Indian Journal of Experimental Biology
Vol. 61, December 2023, pp. 941-945
DOI: 10.56042/ijeb.v61i12.4370

No mutation effect of 50 Hz sinusoidal magnetic field on beta catenin gene phosphorylation site in N-methyl-N-nitrosourea (MNU) induced colon tumor model

Metin Budak[1,2]*, Mahmut Alp Kılıç[3], Tunaya Kalkan[4] & Handan Tuncel[5]

[1]Department of Biophysics, Faculty of Medicine, Trakya University, Edirne, Turkey
[2]Mirko Tos Ear & Hearing Research Center, Trakya University, Edirne, Turkey
[3]Department of Biophysics, School of Medicine, Aydin Adnan Menderes University, Aydin, Turkey
[4]Department of Biophysics, Medical Faculty, Istanbul Aydin University, Istanbul, Turkey
[5]Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University Cerrahpasa, Istanbul, Turkey

Received 06 April 2023; *revised 12 November 2023*

The dysregulation of beta-catenin, a key regulator of cadherin-mediated cell adhesion and crucial for embryonic development and adult tissue processes, has been implicated in various cancers, including colon cancer. Meanwhile, there have been longstanding concerns about the potential carcinogenic effects of magnetic fields. In this study, we investigated the possible relationship between beta-catenin dysfunction and 50 Hz sinusoidal magnetic fields (SMF) using an animal model of N-methyl-N-nitrosourea (MNU)-induced rat colon tumors. To assess beta-catenin phosphorylation, genomic DNA was extracted from 58 samples using a commercial extraction kit, and the target gene region corresponding to an important phosphorylation site of beta-catenin was amplified via polymerase chain reaction (PCR). The amplified samples were subsequently analyzed using the single-strand conformation polymorphism (SSCP) method to detect any differences between the experimental groups. Surprisingly, our results revealed no significant differences in beta-catenin gene phosphorylation sites among the groups. These findings suggest that 50 Hz SMF exposure may not directly impact beta-catenin dysfunction in the context of MNU-induced rat colon tumors. Implications of these results and avenues for further research are discussed.

Keywords: Colorectal cancer, Electromagnetic radiation, Single-strand conformation polymorphism (SSCP)

Fifty Hz sinusoidal magnetic fields (SMFs) are a common type of electromagnetic field emitted from sources such as power lines, electronic devices, and industrial equipment[1]. The potential health effects of exposure to magnetic fields at this frequency, particularly their association with cancer, have been a subject of debate[2,3]. Some epidemiological studies have suggested that exposure to 50 Hz magnetic fields may increase the risk of cancer, including childhood leukemia, brain tumors and lymphoma[4,5]. However, the results of these studies are inconsistent, and a definitive cause-and-effect relationship has not been established due to methodological limitations. Experimental studies have demonstrated that 50 Hz magnetic fields can affect the growth and spread of cancer cells in cell cultures and animal models. However, their direct applicability and clinical significance in humans remain uncertain. Therefore, the findings regarding the association between 50 Hz sinusoidal magnetic fields and cancer are a complex topic that requires further research[6].

Cancers of colon, rectum and anal are grouped as colorectal cancers (CRC)[7]. Unhealthy lifestyles, increasing levels of environmental pollution and occupational hazards, use of contaminated syringes in healthcare settings and infections such as HIV, Hepatitis B and C in addition to genetics and ethnic backgrounds have pushed Asia ahead of the West in total number of cancer cases, particularly lung and colorectal. With a share of 10.6%, colorectal cancer ranks third most common cancer in Asia next only to lung and breast cancer[7]. Epidemiological studies indicate that colorectal cancer is more common in Arab nations[7], precisely, in societies with a diet high in fat and low in fiber[8]. United States report 153,020 estimated new cases of CRC and 52,550 fatalities[9]. Some familial cancer syndromes specifically increase the risk of colon cancer, such as Familial Adenomatous Polyposis Coli (FAP) and Hereditary Non-Polyposis Colon Cancer (HNPCC)[10].

