colonies were equalized and established with two brood combs, two full honey combs, and two empty combs. The Styrofoam hive materials were used due to their transparency to microwave radiation. To further reduce the impacts on the regularity of radiation propagation, all metallic objects in and around the hives were avoided, including removing all the wires traditionally used in pure beeswax combs to provide stability to the frames during honey extraction. Then colonies were split randomly into two groups (EMF, Control) of 8 colonies each.

In the EMF group, these 8 colonies were placed at the optimal angles to the radiation source (0° and 180° at a distance of 2 m; Figs. 1 and 2) to ensure a defined and equal radiation exposure for all colonies. The 8 control colonies were placed in the same pattern at a distance 5 m away from the EMF colonies. The distance between the treatment and control group was set to a distance of 5 m based on the measured radiation pattern (Fig. 2) to prevent unwanted exposure of the control group. The resulting angle, combined with the increased distance of the control group to the emitter, reduced the exposure relative to the treatment group by a factor of >100. Nine additional colonies were used to test the effects of short-term exposures on homing ability of honey bees (Section 2.4). These colonies were also kept in MiniPlus Styrofoam hives and were located at the same apiary as the other experimental hives, but outside the exposure range of the radiation source.

2.2. RF-EMF exposure & technical system

For our experiment we used a high-quality radiation source which generates consistent, well-defined and realistic electromagnetic radiation emissions, designed and constructed for this experiment by the Communications Engineering Lab (CEL) at the Karlsruhe Institute of Technology. This radiation source generates packets which are identical to those used for Wireless LAN (Wi-Fi) transmission at frequencies of 2.450 and 5.805 GHz and which are license-free simulatable. The two different frequencies are generated simultaneously. There is no fundamental technical difference between our

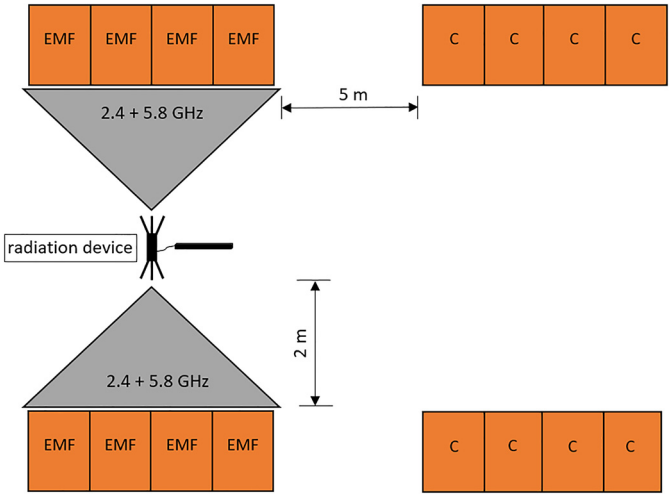


Fig. 1. Experimental setup (bird's eye view) of the RF-EMF exposure setup. The apiary configuration includes 8 colonies with radiation exposure (EMF) and 8 adjacent colonies outside of the radiation pattern as control (C).

radiation source and commercially used Wi-Fi-Devices (IEEE802.11a standard), except that our set-up does not try to establish communication. This is because, over longer time scales, the duration a common Wi-Fi station uses a channel is determined by the amount of data it has to transmit. We could describe this communication-caused changing presence of power as long-term power-varying (i.e. pulsed) transmissions. Since data usage is neither constant nor predictable, this leads to a situation where an experimental setup like ours needs to have adjustability in the percentage of time it is emitting. On a shorter time scale, the methods used to transport data on the radiofrequency signal lead to a short-term irregularly pulsed power transmission. Common Wi-Fi stations, as well as our setup, use such signals with the fine structure of Wi-Fi packets (OFDM). This means that for very short durations (in the range of 50 ns to 100 ns) power peaks occur sparsely, which is typical for Wi-Fi and should be reproduced in our setup with a representative signal.

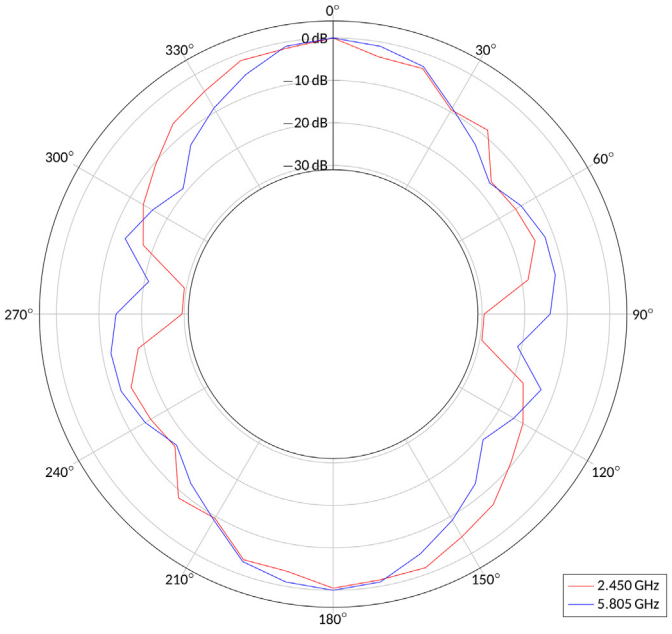


Fig. 2. Radiation pattern of the RF-EMF-System "Beefi" (power density in relation to the main direction in decibels), made by the Communications Engineering Lab (CEL) at the Karlsruhe Institute of Technology. Frequencies: 2.450 and 5.805 GHz. The angles were specified on the device.