*Correspondence:
E-Mail: genomicdna2@yahoo.com

Cell adhesion molecules play a crucial role in development, cell differentiation, and carcinogenesis. Wnt/β-catenin signaling is essential during embryonic development and homeostasis in adult tissues. β-Catenin, encoded by the CTNNB1 gene, is a key protein involved in cell adhesion. Dysregulation of β-catenin leads to abnormal tissue architecture[11-13]. In the absence of Wnt ligands, β-catenin forms a complex called the "β-catenin destruction complex" with other proteins such as glycogen synthesis kinase 3, casein kinase 1, adenomatus polyposis coli and axin[14,15]. Phosphorylation of β-catenin triggers its degradation, while binding of Wnt ligands prevents this degradation and allows β-catenin to accumulate in the nucleus. In the nucleus, β-catenin interacts with T-cell factor/lymphocyte enhancer factor and activates target genes involved in cell growth and survival[16,17]. Alterations in Wnt/β-catenin signaling have been observed in various cancers, including colon cancer[18]. However, the specific role of Wnt/β-catenin signaling in colonospheres remains unknown. Magnetic fields have been a topic of controversy in terms of their potential carcinogenic effects. Some studies suggest a possible association between magnetic fields and certain types of cancer[19,20]. However, the relationship between magnetic fields and colon cancer has not been studied in detail. In this study, we investigated the potential relationship between colon tumors and 50 Hz sinusoidal magnetic fields using the β-catenin gene in an MNU-promoted colon tumor model.

Materials and Methods

Samples

We used 58 formalin-fixed paraffin-embedded *Wistar albino* rat colon tissues that were obtained from a previous study[20]. Briefly, the animals were divided into 4 groups viz.: Control with sample codes M1-M10 (n=10); M1-M24, SMF Group (n=14); M25-M40, SMF+MNU Group (n=16); and M41-M58, MNU Group (n=18). The sinusoidal magnetic fields (SMF) and SMF+MNU groups were exposed to a 5 millitesla (mT), 50 Hertz (Hz) sinusoidal magnetic field for 8 months. The preparation of the rat colon tumor model, paraffin embedding procedure, and magnetic field application are described in the previous study[21].

DNA extraction

Formalin-fixed, paraffin-embedded (FFPE) colon tissues were cut into 4 μm thick slices and placed on microscope slides. Then, the microscope slides were immersed in clean xylene for 10 min twice, to remove the xylene from the samples. Subsequently, the slides were immersed in 100% ethanol for 10 min, twice, to further remove any remaining xylene[22]. After the deparaffinization step, the slides were allowed to dry at room temperature (20-25°C). For DNA extraction, the Qiagen Qiamp DNA FFPE Tissue Kit (Venlo, Netherlands) was used, following the manufacturer's instructions.

PCR amplification

DNA was amplified with primers designed to produce a 150 bp product of the β-catenin gene corresponding to functionally important phosphorylation sites in β-catenin[23]. The forward primer sequence was 5'-ggagttggacatggccatgg-3', and the reverse primer sequence was 5'-tccacatcctcttcctcagg-3'. Briefly, each 50 μL reaction mixture contained 5 μL of 10X DreamTaq™ Green Buffer, 5 μL of 2 mM dNTP Mix, 0.5 μM of each forward and reverse primer, 1 μg of template DNA, and 1.25 U of DreamTaq™ DNA Polymerase (Fermentase - Lithuania). Nuclease-free water was added to bring the total volume to 50 μL. After an initial denaturation at 94°C for 3 min, 30 cycles of PCR were conducted as follows: 94°C for 30 s, 57°C for 30 s, and 72°C for 60 s, followed by a final extension at 72°C for 1 min[24].

Single-strand conformation polymorphism (SSCP)

SSCP is used to observe any differences between the 4 groups. Genomic DNA was isolated as described above. 10 μL of each PCR product was mixed with 10 μL of denaturing buffer (95% formamide, 10 mM NaOH, 0.25% bromphenol blue, and 0.25% xylene cyanol) and denatured by heating at 95°C for 10 min. Subsequently, the samples were rapidly cooled on ice[25]. The denatured PCR samples were run on a 12% acrylamide/bis gel in 0.5X TBE buffer for 3.5 hours at 200 V at room temperature with a water cooling system. After the electrophoresis, the gel was stained using the silver staining method[25,26].