By using Software Defined Radio (SDR)-Technology, the total output power, packet rate, and radiation direction did not change dynamically and was thus consistent, reliable, and quantifiable. The transmitting device was controlled via a notebook (Lenovo ThinkPad T480, Intel Core® i7™ Version) using software based on the popular Software Defined Radio framework GNU Radio designed for the measurement purpose, and the transmitted power could be visualized on this computer. In addition to controlling the transmission intensity, the duty cycle (percentage of time during which Wi-Fi packets are sent), and the duration of the period (ms) over which the duty cycle applies could be set. In this experiment, we chose a duty cycle of 100 % over a period of 100 ms to simulate a heavily loaded access point with multiple connected devices located far away. This is a relevant scenario for outdoor access points, because in most cases the user devices will not be very close to the access point. It is a realistic scenario that honey bee colonies of urban beekeepers, which have grown in popularity and are now quite common, could receive multiple exposures from several Wi-Fi access points or routers. To verify the RF-EMF-System, the output power was measured by using a direction-independent dual-band antenna and a calibrated Signal Analyzer (Rohde&Schwarz FSQ 8), which enabled us to determine the radiation pattern (Fig. 2) and Equivalent Isotropic Radiated Power (EIRP, Table 1). Additionally, the chosen parameter setting and consequently the intensity of the radiation exposure was recorded over the whole trial period. These data were auto-saved on the notebook using comma-separated values (CSV), segregating the details of output for 2.450 and 5.805 GHz. The radiation system ran continuously with the same settings for approximately 12 weeks in 2020 and 14 weeks in 2021 until the experiments were completed. To protect the technical system from the weather, it was covered with a plastic barrel (radiation permeable) and the power supply was provided by a waterproof power cable.

2.3. Brood development

At the beginning of the brood assessment, one brood comb with eggs was chosen from each colony (8 colonies EMF, 8 colonies control) and approximately 200 cells were marked on a transparent acetate sheet (brood area fixing day, BFD) according to Schur et al. (2003). As eggs don't hatch for three days, the exact age of eggs at the begin of documenting their development varied between 1 and 72 h. The development of the brood was subsequently recorded every second day after the BFD for a period of 18 days, when the first bees emerged (3 days as an egg +18 days of development = 21 days). BFD + 16 was chosen for a reliable assessment period for the termination rate of development, because the onset of emergence at BFD + 18 did not permit us to distinguish with certainty whether bees emerged or the cells were cleared out by worker bees at that time. The brood termination rate was calculated according to the proportion of cells that failed to complete brood development and the expected number of successfully developed brood cells.

2.4. Homing ability

To assess the effects on the homing success, two different approaches were used to examine long-term and short-term effects separately (Table 2). The homing experiments require flight activity and the absence of precipitation, so those trials were only performed under good weather conditions.

2.4.1. Long-term exposure

At the start of the homing trials, the colonies had already been irradiated for about 7 weeks and the irradiation was continued throughout the entire trial period. Adult worker bees that had been foraging were collected into plastic specimen containers directly from the hive entrances of four different colonies in each group (EMF, Control). A total of around 160 individual bees were tested in 8 replicate trials with pairwise comparisons of control and EMF bees. In each trial, the homing behavior of bees from one EMF treated colony was simultaneously compared with bees from

Table 1
Equivalent Isotropic Radiated Power (EIRP) and free space loss in relation to the used frequencies. The EIRP defines the radiated power of the transmitting device per side. The free space loss is the reduction of the power density in free space due to the two-meter distance between the honey bee colonies and radiation device.

Frequency	EIRP (on each side)	Free space path loss (α) at a distance of 2 m
2.450 GHz	22.5 dBm = 177.83 mW	$\alpha_{2.450\ GHz} = (40.23 + 20log_{10}(2))\ dB = 46.25$ dB
5.805 GHz	10.0 dBm = 10 mW	$\alpha_{5.805\ GHz} = (47.72 + 20log_{10}(2))\ dB = 53.74$ dB

one control colony (Ø10 bees/colony). The selection of individual colonies per trial was randomized, but with the constraint that each colony must be tested twice in total. The bees of each trial were individually marked using numbered plastic colored disks (one color per group), by briefly immobilizing after the bees post collection at the entrance. We immobilized them by cooling (Human et al., 2013; Scheiner et al., 2013) using a temperature of 2 °C for a period of 10 min. After marking, the bees were fed ad libitum with sugar syrup (Apiinvert®, Südzucker) in ventilated specimen containers (Ø10 bees per group/2 specimen containers) and were positioned in front of their own hives again for a duration of 40 min to ensure appropriate exposure prior to testing (both: EMF/Control). Thus bees taken from colonies with long-term EMF exposure received an additional direct radiation exposure outside the hive, while bees from the control group remained without this EMF exposure.

2.4.2. Short-term exposure

In this experiment we used 9 additional colonies that were located away from the radiation source and collected foraging adult worker bees at the hive entrances. The worker bees were collected into specimen containers directly from the hive entrances. Using a total of around 200 individual bees we performed 11 trials with pairwise comparisons between the treatments. For each trial, both the control bees and the bees that would receive short-term irradiation were collected from the same colony (Ø 18 bees/colony, 9 control, 9 to irradiate). The collected bees were individually marked as described above and fed with sugar syrup in ventilated specimen containers (Ø9 bees per container). Each specimen container of the EMF group with on average 9 bees was irradiated for 40 min at the same location and with the same RF-EMF exposure as the bees experienced in the long-term exposure experiment. The exposure time of 40 min was chosen as a compromise between a realistic foraging time per day for a worker bee in EMF-exposed areas, recovery time after cooling for marking, time for food consumption, and a not too long waiting time. The control bees remained unexposed for the same period of time.

2.4.3. Release site and homing recording

The bees in the specimen containers were transported to a remote release site, about 500 m from the location of the colonies. Previous trials at the same field site have shown that a 500 m return distance results in high rates of successful homing, which is why this distance was chosen. All specimen containers were opened at the same time, the bees released and the timer started. Another observer counted all the returning bees at the hive entrance, noted the individual plate number and recorded the flight duration. After an observation period of at least 45 min (exception: short-term, two trials with a minimum of 37 min) the counting was halted and bees that had not arrived were recorded as "not returned". To prevent unwanted radiation exposures, RFID and harmonic radar technologies could not be used for tracking individual bees in this experiment. The counts were thus recorded visually. In the first year (2020), the return of the homing bees was recorded via observations of hive entrances fitted with transparent tunnels approximately 10 cm in length (Fig. 3). Due to high personnel requirements of observers at each tested hive entrance, the method was adjusted for the second year (2021). Instead of observing the returning bees in real-time, video was recorded with a high resolution camera (GoPro Hero7 silver) and the bees returning successfully were determined from the recording. The percentage rate of homing success was calculated by dividing the number of "bees returned successfully" by the number of "released bees".