Results

The PCR-SSCP analysis to identify potential mutations in the beta-catenin gene in a colon cancer model encountered some technical challenges. A total of 15 samples from the experimental and control groups failed PCR amplification due to issues arising from the paraffin fixation methods. These failures were eliminated from further evaluations. PCR amplification was successfully performed on the remaining 43 samples. However, no significant difference was observed in the SSCP analysis among

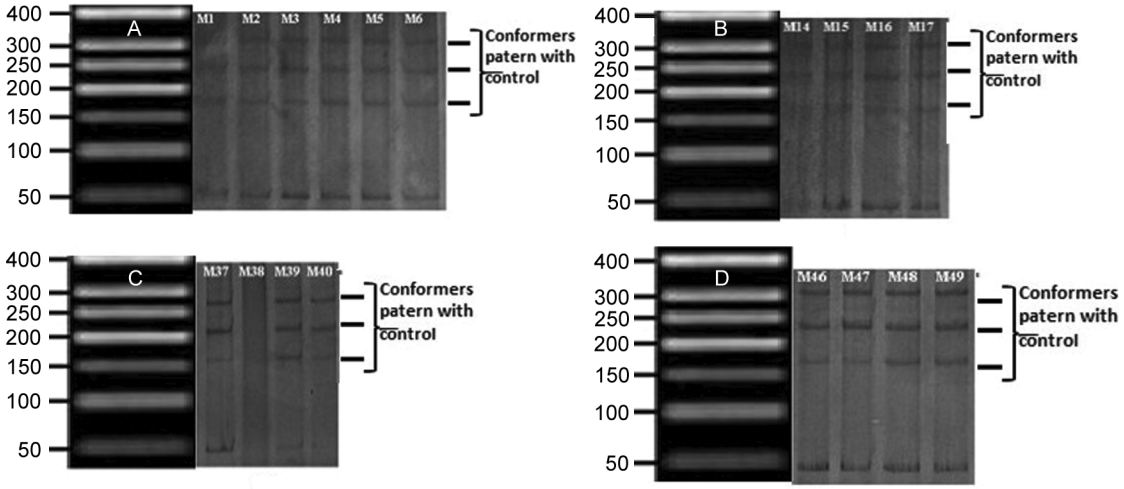

Fig. 1 — single-strand conformation polymorphism (SSCP) results of control and experiment groups; (A) Control Group; (B) SMF Group; (C) SMF+MNU Group; and (D) MNU Group (First Lines is control lines).

these samples. The absence of a notable difference between the groups was considered a crucial finding to enhance the reliability of the procedures conducted. The final distribution of the groups is as follows: Control, 7 samples; 11 each for SMF and SMF+MNU Groups; and 14 samples in MNU Group. SSCP results are visualized in Fig. 1. Despite the technical challenges of the PCR-SSCP method used to detect mutations in the beta-catenin gene under the influence of a 50 Hz magnetic field, these findings support the reliability of the obtained data.

Discussion

Colon cancer is one of the most commonly reported disorders associated with β-Catenin dysfunction. Nuclear accumulation and excessive activation of β-catenin play a critical role in the pathogenesis of colon cancer. Activating mutations disrupt the Wnt/β-catenin signaling pathway, leading to aberrant cell proliferation and promoting the growth of cancer cells[27-29]. β-Catenin protein, the product of the *CTNNB1* gene, is an important regulatory adhesion molecule that helps define cell proliferation[30,31]. β-Catenin protein is known as a regulator of cadherin-mediated cell adhesion, which is an intercellular adhesion system[32]. The *CTNNB1* gene has been identified as the gene that encodes the β-catenin protein. This protein plays a significant role in cell proliferation, differentiation, and vital developmental processes[33-35]. However, abnormal functions of β-catenin are frequently observed in various cancer types. Additionally, E-cadherin is an important adhesion molecule that binds with β-catenin in adherens junctions. Miscoding or dysfunction of the *CTNNB1* gene has been reported in various types of cancer, and it is frequently detected in human colon cancer cases[21,35,36]. N-Methyl-N-nitrosourea (MNU) is a chemical agent widely used in experimental cancer models. MNU can induce cancer formation by alkylating DNA. The use of MNU in colon cancer models provides an opportunity to investigate the relationship between β-catenin dysfunction and cancer development[37,38]. Several studies have reported frequent observations of excessive activation of β-catenin in MNU-induced colon cancer models. Mutations in the β-catenin gene, in particular, can lead to the overactivation of the Wnt/β-catenin signaling pathway and may play a significant role in the pathogenesis of colon cancer[39,40].

In this paper, we explored the relationship between the β-catenin gene and the potential cancer-inducing effect of magnetic fields on N-methyl-N-nitrosourea (MNU)-induced rat colon tumor models. In a previous study by our group, elevated E-cadherin expression was observed in the nucleus and cytoplasm of the SMF+MNU group[21]. However, in the present study, no differences were observed in the investigated phosphorylation site of the β-catenin gene between these groups and the control group. Shimizu and colleagues investigated another phosphorylation site of the β-catenin gene in MNU-induced gastric carcinomas[41]. According to their study, MNU-induced gastric carcinomas showed no changes in the investigated phosphorylation site. As a result, the MNU-induced colon tumor model that we used may not be suitable for investigating β-catenin disorders.

In this study, we conducted a comprehensive investigation on the potential effects of 50 Hz electromagnetic waves, a non-ionizing radiation type, on the β-catenin gene in rat colon tumor models. Our findings clearly demonstrated that exposure to these electromagnetic waves did not result in any significant alterations or disruptions in the expression or functionality of the β-catenin gene within the tumor models. Neither SMF nor MNU+SMF had an effect on the investigated phosphorylation site of the β-catenin gene. Some epidemiological studies propose that exposure to 50 Hz magnetic fields may increase the risk of cancer, such as childhood leukemia, brain tumors and lymphoma. However, due to methodological limitations and conflicting findings in these studies, the results are not sufficiently conclusive[4,42]. Experimental studies conducted on cell cultures and animal models have demonstrated that exposure to 50 Hz magnetic fields can influence the growth and spread of cancer cells. However, the direct applicability of these findings to humans remains uncertain[43,44].

Conclusion

In this study, exposure to 50 Hz electromagnetic waves did not induce significant alterations or disruptions in the expression or functionality of the β-catenin gene within the tumor models. Neither SMF nor MNU+SMF had an effect on the investigated phosphorylation site of the β-catenin gene. Though the results of this study provide valuable insights into the safety profile of 50 Hz electromagnetic waves regarding their impact on the molecular mechanisms associated with the β-catenin gene, it only represents a preliminary step in understanding the relationship between electromagnetic waves and the β-catenin gene. Further in-depth investigations are warranted to explore potential interactions, mechanisms and long-term effects. Future studies can focus on elucidating other molecular pathways, evaluating different exposure durations or intensities, and examining additional cancer models to enhance our understanding of the broader implications and mechanisms underlying the effects of electromagnetic waves on genetic regulation.

Acknowledgement

This study was funded by Istanbul University Research Fund (Project No: 10808 and BYP-415/060504 and) Cerrahpasa Medicine Faculty Ethics Committee Permission no: 790-231)

Conflict of Interest

Authors declare no competing interests.

References

1 Calabrò E & Magazu S, Infrared spectroscopic demonstration of magnetic orientation in SH-SY5Y neuronal-like cells induced by static or 50 Hz magnetic fields. *Int J Radiat Biol*, 95 (2019) 781.
2 Lundberg L, Sienkiewicz Z, Anthony DC & Broom KA, Effects of 50 Hz magnetic fields on circadian rhythm control in mice. *Bioelectromagnetics*, 40 (2019) 250.
3 Sztafrowski D & Jakubaszko J, Survey identification of impact the 50 Hz magnetic field on selected biochemical parameters of human blood. *J Phys Conf Ser*, 1782 (2021) 012038.
4 Touitou Y & Selmaoui B, The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. *Dialogues Clin Neurosci*, 4 (2022) 381.
5 Takeshima H & Ushijima T, Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. *NPJ Precis Oncol*, 3 (2019) 7.
6 Fei Y, Su L, Lou H, Zhao C, Wang Y & Chen G, The effects of 50 Hz magnetic field–exposed cell culture medium on cellular functions in FL cells. *J Radiat Res*, 60 (2019) 424.
7 Rajappa S, Singh M, Uehara R, Schachterle SE & Setia S. Cancer incidence and mortality trends in Asia based on regions and human development index levels: an analyses from GLOBOCAN 2020. *Curr Med Res Opin*, 39 (2023) 1127. doi: 10.1080/03007995.2023.2231761.
8 Huang P & Liu Y, A reasonable diet promotes balance of intestinal microbiota: prevention of precolorectal cancer. *BioMed Res Int*, 1 (2019). 3405278
9 Siegel RL, Miller KD, Wagle NS & Jemal A, Cancer statistics, 2023. *CA Cancer J Clin*, 73 (2023) 17. doi: 10.3322/caac.21763.
10 Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S & Al Moustafa A-E, Molecular mechanisms of colon cancer progression and metastasis: recent insights and advancements. *Int J Mol Sci*, 22 (2020) 130.
11 Schaefer KN & Peifer M, Wnt/Beta-catenin signaling regulation and a role for biomolecular condensates. *Dev Cell*, 48 (2019) 429.
12 Gires O, Pan M, Schinke H, Canis M & Baeuerle PA, Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? *Cancer Metastasis Rev*, 39 (2020) 969.
13 Prasannaraj G & Prabhakaran V, Mild overexpression of a novel transcription factor Grainy head like-2 (Grhl2) elicits hybrid phenotype in human astrocytoma cells. *Indian J Exp Biol*, 60 (2022) 386.
14 Jung Y-S & Park J-I, Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond β-catenin and the destruction complex. *Exp Mol Med*, 52 (2020) 183.
15 Zhang X, Dong NZ & Hu XY, Wnt/β-catenin Signaling Inhibitors. *Curr Top Med Chem*, 23 (2023) 896.
16 Keshavarzi M, Moradbeygi F, Mobini K, Ghaffarian AB, Mohammadi P, Ghaedi A & Mohammadi-Bardbor A, The interplay of aryl hydrocarbon receptor/WNT/CTNNB1/ Notch signaling pathways regulate amyloid beta precursor