Table 2
Treatment variants for homing success examinations. The continuous exposure (long-term variant) began about 7 weeks before the start of the experiment and continued without interruption for the entire experimental period. The sampled bees received the (additional) 40 min exposure as direct exposure outside the hives.

Variant	Exposure time	Number of trials	Number of hives	Flight distance
Long-term	Continuously +40 min	8	8 (4 EMF, 4 Control)	500 m
Short-term	40 min	11	9 (EMF and Control)	500 m

2.5. Longevity

Newly emerged worker bees were collected from four brood combs pulled from two different colonies in another apiary that contained sufficient brood stages (20/21 days old) from which young bees would soon emerge. These brood combs were placed in a cage in an incubator for about 24 h until approximately 1500 bees emerged. These bees were mixed, divided into groups, and then marked with eight different colors, either on the thorax or the abdomen. Unfortunately, some colors (gold, silver, purple) were not permanent and could be poorly recognized as bees aged, so these groups had to be excluded from the analysis. Thus 675 newly emerged worker bees in 9 groups (5 EMF, 4 control) with 75 bees each were followed for lifespan, by being introduced into one of the 16 experimental hives (8 irradiated and 8 control colonies). One day post introduction we started tracking lifespan by verifying their continued presence in the hive by counting the number of marked bees that had been accepted by the colony. This value served as the initial population for the longevity evaluations. The total observation period was 28 days, with observations made on 11 days. On each counting day, all frames of the colonies and the inner walls and bottom of the hives were carefully inspected and the total number of marked bees on the frames and on the interior walls of the hive was counted. The counted frames were temporarily removed into a separate box to minimize the possibility of double counting an individual. These counts were performed early in the morning before flight activity commenced so that all bees including foragers could be documented.

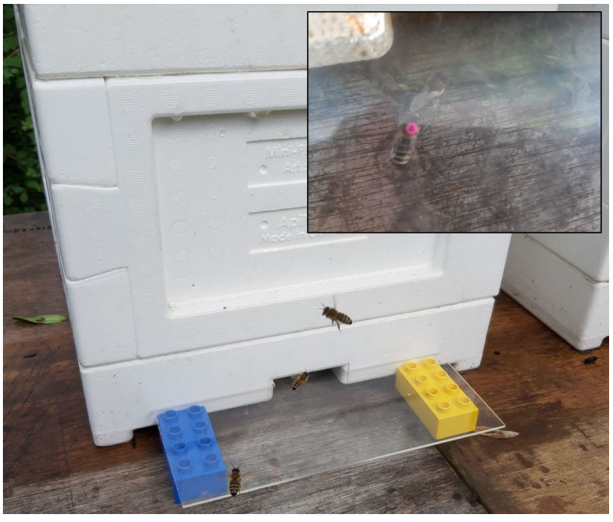


Fig. 3. Transparent tunnels of about 10 cm on the mini-hives entrances to facilitate the observations of the homing honey bees (individual marked).

2.6. Statistical analysis

The data was analyzed using the IBM SPSS Statistics 26 (IBM, Armonk, USA) and JMP Pro 16 (SAS Institute, Cary, USA) computer software.

Brood development data-samples were checked with a Shapiro-Wilk test to examine normal distribution (rejecting = $p < 0.05$). Because of parametric characteristics of the data, we used the *t*-test for independent samples ($\alpha = 0.05$) for the comparison.

To analyze the homing ability data sets, we used a generalized linear mixed model (GLMM) with binomial (homing rate: 1 = returned, 0 = not returned) and inverse Gaussian (duration, min) distribution, which allowed us to include inter-colony and trial effects of the experiments. The model was designed setting homing rate and homing duration as variables, treatment as a fixed factor, and hive and trial number as random factors.

Brood mortality and bee longevity data were analyzed by using the Kaplan-Meier-Survival analysis including a pairwise comparison and test for significance with the commonly used Log-Rank (Cox-Mantel) test ($\alpha = 0.05$). In addition to the analysis of the longevity data for the entire lifespan, we also analyzed this dataset for the period when bees typically transition to foraging (day 11 to 29). For this subset analysis, we used the Wilcoxon test ($\alpha = 0.05$) to compare treatment groups because of its sensitivity to early differences between survival curves.

3. Results

3.1. Brood development

We investigated brood development, comparing colonies by following bee development from egg to emergence. The brood termination rate indicates the percentage of brood not reared to completion. The final brood termination rate at day 16 post BFD, the day when eggs are recorded, was not significantly different ($p = 0.862$) between the group exposed to radiation (33.5 %) and the untreated control (35.4 %) (Fig. 4). The number of bees that developed all the way through to BFD + 16 did not differ, confirming an equal survival probability over time (Kaplan-Meier-Survival with the Log-Rank: $p = 0.262$) (Fig. 5). Our results thus do not indicate any negative effects of the tested RF-EMF on the development of honey bee brood and adult bee emergence.

3.2. Homing ability

We investigated the impacts on homing ability by transporting bees 500 m away from their colonies, then comparing the number of successful return flights of treated and control bees.

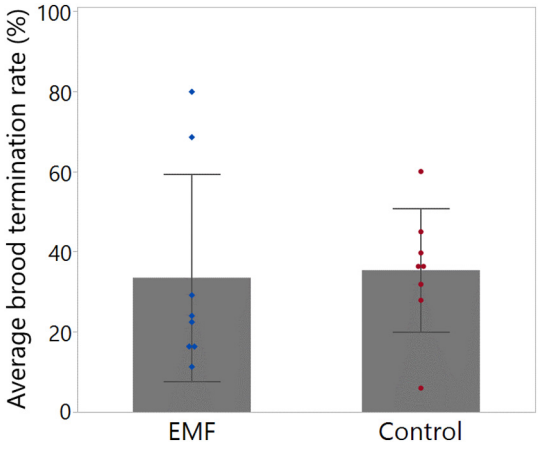


Fig. 4. The average brood termination rate at day 16 after BFD ± SD revealed no significant differences ($p = 0.862$). We used *t*-test for independent samples ($\alpha = 0.05$) to compare the two groups (EMF, Control). n = 8 colonies/each group.