mRNA/protein expression and effected the learning and memory of mice. *Toxicol Res*, 11 (2022) 147.
17 Nakayama M, Oshima H & Oshima M, Gain-of-function mutant p53 hyperactivates Wnt signaling in colon cancer cells. *Can Sci*, 113 (2022) 958.
18 Wang B, Li X, Liu L & Wang M, β-Catenin: oncogenic role and therapeutic target in cervical cancer. *Biol Res*, 53 (2020) 33.
19 Li P, McLaughlin J & Infante-Rivard C, Maternal occupational exposure to extremely low frequency magnetic fields and the risk of brain cancer in the offspring. *CCC*, 20 (2009) 945.
20 Seomun G, Lee J & Park J, Exposure to extremely low-frequency magnetic fields and childhood cancer: A systematic review and meta-analysis. *Plos One*, 16 (2021) 1.
21 Budak M, Korpinar M, Kalkan T & Tuncel H, Mutation detection in the promoter region of survivin gene on N-methyl-N-nitrosourea induced colon tumor model in experiment. *Bratisl Lek Listy*, 115 (2014) 554.
22 Topyalin N, Budak M, Ozbay N, Yildiz M, Kaner T, Aydin A & Gezen AF, A comparative histopathological and immunohistochemical study of Survivin and Ki-67 proteins in glial tumours. *Biotechnol Biotechnol Equip*, 33 (2019) 504.
23 van der Wal T & van Amerongen R, Walking the tight wire between cell adhesion and WNT signalling: A balancing act for β-catenin. *Open Biol*, 10 (2020) 200267.
24 Magnuson B, South E, Exon J, Dashwood RH, Xu M, Hendrix K & Hubele S, Increased susceptibility of adult rats to azoxymethane-induced aberrant crypt foci. *Cancer Lett*, 161 (2000) 185.
25 Dorjay C & Abraham BL, Association of POU1F1 gene polymorphism with pre-pubertal body weight in Attappady Black goats of India. *Small Rumin Res*, 211 (2022) 106681.
26 Mayta ML, Dotto M, Orellano EG & Krapp AR, An experimental protocol for teaching CRISPR/Cas9 in a post-graduate plant laboratory course: An analysis of mutant-edited plants without sequencing. *Biochem Mol Biol Educ*, 50 (2022) 537.
27 Yan R, Zhu H, Huang P, Zhang W, Hao P & Qu Y, Liquidambaric acid inhibits Wnt/β-catenin signaling and colon cancer via targeting TNF receptor-associated factor 2. *Cell Rep*, 38 (2022) 110319.
28 Harini KS & Ezhilarasan D, Wnt/beta-catenin signaling and its modulators in nonalcoholic fatty liver diseases. *Hepatobiliary Pancreat Dis Int*, 22 (2022) 333.
29 Yildiz F, Eciroglu H, Övey IS & Avnioglu S, Effect of combination treatment of protocatechuic acid with 5-fluorouracil and oxaliplatin on colon cancer Caco-2 cell line. *Indian J Exp Biol*, 61 (2023) 151.
30 Nie Q, Peng WW, Wang Y, Zhong L, Zhang X & Zeng L, β-catenin correlates with the progression of colon cancers and berberine inhibits the proliferation of colon cancer cells by regulating the β-catenin signaling pathway. *Gene*, 818 (2022) 146207.
31 Schmidt A, da Silva Brito WA, Singer D, Mühl M, Berner J, Saadati F, Wolff C, Miebach L, Wende K & Bekeschus S, Short-and long-term polystyrene nano-and microplastic exposure promotes oxidative stress and divergently affects skin cell architecture and Wnt/beta-catenin signaling. *Part Fibre Toxicol*, 20 (2023) 1.
32 Gehren AS, de Souza WF, Sousa-Squiavinato AC, Diego AAR, Bruno RBP, Eliana SFWA & Jose AMD, Parthenolide inhibits proliferation and invasion, promotes apoptosis, and reverts the cell–cell adhesion loss through downregulation of NF-κB pathway TNF-α-activated in colorectal cancer cells. *Cell Biol Int*, 9 (2023) 1638.
33 Zhao P, Sun L & Zhao C, TCF1/LEF1 triggers Wnt-dependent chemokine/cytokine-induced inflammation and cadherin pathways to drive T-ALL cell migration. *Biochem Biophys Rep*, 34 (2023) 101457.
34 Parker J, Hockney S, Blaschuk OW & Pal D, Targeting N-cadherin (CDH2) and the malignant bone marrow microenvironment in acute leukaemia. *Expert Rev Mol Med*, 25 (2023) 1.
35 Dumitru CS, Ceausu AR, Comsa S & Raica M, Loss of E-Cadherin expression correlates With Ki-67 in head and neck squamous cell carcinoma. *In Vivo*, 36 (2022) 1150.
36 Pal I, Rajesh Y, Banik P, Dey G, Dey KK, Bharti R, Naskar D, Chakraborty S, Ghosh SK, Das SK, Emdad L, Kundu SC, Fisher PB & Mandal M, Prevention of epithelial to mesenchymal transition in colorectal carcinoma by regulation of the E-cadherin-β-catenin-vinculin axis. *Cancer Lett*, 452 (2019) 254.
37 Moon RC & Mehta RG, Cancer chemoprevention by retinoids: animal models. *Meth Enzymol*, 190 (1990) 395.
38 Yusuf A, Odeh OE, Alhassan SO & Atawodi SEO, Evaluation of the preventive potential of graded dietary inclusion of Hyphaene thebaica (Linn) fruit in rat model of colon carcinogenesis. *J Food Biochem*, 46 (2022) e14446.
39 Takasu S, Tsukamoto T, Cao XY, Toyoda T, Hirata A, Ban H, Yamamoto M, Sakai H, Yanai T, Masegi T, Oshima M & Tatematsu M, Roles of cyclooxygenase-2 and microsomal prostaglandin E synthase-1 expression and β-catenin activation in gastric carcinogenesis in N-methyl-N-nitrosourea-treated K19-C2mE transgenic mice. *Cancer Sci*, 99 (2008) 2356.
40 Ji L, Qian W, Gui L, Ji Z, Yin P, Lin GN, Wang Y, Ma B & Gao WQ, Blockade of β-catenin–induced CCL28 suppresses gastric cancer progression via inhibition of Treg cell infiltration. *Cancer Res*, 80 (2020) 2004.
41 Shimizu M, Suzui M, Moriwaki H, Mori H & Yoshimi N, No involvement of β-catenin gene mutation in gastric carcinomas induced by N-methyl-N-nitrosourea in male F344 rats. *Cancer Lett*, 195 (2003) 147.
42 Swanson J, Kheifets L & Vergara X, Changes over time in the reported risk for childhood leukaemia and magnetic fields. *J Radioll Prot*, 39 (2019) 470.
43 Bodewein L, Schmiedchen K, Dechent D, Stunder D, Graefrath D, Winter L, Kraus T & Driessen S, Systematic review on the biological effects of electric, magnetic and electromagnetic fields in the intermediate frequency range (300 Hz to 1 MHz). *Environ Res*, 171 (2019) 247.
44 Zadeh-Haghighi H & Simon C, Magnetic field effects in biology from the perspective of the radical pair mechanism. *J R Soc Interface*, 19 (2022) 20220325.