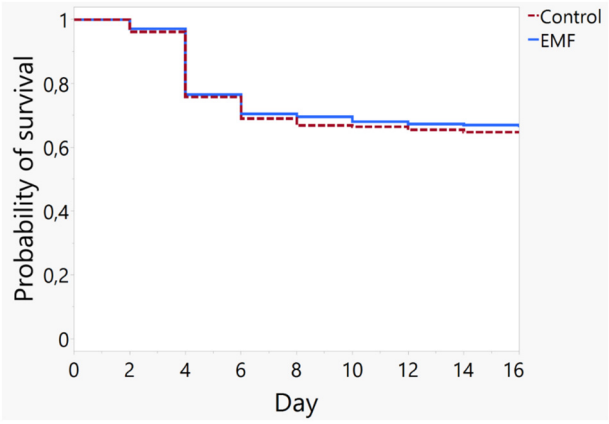


Fig. 5. The Kaplan-Meier-Survival analysis including a pairwise comparison and test for significance with Log-Rank (Cox-Mantel) test ($\alpha = 0.05$) revealed no significant differences of brood mortality over time between the two groups (EMF, Control). n = 8 colonies/each group.

Long-term RF-EMF exposure had a clear negative impact on the orientation ability of foraging honey bees (GLMM, $p = 0.0064$) (Fig. 6a + b). The number of bees that successfully found their way back to their hives differed significantly, with 95.2 % of the bees in the control group returning home successfully compared to only 78.6 % of the bees in the treatment group. The long-term treated bees also required approximately 20 % more time on their return homing flights compared to the controls (median: EMF 9:58 min vs. Control 8:14 min), however these differences were not significant (GLMM, $p = 0.4224$) (Fig. 6). When honey bees were only irradiated for a short time (40 min) and not for the entire brood and imaginal developmental period, the RF-EMF had no effect on the number of successfully returning bees (average: EMF 90.0 %, Control 86.6 %; GLMM, $p = 0.4696$), nor did it impact the time needed for the bees to return to their colony (GLMM, $p = 0.0596$) (Fig. 6, Table 3).

3.3. Longevity

To understand the impact of RF-EMF on adult bees, we tracked the longevity of newly emerged adults that were reared in other colonies without exposure during development and then placed into the treatment and control hives. The bees were accepted at the start of the experiment at similar rates (72.3 % control vs. 74.7 % EMF). Bees in the RF-EMF treated hives lived on average 17.7 ± 0.61 days compared to the slightly longer-lived controls with 19.0 ± 0.68 days. However, survival analysis over their entire lifespan of 28 days showed no significant differences between the treated and untreated group (Kaplan-Meier-Survival with the Log-Rank: $p = 0.102$), which indicates that the tested RF-EMF has no negative impact on overall life span. Honey bees typically spend the first 7–10 days engaged in in-hive tasks, in the relatively safe environment of the colony. As we showed above RF-EMF impacts homing activity, and thus the impact on longevity might only become noticeable once bees start foraging. We thus compared survival during the normal window when bees begin foraging (day 11 to 29) and found significant differences between the two groups ($p = 0.042$, Wilcoxon) (Fig. 7).

4. Discussion

Anthropogenic electromagnetic radiation is a form of environmental pollution that is suspected of having negative effects on flying insects including bees. We currently lack high-quality studies on the precise impact of electromagnetic fields (EMF) on bees. Broad consensus demands more robust research to understand the potential impacts of EMF on bee health coupled with an appropriately controlled setup of quantifiable exposure (Balmori, 2021; Malkemper et al., 2018; Vanbergen et al., 2019). We sought to help fill some of these knowledge gaps with our comprehensive

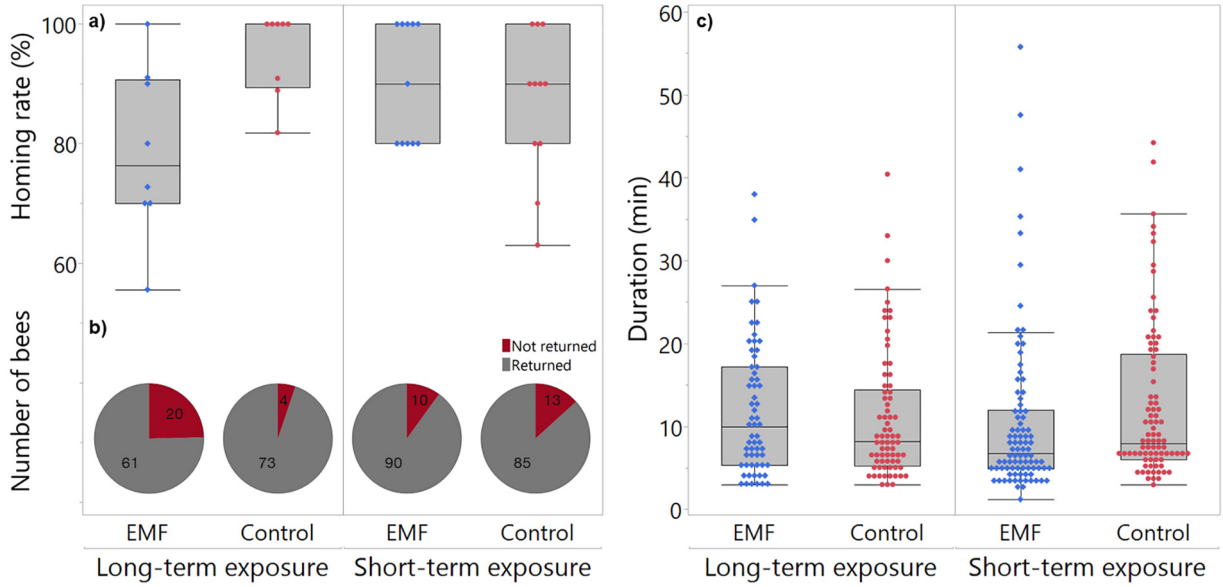


Fig. 6. The results show a) the homing rate, b) the absolute number of homing bees as well as c) the homing duration of the long-term and short-term exposure variants. a) Homing rate is defined as the return rate of released bees (%) per trial (long-term n = 8, short-term n = 11). c) The homing duration refers to the time (min) for individual bees to return to the colony. The absolute number of returned and missing bees can be found in the pie charts b).

study on the effects of field-realistic electromagnetic radiation at frequencies commonly used in Wi-Fi signals on honey bees, by evaluating how simultaneously exposures to 2.4 and 5.8 GHz frequencies affected homing ability, longevity, and brood development of honey bees under field conditions. Such exposure frequencies are fairly common in Wi-Fi devices (Khorov et al., 2020), ubiquitously present in the environment (Sagar et al., 2018), and are considered harmless to human health. But what are their impacts on honey bees? We chose these three key markers of health, because brood development gives insight into the potential growth of a colony, adult longevity allows us to determine if exposure could reduce lifespan and thus potentially lead to reduced productivity and if severe, colony collapse, and finally homing behavior, as the successful return of foragers to their colony is critical for normal colony development and provides insight into potential negative impacts on insect cognition and memory.

Much prior research into EMF used unpredictable and irregular radiation sources, which make the results difficult to evaluate. In our experiment, we used a high-end radiation source which generates a consistent and definable electromagnetic radiation developed in an interdisciplinary cooperation with electrical engineers (see methods for details). This source allowed a precise alignment of the radiation so that only the eight EMF colonies were impacted, eliminating site differences common in other EMF research where an exposure and a control location are compared. Our results clearly demonstrate a significant negative effect on the homing behavior of foraging bees, which could act as an additional stressor with potential consequences on healthy colony development.

Possible negative effects of EMF on animal orientation were already supposed in the review of Vanbergen et al. (2019) and summarized by Balmori (2015). Some animals can perceive electric or magnetic fields and EMF exposure might thus modify their orientation behavior as it interferes with their perception of the naturally occurring fields (Bae et al., 2016; Lambinet et al., 2017; Liang et al., 2016; Sutton et al., 2016; Zhang and Pan, 2021). To date little evidence exists on the effect of EMF on the orientation or homing behavior of wild bees or honey bees. Taye et al. (2017) showed an effect on returning ability (number of returning bees), but in the experiment they simply varied the distance of the colony location to a single mobile tower. In the non-peer reviewed study of Kimmel et al. (2007) the effects of 1900 MHz (DECT base stations, average power used in stand-by modus: 2.5 mW) on homing behavior were examined and a significant difference of approximately 14 % in homing duration and rate (calculated in one index) of individual marked bees was found between the treatment group and control. However, long-term and close exposure of bee colonies to the direct radiation from DECT base stations is not field realistic, as they only exist in homes and pollinators would have no exposure to them in the field. Despite the limitations of this study and the use of a different exposure scenario, we show similar negative impacts on homing ability, although bees with long-term RF-EMF exposure in our experiment had a reduced homing success of approximately 17 % compared to controls. More than 95 % of our control bees were able to successfully return to their colonies, confirming that the handling of the bees had a negligible effect. Although not significantly different, long-term RF-EMF exposed bees that did successfully return home required 1:44 min (median) longer than their control counterparts. The short-term exposure of only 40 min directly before the tests did not negatively impact homing rate. Because the long-term irradiated bees also received renewed irradiation for about 40 min before the homing flight, the exposure of the two variants (long-term/short-term) differed only in terms of the long-term exposure during development and in their early life as in-hive bees. Our results indicate that an exposure of 2.4 and 5.8 GHz over days or even weeks is

Table 3
Results of the GLMM with binomial (homing rate) and inverse Gaussian (duration, min) distribution. A model was estimated for the homing rate and homing duration of each exposure variant. Long-term: n = 8 trials, short-term: n = 11 trials. Estimate and p-value (*p < 0.05, **p < 0.01, ***p < 0.001) of the intercept and treatment (EMF, Control). Significant effects of the treatment are highlighted in bold.

Exposure variant	Coefficients	Homing rate	Homing duration
Long-term	Intercept	2.017*** (p = 0.0001)	0.008** (p = 0.0041)
	Treatment [EMF]	**−0.897** (p = 0.0064)**	−0.001 (p = 0.4224)
Short-term	Intercept	1.977*** (p = 0.0001)	0.009** (p = 0.0015)
	Treatment [EMF]	0.162 (p = 0.4696)	0.002 (p = 0.0596)

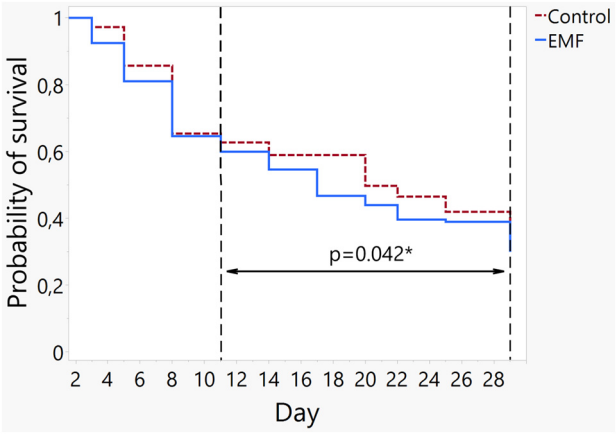


Fig. 7. The Kaplan-Meier-Survival analysis including a pairwise comparison and test for significance with Log-Rank (Cox-Mantel) test ($\alpha = 0.05$) revealed no significant differences in overall longevity between the two groups (EMF, Control). The partial analysis for the period from day 11 to 29 (possible flight period) with Wilcoxon test ($\alpha = 0.05$) shows significant differences between the two groups ($p = 0.042$). EMF n = 280 bees, Control n = 217 bees.

necessary for bees to lose significant homing ability and that there is no habituation effect to the radiation. This suggests that free-flying foragers from colonies not directly exposed to EMF would continue to return home successfully, even if their foraging flight trajectories took them through exposure fields for short durations. Prior studies have also shown effects on short-term memory in bees (Lopatina et al., 2019; Shepherd et al., 2018), which could translate into lower homing rates of foraging bees, as they need good spatial memory to navigate home. Exactly how EMF exposure impacts learning and memory is still unclear and would benefit from in-depth research into changes in neurochemical signaling, memory retention, learning acquisition, and gene expression patterns.

In our studies, RF-EMF did not impair overall longevity, confirming the laboratory results demonstrated by Westerdahl and Gary (1981) under field conditions. They found no effect of 2.45 GHz on longevity of honey bees, regardless of the different intensities of 3, 6, 12, 25, and 50 mW/cm^2 tested (Westerdahl and Gary, 1981). Another experiment that tested magnetic fields increased longevity, but this was accompanied with reduced flight activity (Martin et al., 1989), which inherently increases longevity (Neukirch, 1982). Since colonies exposed to RF-EMF had lower homing ability, we would expect this increased failure to return home to cascade into reduced longevity of the bees once they begin foraging. When survival was examined during this later life stage when flight activity is presumed to begin for the majority of bees (day 11 to 29), we see a significantly elevated death rate among the treatment bees compared to the controls. As losses did not occur in the early in-hive stages, we see this increased mortality later in life as a validation of reduced homing success in RF-EMF treated bees.

The development of healthy brood is critical for colony growth and consequently for the survival of honey bee colonies (Medrzycki et al., 2013). The impacts of electromagnetic radiation on brood development in *Drosophila* have been mixed with some studies showing reduced eclosion rates and delayed development, while others demonstrated a shortened development time (Agrawal et al., 2021; Wang et al., 2022; Zmejkoski et al., 2017). One study on honey bee development focused on queens and showed an increase in queen mortality during pupation under 900 MHz irradiation, however, the authors note that their testing scenario mimicked a worst-case situation and was not a realistic exposure (Odemer and Odemer, 2019). Even if the EMF of this frequency influence the oxidative stress response by changing antioxidant enzyme activity (Vilić et al., 2021), to our knowledge there is no further evidence of larval or pupal mortality of honey bees. We investigated the brood mortality rate of around 1600 individual bees per treatment in free-flying colonies based on an assessment method commonly used to document pesticide impacts on preimaginal stages of honey bees (Medrzycki et al., 2013; Schur et al., 2003) and found no difference in brood termination rates, demonstrating that RF-EMF had no measurable effect on ontogenesis. Future research should investigate the long-term effects on the total amount of brood produced and thus colony growth, as this was not addressed in our current experiment.

Honey bee colonies are robust and adaptable, because they have a large work force that can buffer and compensate some losses, reallocating workers to different tasks as needed. Unfortunately, solitary bees lack this buffer workforce (Straub et al., 2015) and may therefore be more affected by EMF. Future studies should investigate impacts on wild bees, especially those with solitary lifestyles and above-ground nesting species. Underground nests, such as those of *Bombus terrestris*, should be shielded from radiation and thus their brood would be less exposed, with lower potential for long-term impacts on homing success once they become active as adults, while those nesting above-ground would receive much higher exposure. As radiation clearly impacted homing ability in honey bees, we must investigate if the orientation and homing ability of solitary bees are negatively influenced by RF-EMF radiation.

It is worth noting that modern cellular mobile communications technology, i.e. 4G/LTE and 5G, use the same method of transporting data (OFDM), albeit at different parameterizations. It is reasonable to assume that a 4G/5G system working on the same frequency band as Wi-Fi will have similar effects, but the fact that the current spectrum allocations are in bands with frequencies ranging from the lower microwave region (412 MHz, LTE band 88) with wavelengths roughly six times that of the 2.4 GHz band used by Wi-Fi, to a 1/3 shorter wavelength (3.6 GHz, LTE band 43) (3GPP, 2023a) implies that inferring the effects of existing cellular infrastructure on organisms from the results of this study should be done under the premise of experimental verification. 5G opened up further bands for operation: While the New Radio (NR) bands of Frequency Range 1 (3GPP, 2023b) cover similar bands as LTE, they are especially utilized by the growing number of so-called campus networks. These are private installations of cellular infrastructure, especially in the context of industrial automatization, in bands very close in frequency to the 2.4 GHz band used by Wi-Fi. Here, further investigation using a similar experimental setup appears warranted. The bands of Frequency Range 2 (3GPP, 2023c) are above 20 GHz, and the physiological effects of these frequencies can be expected to differ from the effects causing the results of this study. As a general tendency, higher frequencies are more readily absorbed by the atmosphere, vegetation, etc., so spatial separation of the bee colony from the emitter will reduce exposure more drastically than in this study.

The effect of RF-EMF depends heavily on output power and frequency, as well as the consistency of the body absorbing the radiation. The type of frequency used influences the tissue penetration depth and which organisms can best absorb it depending on their body size (resonance). The resonance frequency specifies that a body whose size is close to half the length of the emitted frequency will have the highest specific energy absorption rate (ICNIRP, International Commission on Non-Ionizing Radiation Protection, 2020). For example, we can calculate that a bee is a much better receiver for 5.8 GHz (half wavelength = 2.6 cm) than bigger animals or humans and therefore absorbs more radiation energy. As body size also strongly influences absorption rate, results from one study organism cannot be easily transferred to another, highlighting that we need additional studies with different types of electromagnetic fields and their impacts on various insects.

Our study looked at the impacts of EMF exposure during development, and its influence in three key elements of honey bee health. However, we did not track the potential long-term consequences of continuous exposure on colony development throughout the beekeeping year and if sublethal losses of returning foragers impact colony overwintering strength, a key marker of winter survival. The topic should be investigated in long-term studies that track colony populations over time. Due to the intricacies of working with RF-EMF, we highly encourage interdisciplinary cooperations that overcome the high technical hurdles of a standardizable set up.

5. Conclusion

The complex effects of radiofrequency electromagnetic fields (RF-EMF) on insects needs increased attention. Our results suggest that there are no lethal or highly obvious impacts on honey bee health, however RF-EMF acts as a sublethal stressor, reducing the homing success of honey bees and thus generating a loss of foragers. This disorientation only occurs in colonies continuously exposed to the tested and field realistic RF-EMF, a signal commonly used for Wi-Fi. RF-EMF did not impact other important fitness parameters, such as the overall development of healthy brood or longevity. Due to their different nesting habitats and degrees of sociality, wild bees may be more susceptible to negative effects of electromagnetic radiation. These knowledge gaps must be urgently addressed, as many wild bee species are threatened by extinction. In our globalized and interconnected world, few environments are free of electromagnetic radiation. A better understanding of the sublethal effects of electromagnetic radiation exposure on communication, learning, memory, and development of bees and insects are urgently needed.

Funding

This work was funded by the Ministerium für Ernährung, Ländlichen Raum und Verbraucherschutz Baden-Württemberg.

CRediT authorship contribution statement

Manuel Treder: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Marcus Müller:** Methodology, Software, Resources, Writing – review & editing. **Larissa Fellner:** Investigation, Data curation. **Kirsten Traynor:** Validation, Writing – review & editing. **Peter Rosenkranz:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Our special thanks go to Laurent Schmalen and David Winter from the Communications Engineering Lab (CEL) at the Karlsruhe Institute of Technology for the advice and for providing the necessary technical equipment. We would also like to thank Vera Joedecke and Ute Ruttensperger from the State Horticultural College and Research Institute (LVG) Heidelberg for their support of our work, as well as the employees of the State Institute of Bee Research, Hohenheim, Germany.

References

3GPP, 2023a. TS 36.101: Evolved Universal Terrestrial Radio Access (E-UTRA); User Equipment (UE) radio transmission and reception. https://portal.3gpp.org/desktopmodules/Specifications/SpecificationDetails.aspx?specificationId=2411 (accessed 2023).

3GPP, 2023b. TS 38.101-1: NR; User Equipment (UE) radio transmission and reception; Part 1: Range 1 Standalone. https://portal.3gpp.org/desktopmodules/Specifications/SpecificationDetails.aspx?specificationId=3283 (accessed 2023).

3GPP, 2023c. TS 38.101-2: NR; User Equipment (UE) radio transmission and reception; Part 2: Range 2 Standalone. https://portal.3gpp.org/desktopmodules/Specifications/SpecificationDetails.aspx?specificationId=3284 (accessed 2023).

Agrawal, N., Verma, K., Baghel, D., Chauhan, A., Prasad, D.N., Sharma, S.K., et al., 2021. Effects of extremely low-frequency electromagnetic field on different developmental stages of Drosophila melanogaster. Int. J. Radiat. Biol. 97 (11), 1606–1616.

Bae, J.-E., Bang, S., Min, S., Lee, S.-H., Kwon, S.-H., Lee, Y., et al., 2016. Positive geotactic behaviors induced by geomagnetic field in Drosophila. Mol. Brain 9 (1), 55.

Balmori, A., 2015. Anthropogenic radiofrequency electromagnetic fields as an emerging threat to wildlife orientation. Sci. Total Environ. 518-519, 58–60.

Balmori, A., 2021. Electromagnetic radiation as an emerging driver factor for the decline of insects. Sci. Total Environ. 767, 144913.

Bandara, P., Carpenter, D.O., 2018. Planetary electromagnetic pollution: it is time to assess its impact. Lancet Planetary Health 2 (12), e512–e514.

Engels, S., Schneider, N.-L., Lefeldt, N., Hein, C.M., Zapka, M., Michalik, A., et al., 2014. Anthropogenic electromagnetic noise disrupts magnetic compass orientation in a migratory bird. Nature 509 (7500), 353–356.

Favre, D., 2011. Mobile phone-induced honeybee worker piping. Apidologie 42 (3), 270–279.

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, E., et al., 2013. Miscellaneous standard methods for Apis mellifera research. J. Apic. Res. 52 (4), 1–53.

ICNIRP, International Commission on Non-Ionizing Radiation Protection, 2020. Guidelines for limiting exposure to electromagnetic fields (100 kHz to 300 GHz). Health Phys. 118 (5), 483–524.

International Telecommunication Union, 2021. Facts and Figures: Measuring Digital Development. p. 2021.

Khorov, E., Levitsky, I., Akyildiz, I.F., 2020. Current Status and Directions of IEEE 802.11be, the Future Wi-Fi 7. IEEE Access. 8 pp. 88664–88688.

Kimmel, S., Kuhn, J., Harst, W., Stever, H., . Electromagnetic radiation: influences on honeybees (*Apis mellifera*) https://www.researchgate.net/publication/292405747 https://www.researchgate.net/publication/292405747 ILIAS-InterSymp Conference.

Lambinet, V., Hayden, M.E., Reigl, K., Gomis, S., Gries, G., 2017. Linking magnetite in the abdomen of honey bees to a magnetoreceptive function. Proc. Biol. Sci. 284 (1851).

Lázaro, A., Chroni, A., Tscheulin, T., Devalez, J., Matsoukas, C., Petanidou, T., 2016. Electromagnetic radiation of mobile telecommunication antennas affects the abundance and composition of wild pollinators. J. Insect Conserv. 20 (2), 315–324.

Liang, C.-H., Chuang, C.-L., Jiang, J.-A., Yang, E.-C., 2016. Magnetic sensing through the abdomen of the honey bee. Sci. Rep. 6, 23657.

Lopatina, N.G., Zachepilo, T.G., Kamyshev, N.G., Dyuzhikova, N.A., Serov, I.N., 2019. Effect of non-ionizing electromagnetic radiation on behavior of the honeybee, Apis mellifera L. (Hymenoptera, Apidae). Entmol. Rev. 99 (1), 24–29.

Malkemper, E.P., Tscheulin, T., Vanbergen, A.J., Vian, A., 2018. The impacts of artificial electromagnetic radiation on wildlife (flora and fauna).: current knowledge overview: a background document to the web conference. A Report of the EKLIPSE Project.

Martin, H., Korall, H., Föster, B., 1989. Magnetic field effects on activity and ageing in honeybees. J. Comp. Physiol. A 164, 423–431.

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L.P., Chauzat, M.-P., Claßen, C., et al., 2013. Standard methods for toxicology research in Apis mellifera. J. Apic. Res. 52 (4), 1–60.

Migdał, P., Murawska, A., Bieńkowski, P., Berbeć, E., Roman, A., 2021. Changes in honeybee behavior parameters under the influence of the E-field at 50 Hz and variable intensity. Animals 11 (2).

Migdał, P., Berbeć, E., Bieńkowski, P., Plotnik, M., Murawska, A., Latarowski, K., 2022. Exposure to magnetic fields changes the behavioral pattern in honeybees (Apis mellifera L.) under laboratory conditions. Animals 12 (7).

Molina-Montenegro, M.A., Acuña-Rodríguez, I.S., Ballesteros, G.I., Baldelomar, M., Torres-Díaz, C., Broitman, B.R., et al., 2023. Electromagnetic fields disrupt the pollination service by honeybees. Sci. Adv. 9(19):eadh1455.

Neukirch, A., 1982. Dependence of the life span of the honeybee (Apis mellifica) upon flight performance and energy consumption. J. Comp. Physiol. B. 146 (1), 35–40.

Odemer, R., Odemer, F., 2019. Effects of radiofrequency electromagnetic radiation (RF-EMF) on honey bee queen development and mating success. Sci. Total Environ. 661, 553–562.

Russell, C.L., 2018. 5 G wireless telecommunications expansion: public health and environmental implications. Environ. Res. 165, 484–495.

Sagar, S., Dongus, S., Schoeni, A., Roser, K., Eeftens, M., Struchen, B., et al., 2018. Radiofrequency electromagnetic field exposure in everyday microenvironments in Europe: a systematic literature review. J. Expo. Sci. Environ. Epidemiol. 28 (2), 147–160.

Scheiner, R., Abramson, C.I., Brodschneider, R., Crailsheim, K., Farina, W.M., Fuchs, S., et al., 2013. Standard methods for behavioural studies of Apis mellifera. J. Apic. Res. 52 (4), 1–58.

Schur, A., Tornier, I., Brasse, D., Mühlen, W., Ohe, W. von der, Wallner, K., et al., 2003. Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions. Bull. Insectology 56, 91–96.

Shepherd, S., Lima, M.A.P., Oliveira, E.E., Sharkh, S.M., Jackson, C.W., Newland, P.L., 2018. Extremely low frequency electromagnetic fields impair the cognitive and motor abilities of honey bees. Sci. Rep. 8 (1), 7932.

Shepherd, S., Hollands, G., Godley, V.C., Sharkh, S.M., Jackson, C.W., Newland, P.L., 2019. Increased aggression and reduced aversive learning in honey bees exposed to extremely low frequency electromagnetic fields. PLoS One 14 (10), e0223614.

Straub, L., Williams, G.R., Pettis, J., Fries, I., Neumann, P., 2015. Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. Curr. Opin. Insect Sci. 12, 109–112.

Sutherland, W.J., Butchart, S.H.M., Connor, B., Culshaw, C., Dicks, L.V., Dinsdale, J., et al., 2018. A 2018 horizon scan of emerging issues for global conservation and biological diversity. Trends Ecol. Evol. 33 (1), 47–58.

Sutton, G.P., Clarke, D., Morley, E.L., Robert, D., 2016. Mechanosensory hairs in bumblebees (*Bombus terrestris*) detect weak electric fields. Proc. Natl. Acad. Sci. U. S. A. 113 (26), 7261–7265.

Taye, R.R., Deka, M.K., Rahman, A., Bathari, M., 2017. Effect of electromagnetic radiation of cell phone tower on foraging behaviour of Asiatic honey bee, *Apis cerana* F. (Hymenoptera: Apidae). J. Entomol. Zool. 5 (3), 1527–1529.

Thielens, A., Greco, M.K., Verloock, L., Martens, L., Joseph, W., 2020. Radio-frequency electromagnetic field exposure of Western honey bees. Sci. Rep. 10 (1), 461.

Toribio, D., Joseph, W., Thielens, A., 2022. Near field radio frequency electromagnetic field exposure of a Western honey bee. IEEE Trans. Antennas Propagat. 70 (2), 1320–1327.

Vanbergen, A.J., Potts, S.G., Vian, A., Malkemper, E.P., Young, J., Tscheulin, T., 2019. Risk to pollinators from anthropogenic electro-magnetic radiation (EMR): evidence and knowledge gaps. Sci. Total Environ. 695, 133833.

Vijver, M.G., Bolte, J.F.B., Evans, T.R., Tamis, W.L.M., Peijnenburg, W.J.G.M., Musters, C.J.M., et al., 2014. Investigating short-term exposure to electromagnetic fields on reproductive capacity of invertebrates in the field situation. Electromagn. Biol. Med. 33 (1), 21–28.

Vilić, M., Tlak Gajger, I., Tucak, P., Štambuk, A., Šrut, M., Klobučar, G., et al., 2017. Effects of short-term exposure to mobile phone radiofrequency (900 MHz) on the oxidative response and genotoxicity in honey bee larvae. J. Apic. Res. 56 (4), 430–438.

Vilić, M., Žura Žaja, I., Tkalec, M., Štambuk, A., Šrut, M., Klobučar, G., et al., 2021. Effects of a radio frequency electromagnetic field on honey bee larvae (Apis mellifera) differ in relation to the experimental study design. Vet. Arhiv. 91 (4), 427–435.

Wang, Y., Jiang, Z., Zhang, L., Zhang, Z., Liao, Y., Cai, P., 2022. 3.5-GHz radiofrequency electromagnetic radiation promotes the development of Drosophila melanogaster. Environ. Poll. (Barking, Essex 1987) (294), 118646.

Westerdahl, B.B., Gary, N.E., 1981. Longevity and food consumption of microwave-treated (2.45 GHz CW) honeybees in the laboratory. Bioelectromagnetics 2 (4), 305–314.

Zhang, Y., Pan, W., 2021. Removal or component reversal of local geomagnetic field affects foraging orientation preference in migratory insect brown planthopper Nilaparvata lugens. PeerJ 9, e12351.

Zmejkoski, D., Petković, B., Pavković-Lučić, S., Prolić, Z., Anđelković, M., Savić, T., 2017. Different responses of Drosophila subobscura isofemale lines to extremely low frequency magnetic field (50 Hz, 0.5 mT): fitness components and locomotor activity. Int. J. Radiat. Biol. 93 (5), 544–552.